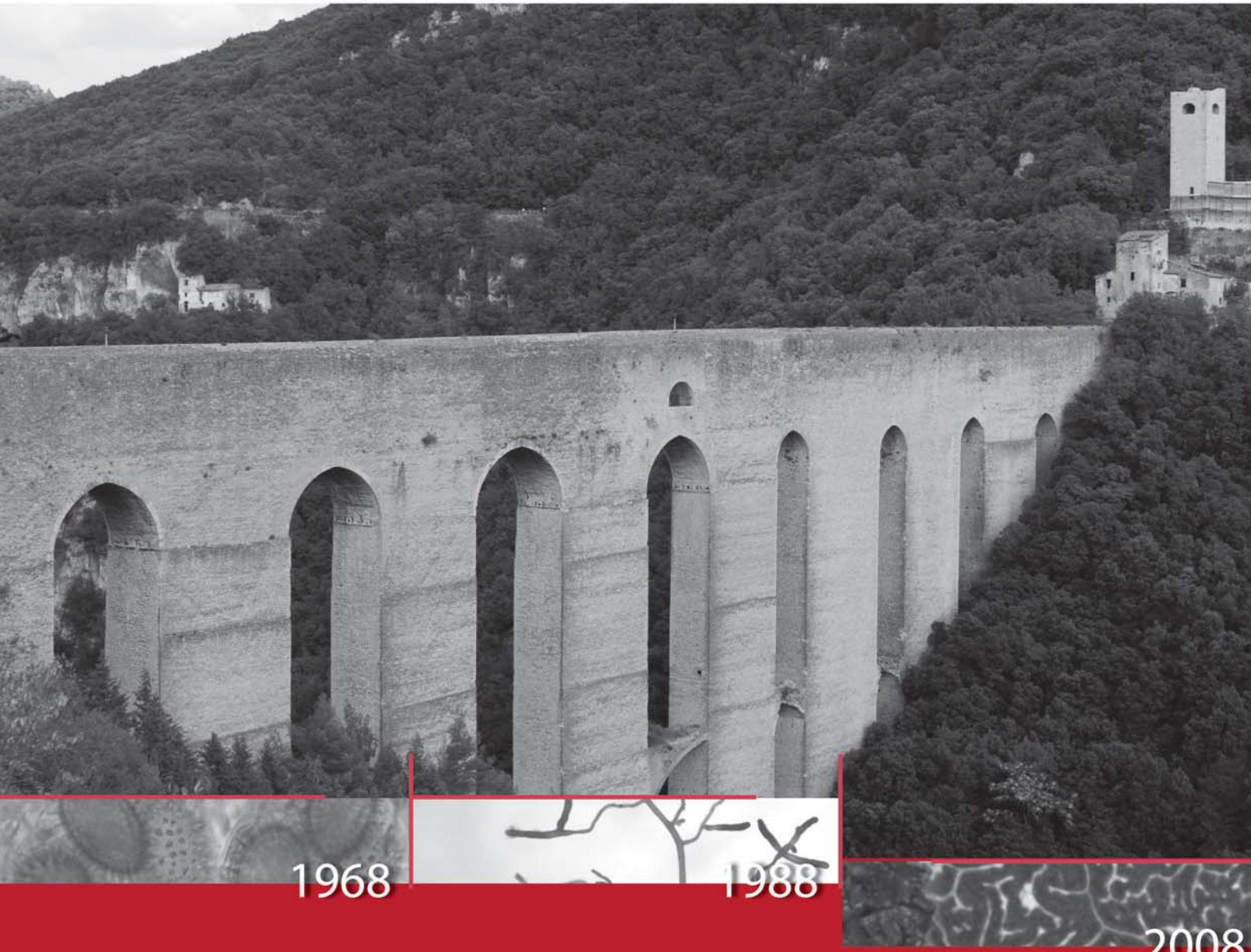
SPOLETO
25-28 NOVEMBRE 2008
CHIOSTRO DI SAN NICOLO'

TUBER 2008

ATTI
3° CONGRESSO INTERNAZIONALE DI SPOLETO SUL TARTUFO



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Comunità Montana
dei Monti Martani, Serano e Subasio

Università degli Studi di Perugia

Comunità Montana dei Monti Martani Serano e Subasio

3° CONGRESSO INTERNAZIONALE DI SPOLETO SUL TARTUFO

SPOLETO, 25-28 NOVEMBRE 2008
CHIOSTRO DI SAN NICOLO'

a cura di: Donnini Domizia
con la collaborazione di Baciarelli Falini Leonardo, Bencivenga Mattia, Di Massimo Gabriella

Comunità Montana dei Monti Martani Serano e Subasio

Università degli Studi di Perugia

ORGANIZZAZIONE DEL CONGRESSO

Comunità Montana dei Monti Martani e del Serano in collaborazione con il Dipartimento di Biologia Applicata dell'Università degli Studi di Perugia.

UFFICIO DI PRESIDENZA

Presidente: Vincenza Campagnani
Vicepresidente: Mattia Bencivenga
Segretario: Alvaro Paggi

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Prefazione

A 20 anni di distanza dal Secondo Congresso Internazionale sul Tartufo, che si tenne a Spoleto nel 1988, il settore tartufi e tartuficoltura ha avuto un grande sviluppo che ha riguardato l'intera filiera.
Lo sviluppo dell'interesse scientifico viene dimostrato dai simposi tra gli esperti di vari paesi che si sono tenuti nell'ultimo ventennio: il Congresso Internazionale di Aix-en-Provence (1999) che ha visto la presentazione di 126 relazioni e comunicazioni da parte di studiosi provenienti da tutte le parti del mondo, i simposi biennali (International Workshop on Edible Mycorrhizal Mushrooms) che si sono tenuti in Svezia nel 1988, in Nuova Zelanda nel 2001, in Canada nel 2003, in Spagna nel 2005, in Cina nel 2007 e la conferenza mondiale sulla conservazione e l'uso sostenibile dei funghi spontanei che si è tenuta a Cordoba nel 2007. Vanno ricordati poi i convegni a carattere nazionale, regionale e locale che si tengono continuamente in Italia e nelle altre nazioni tartufigene. Il tartufo ha richiamato l'interesse di nuovi settori scientifici, come la genetica che ha applicato le tecniche molecolari per identificare i tartufi, i miceli e le micorrize ed ha effettuato ricerche che hanno consentito il sequenziamento del genoma del tartufo nero.
È aumentato l'interesse economico che ha visto sorgere numerose aziende di conservazione e trasformazione dei tartufi (in Umbria nel 2007 erano 63), vivai che producono le piante micorrizate, agricoltori che si interessano alla coltivazione dei tartufi e raccoglitori che si dedicano alla ricerca dei tartufi spontanei.
Sono sorti nuovi problemi, come la diffusione dei surrogati del tartufo, che hanno consentito un aumento del consumo pur essendo diminuita la produzione di questi pregiati funghi. L'interesse economico ha spinto i raccoglitori ed i commercianti a ricercare nuove aree di produzione sia in Europa che in altri continenti: ricerche che hanno consentito di individuare ottime zone di produzione nell'est europeo, nel nord Africa ed in Oriente. Queste scoperte hanno creato problemi legislativi in quanto alcune specie di tartufo non europee sono diverse da quelle autoctone: alcuni paesi hanno inserito tali specie nell'elenco dei tartufi commerciabili, altri non si sono adeguati con la conseguenza che queste risultano illegali. Nell'ambito del territorio europeo esistono problemi commerciali perché in alcune nazioni il tartufo appartiene al proprietario del terreno, è considerato un prodotto agricolo e come tale gli viene applicata un'imposta diversa rispetto ai paesi in cui il tartufo è annoverato tra i prodotti spontanei. Non sono sufficientemente sperimentate le tecniche di coltivazione in campo delle piante tartufigene: sono parzialmente note solo quelle relative al tartufo nero.
Da questa situazione e convinto di avviare a soluzione soprattutto i problemi tecnici e giuridici del tartufo, ho accolto l'invito della Comunità Montana dei Monti Martani e del Serano ad organizzare il terzo Congresso Internazionale di Spoleto sul Tartufo. Con entusiasmo, ma anche con scarsissime risorse, sono stati istituiti un Comitato organizzatore ed un Comitato scientifico che hanno consentito la realizzazione del Congresso che ha visto la partecipazione di oltre 300 esperti provenienti da 23 nazioni dislocate in tutti i continenti e la presentazione di 132 relazioni, comunicazioni e poster.
La pubblicazione degli atti, a causa del grande onere finanziario aggravato dalla crisi economica generale, per il momento viene fatta solo su base informatica.
Sono particolarmente grato ai componenti dei vari comitati il cui lavoro ha consentito la realizzazione del Congresso ed ai "Veterani del tartufo", che scherzosamente abbiamo chiamato "dinosauri", il cui impegno ha permesso di rendere la tartuficoltura un'attività agricola con solide basi scientifiche: Anna Fontana, Bruno Granetti, Gérard Chevalier, Mario Palenzona.
Mi auguro che la ricerca sul tartufo, che ha avuto grandi successi negli ultimi decenni, venga ulteriormente stimolata anche mediante la costituzione di un "Centro Internazionale di Ricerche sul Tartufo", proposto nel 1988 durante la conclusione del Secondo Congresso Internazionale di Spoleto e mai realizzato. Spero che l'impegno assunto dalle Autorità locali e regionali di ripetere ogni 20 anni il Congresso di Spoleto venga mantenuto allo scopo di tenere vive le ricerche di idnologia necessarie per consentire la sopravvivenza del tartufo naturale e risolvere i numerosi problemi della tartuficoltura.

Mattia Bencivenga

Preface

Twenty years after the Second International Conference on Truffles, which was held in Spoleto in 1988, the truffle and truffle cultivation sector has developed widely in every aspect. The increase in scientific interest has been demonstrated by the symposiums held by experts from various countries in the last two decades: The International Congress in Aix-en-Provence (1999) in which 126 speakers took part, and for which scholars from every corner of the world wrote, the biennial symposiums (International Workshop on Edible Mycorrhizal Mushrooms) held in Sweden in 1988 and New Zealand in 2001, in Canada in 2003 and Spain in 2005, in China in 2007, and the International Conference on the conservation and sustainable use of wild mushrooms held in Cordoba in 2007. Worthy of note, too, are the national, regional, and local conferences that are held frequently in Italian and other truffle-producing nations. The truffle has also attracted the interested of scientific fields such as genetics, what has applied molecular techniques to the identification of truffles, mycelia, and mycorrhizae and has done research which has made possible the sequencing of the genome of the black truffle.

Economic interest has also increased, and is evident in the birth of numerous businesses devoted to the conservation and transformation of truffles (in Umbria, in 2007 these numbered 63), nurseries that produce mycorrhizal plants, farmers who cultivate truffles, and hunters dedicated to the search for wild truffles.

New problems have arisen, such as the pervasiveness of truffle surrogates which have made an increase in consumption possible, even though the production of these valuable mushrooms has decreased. Economic interest has lead hunters and businessmen to look for new production areas both in Europe and in other continents, with the result that excellent areas for production in Eastern Europe, North Africa, and in the Orient have been identified. These discoveries have created legal problems in that some non-European species of truffles belong to a species different from the autocthonous ones; some countries have included these species in the list of marketable truffles; others did not conform, with the result that these species are illegal. In the context of Europe, there are commercial problems because in some countries the truffle belongs to the owner of the terrain; it is considered an agricultural product and, as such, is taxed differently from the way it is taxed in countries where the truffle is considered a wild product. More experimentation needs to be done with regards to techniques of truffle-producing plant cultivation in the field; we have partial information only about those related to the black truffle.

Because of this situation, and because I was convinced that it would be possible to work towards solutions, above all, for the technical and legal problems related to the truffles, I accepted the Comunità Montana (local authority dedicated to the conservation of mountain areas) of Monti Martani and of Serano's invitation to organize the third Spoleto International Congress on Truffles. With enthusiasm, but with limited resources, both an organizing Committee and a scientific Committee were formed, which in turn made it possible for the Conference to take place, a conference which was attended by more than 300 experts from 23 nations from all the continents, and at which 132 talks, communications and posters were presented. Because of onerous financial burdens exacerbated by the widespread economic crisis, the Conference Proceedings have been published, for the time being, only on the web.

I am particularly grateful to the members of the various committees whose work made it possible for the conference to be held, and to the "veterans of the truffle" who we have nicknamed, affectionately, the dinosaurs, whose dedication has transformed truffle cultivation into an agricultural activity with a solid scientific base: Anna Fontana, Bruno Granetti, Gérard Chevalier, Mario Palenzona. I hope that the research on truffles which has had such success in recent decades with be further stimulated by the creation of an International Center for Truffle Research proposed in 1988 at the conclusion of the Second International Conference in Spoleto, but never realized. I hope that the commitment on the part of the local and regional Authorities to repeat the Spoleto Conference every twenty years will be brought to fruition, so as to keep alive an interest in the research on idnologia necessary to safeguard the survival of the natural truffle, and to resolve the numerous problems of truffle cultivation.

Mattia Bencivenga

COMITATO SCIENTIFICO

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Sergio Arcioni
CNR - Istituto di Genetica Vegetale - Perugia

Wang Yun
University of Science and Technology of China

PROGRAMMA

24/11/2008
H 15.30 – 19.00 - Apertura della segreteria

25/11/2008
H 08.00 – Apertura della segreteria
H 10.00
Apertura del Congresso e saluto delle Autorità

H 10.30 - **Relazioni di apertura:**
Chairman Mattia Bencivenga
H 10.30 - 11.00: **Bruno Granetti** - The main areas of truffle research since the congress in Aix-En-Provence

H 11.00 – 11.30: Coffee break

H 11.30 – 12.00: **Wang Yun** - Truffle research and cultivation in China
H 12.00 – 12.30: **Charles Lefevre** - Truffles and truffle cultivation in North America
H 12.30 – 13.00: **Gerard Chevalier** - Truffles and truffle cultivation in Europe

25/11 – H 15.00
Genomic and cell biology session
H 15.00 - 15.30
Chairman Sergio Arcioni
Martin Francis - The genome of the Perigord truffle *(Tuber melanosporum)* reveals evolutionary insights into mycorrhizal symbiosis

H 15.30 – 17.00
Balestrini R., Zampieri E., Abbà S., Faccio A., Roberson R., Bonfante P. - An integrated cell and molecular view point of truffle biology
Paolocci F., Riccioni C., Belfiori B., Passeri V., Arcioni S., Rubini A. - New insights into truffle life cycle and reproductive mode: from basic research to the field
Rubini A., Riccioni C., Belfiori B., Passeri V., Arcioni S., Paolocci F. - Tracking the origin of *Tuber magnatum* and *T. melanosporum* truffles by molecular markers
Napoli C., Mello A, Vizzini A., Sourzat P., Smalla K., Bonfante P. – The microbial complexity of *Tuber melanosporum* trufflegrounds
Murat C., Rubini A., Bonfante P., Winckler P., Martin F. - Identification of microsatellites in the *Tuber melanosporum* Vittad. genome

H 17.00 – 17.30: Coffee break

PROGRAM

11/24/2008
H 03.30 p.m. – 07.00 p.m. – Secretariat Opening

11/25/2008
H 08.00 a.m. – Secretariat Opening
H 10.00 a.m. **– Official Opening of the Congress – Institutional welcome**

H 10.30 a.m.- **Opening talks**
Chairman Mattia Bencivenga
H 10.30 - 11.00 a.m.: **Bruno Granetti** - The main areas of truffle research since the congress in Aix-En-Provence

H 11.00 a.m. – 11.30 a.m. - Coffee break

H 11.30 a.m. – 12.00 p.m.: **Wang Yun** - Truffle research and cultivation in China
H 12.00 – 12.30 p.m.: **Charles Lefevre** - Truffles and truffle cultivation in North America
H 12.30 – 01.00 p.m.: **Gerard Chevalier** - Truffles and truffle cultivation in Europe

11/25 – H 03.00 p.m.
Genomic and cell biology session
H 03.00 – 03.30 p.m.
Chairman Sergio Arcioni
Martin Francis - The genome of the Perigord truffle *(Tuber melanosporum)* reveals evolutionary insights into mycorrhizal symbiosis

H 03.30 – 05.00 p.m.
Balestrini R., Zampieri E., Abbà S., Faccio A., Roberson R., Bonfante P. - An integrated cell and molecular view point of truffle biology
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H 05.00 – 05.30 p.m. : Coffee break

H 17.30 – 18.30
Chairwoman Christina Wedén

Zambonelli A., Amicucci A., Menotta M., Polidori E., Iotti M., Saltarelli R., Potenza L., Stocchi V. - Influence of host plant and different nutritional supplies on morphology and gene expression in *Tuber borchii*

Zampieri E., Murat C., Mello A., Cagnasso M., Bonfante P. - Use of β-tubuline as a DNA barcode to identify *Tuber* sp.

Ceccaroli P., Zeppa S., Zambonelli A., Guidi C., Guescini M., Potenza L., Stocchi V. - Gene expression during *Tuber borchii* fruit body maturation

Kagan-Zur V., Sitrit Y., Roth-Bejerano N., Zaretsky M. - A fungal gene potentially specific to mycorrhiza establishment between *Terfezia boudieri* isolates and *Cistus incanus* hairy roots

H 18.30 – 19.30 - Riunione Commissione Coltivazione *Tuber aestivum* Vittad.

26/11/2008

H 9.00

Ecology and population biology session

H 9.00 – 9.40
Chairwoman Ana Marìa De Miguel

Bragato G. - Soils suitable for the white truffle

Raglione M., Lorenzoni P., Owczarek M., Bonifazi A. - Soils suitable for the black truffles

H 9.40 – 11.00

González Armada B., De Miguel Velasco A.M., Cavero Remón R.Y. - Study of the above and below ground ecology (vascular and mycorrhizal flora) in truffle brûles in Navarra

Bonito G., Brenneman T., Vilgalys R. - Ectomycorrhizal associations of pecan trees *Carya illinoinensis* grown under monoculture and naturalized by truffles (*Tuber lyonii*)

Ławrynowicz M. - Hypogeous fungi in the anthropogenic sites in Poland

Ciaschetti G., Lena B., Paolanti M., Spinelli D., De Laurentiis G., Pacioni G. - Ecological characterization of natural sites with *Tuber melanosporum* Vittad. and *Tuber magnatum* Pico in the Abruzzo region (Central Italy)

Gógán Csorbainé A., Bratek Z., Illyés Z., Dimény J. - Studies on *Tuber macrosporum* natural truffle habitats in the Carpatho-Pannon Region

H 11.00 – 11.30 Coffee break

H 05.30 – 06.30 p.m.
Chairwoman Christina Wedén

Zambonelli A., Amicucci A., Menotta M., Polidori E., Iotti M., Saltarelli R., Potenza L., Stocchi V. - Influence of host plant and different supplies on morphology and gene expression in *Tuber borchii*

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H 06.30 – 07.30 p.m. - Committee Meeting: Cultivation of *Tuber aestivum* Vittad.

11/26/2008

H 9.00 a.m.

Ecology and population biology session

H 9.00 a.m. – 9.40 a.m.
Chairwoman Ana Marìa De Miguel

Bragato G. - Soils suitable for the white truffle

Raglione M., Lorenzoni P., Owczarek M., Bonifazi A. - Soils suitable for the black truffles

H 9.40 a.m. – 11.00 a.m.

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H 11.00 – 11.30 a.m. - Coffee break

H 11.30 – 13.00
Chairman Gianluigi Gregori

Honrubia M., Alfaro R., Fernández A., Suz L.M., Morte A. - Location and ecological characterization of natural truffle grounds in the region of Murcia (south east of Spain)

Marjanović Ž., Saljnikov E., Milenković M., Nikolić N., Grebenc T. - Ecological features of *Tuber magnatum* Pico in the conditions of West Balkans – Soil characterization

Albertini E., Bencivenga M., Benucci G. M. N., Di Massimo G., Gigliotti G., Raggi L. - Ecology of *Tuber magnatum* Pico in the high Valley of Chiascio (Central Italy)

Salerni E., Baglioni F., Mazzei T., Perini C. - Relationship between the management of forest vegetation and the production of *Tuber aestivum* Vittad. in a natural truffle ground on Monte Amiata (Tuscany, Italy): preliminary results

Wedén C., Bai G., Göransson U., Borg-Karlsson A.K., Backlund A. - The swedish populations of *Tuber aestivum*

26/11 – H 15.00

Taxonomy, Biology and Ectomycorrhizae session

H 15.00 – 15.20
Chairman José Luìs Manjon

Zambonelli A., Iotti M., Bonuso E., Hall I. - Taxonomical and commercial problems in the valuable truffle *T. borchii*

H 15.20 – 17.00

Zarivi O., Bonfigli A., Colafarina S., Ragnelli A.M., Aimola P., Pacioni G., Miranda M. – Is tyrosinase expression during *Tuber melanosporum* development due to enzyme inhibition or gene switching off?

Águeda B., Agerer R., De Miguel A.M., Parladé J., - *Quercirhiza quadratum*: a revision of the characters and identity of the AD type ectomycorrhiza

Angelini P., Venanzoni R., Pagiotti R., Tirillini B., Granetti B., Donnini D. - Biological activities of methanolic extract from *Tuber* species

Barry-Etienne D., Ricard J.M., Diette S., Moundy P.J., Chandioux O., Fiorese D., Jaillard B., Serre F., Jourdan C. - Distribution of *Tuber melanosporum* mycorrhizas on rootstocks of holm-oaks (*Quercus ilex*) in production

Ceccaroli P., Saltarelli R., Buffalini M., Polidori E., Guescini M., Stocchi V. - Role of *Tuber borchii* mannitol dehydrogenase in key environmental and metabolic responses

H 11.30 – 01.00 p.m.
Chairman Gianluigi Gregori

Honrubia M., Alfaro R., Fernández A., Suz L.M., Morte A. - Location and ecological characterization of natural truffle grounds in the region of Murcia (south east of Spain)

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Wedén C., Bai G., Göransson U., Borg-Karlsson A.K., Backlund A. - The swedish populations of *Tuber aestivum*

11 / 26 – H 03.00 p.m.

Taxonomy, Biology and Ectomycorrhizae session

H 03.00 – 03.20 p.m.
Chairman José Luìs Manjon

Zambonelli A., Iotti M., Bonuso E., Hall I. - Taxonomical and commercial problems in the valuable truffle *T. borchii*

H 03.20 – 05.00 p.m.

Zarivi O., Bonfigli A., Colafarina S., Ragnelli A.M., Aimola P., Pacioni G., Miranda M. – Is tyrosinase expression during *Tuber melanosporum* development due to enzyme inhibition or gene switching off?

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Ceccaroli P., Saltarelli R., Buffalini M., Polidori E., Guescini M., Stocchi V. - Role of *Tuber borchii* mannitol dehydrogenase in key environmental and metabolic responses

Piltaver A., Ratoša I. - Hypogeous fungi from Slovenia

H 17.00 – 17.30: Coffee break

H 17.30 – 18.30
Chairman Giovanni Pacioni

Sbissi I., Cherif A., Neffati M. - Chemical characterisation and antibacterial activity of some *Terfezia boudieri* extracts

Slama A., Fortas Z., Boudabous A., Neffati M. - Mycorrhization between *Terfezia boudieri* Chatin and *Helianthemum sessiliflorum* Desf. in Tunisia

Pargney J. C., Boumaza O., Toutain F. - *In situ* micro- and ultrastructural study of ascocarp development in *Tuber mesentericum.*

Donnini D., Baciarelli Falini L., Di Massimo G., Benucci G.M.N., Bencivenga M. - Competition between *Pisolithus arhizus* (Scop.) Rauschert and *Scleroderma verrucosum* (Bull.) Pers. and different species of *Tuber*

H 18.30 – 19.00
Riunione Commissione: "Metodo di analisi e certificazione delle piante micorrizate"

27/11/2008

H 9.00 - Conservation, commerce and valorisation session

H 9.00 – 9.30
Chairwoman Elena Giovagnotti

Urbani O. – Conservation and commerce of truffles in Italy

H 9.30 – 09.50 Interventi sulla relazione precedente

H 09.50 – 11.00

Zampi S., Giovagnotti E., Rosi E. - Regione Umbria: actions for the regional truffle resource conservation and development.

Sibille B. - From collection to preservation: the commitment of Piedmont Region in the truffle chain – a natural and economical heritage Piedmont stacks to create an opportunity of development under equity and sustainability criteria

Paggi A., De Angelis V., Filippucci R. – Truffles and truffle cultivation in "Comunità Montana dei Monti Martani e del Serano"

Galluzzo N. - The willingness to pay of the consumer for food quality approach as the truffle with an application of a choice model

Rivera C.S., Venturini M.E., Blanco D. Tolerance of fresh summer truffles (*Tuber aestivum*) to different levels of O_2 and CO_2

Piltaver A., Ratoša I. - Hypogeous fungi from Slovenia

H 05.00 – 05.30 p.m. : Coffee break

H 05.30 – 06.30 p.m.
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Slama A., Fortas Z., Boudabous A., Neffati M. - Mycorrhization between *Terfezia boudieri* Chatin and *Helianthemum sessiliflorum* Desf. in Tunisia

Pargney J. C., Boumaza O., Toutain F. - *In situ* micro- and ultrastructural study of ascocarp development in *Tuber mesentericum.*

Donnini D., Baciarelli Falini L., Di Massimo G., Benucci G.M.N., Bencivenga M. - Competition between *Pisolithus arhizus* (Scop.) Rauschert and *Scleroderma verrucosum* (Bull.) Pers. and different species of *Tuber*

H 06.30 – 07.00 p.m. – Committee Meeting for the study of "Analysis methods and certification of mycorrhized plants"

11/27/2008

H 09.00 a.m.

Conservation, commerce and valorisation session

H 09.00 – 9.30 a.m.
Chairwoman Elena Giovagnotti

Urbani O. – Conservation and commerce of truffles in Italy

H 09.30 – 09.50 a.m. Discussion

H 09.50 – 11.00 a.m.

Zampi S., Giovagnotti E., Rosi E. – Umbria Region: actions for the regional truffle resource conservation and development.

Sibille B. - From collection to preservation: the commitment of Piedmont Region in the truffle chain – a natural and economical heritage Piedmont stacks to create an opportunity of development under equity and sustainability criteria

Paggi A., De Angelis V., Filippucci R. – Truffles and truffle cultivation in "Comunità Montana dei Monti Martani e del Serano".

Galluzzo N. - The willingness to pay of the consumer for food quality approach as the truffle with an application of a choice model

Rivera C.S., Venturini M.E., Blanco D. - Tolerance of fresh summer truffles (*Tuber aestivum*) to different levels of O_2 and CO_2

Spanier T., K. Kambell - The growing-up of Oregon truffes *(Tuber oregonense, Tuber gibbosum, Leucangium carthusianum)*

H 11.00 – 11.30 Coffee break

H 11.30 - Cultivation session
H 11.30 – 11.50
Chairman Mario Honrubia
Chevalier G., Palenzona M. - Implications of a 40-years research on truffles: future prospects

H 11.50 – 13.00
Shamekh S., Leisola M. - Truffle orchards in Finland
Colinas C., Oliach, D., Fischer C. R., Olivera A., Martínez de Aragón J., Bonet J. A. - Longterm regional land planning to promote truffle cultivation based on soil and weather models in Catalonia, NE Spain
De Miguel A. M., Sáez R., González B. - Truffle growing activity in Navarra. Questions without an answer
Moundy P.J., Diette S., Barry-Etienne D. - An innovative technique for soil inoculum determination applied to truffle cultivation

27/11 - H 15.00 – 17.00
Chairwoman Anna Maria Ferrara
Sourzat P., Gregori G. - Truffle cultivation in France and Italy
Fischer C. R., Martínez de Aragón J., Oliach D., Olivera A., Bonet J. A., Colinas C. - Can burnt forest lands be used for black truffle cultivation?
Baciarelli Falini L., Bencivenga M., Donnini D., Di Massimo G. – Research results in truffle beds of the Umbria Region
Gregori G., Ponzio G., Sbrissa F., Verlato G., Giomaro G., Sisti D. - Auxometry of the *Tuber aestivum/uncinatum* carpophore
Hall I.R., Zambonelli A. - A quarter century of truffle cultivation in New Zealand – successes and problems
Reyna S., Garcia-Barreda S. - Feasibility of truffle plantations in firebreaks

H 17.00 – 17.30: Coffee break

H 17.30 – 19.00
Chairman Pierre Sourzat
Palazón C., Barriuso J. J., Sánchez-Durán S. - The project "Desarrollo integral de la truficultura de Teruel (Spain)"
Pardo C., Lafon S., Guillon A. - The territorial analysis of the truffle production:

Spanier T., K. Kambell - The growing up of Oregon truffes *(Tuber oregonense, Tuber gibbosum, Leucangium carthusianum)*

H 11.00 – 11.30 a.m. Coffee break

H 11.30 a.m. – Cultivation session
H 11.30 – 11.50 a.m.
Chairman Mario Honrubia
Chevalier G., Palenzona M. – Implications of a 40-years research on truffles: future prospects

H 11.50 – 01.00 p.m.
Shamekh S., Leisola M. – Truffle orchards in Finland
Colinas C., Oliach, D., Fischer C. R., Olivera A., Martínez de Aragón J., Bonet J. A. - Longterm regional land planning to promote truffle cultivation based on soil and weather models in Catalonia, NE Spain
De Miguel A. M., Sáez R., González B. - Truffle growing activity in Navarra. Questions without an answer
Moundy P.J., Diette S., Barry-Etienne D. - An innovative technique for soil inoculum determination applied to truffle cultivation

11/ 27 – H 03.00 – 05.00 p.m. Chairwoman Anna Maria Ferrara
Sourzat P., Gregori G. - Truffle cultivation in France and Italy
Fischer C. R., Martínez de Aragón J., Oliach D., Olivera A., Bonet J. A., Colinas C. - Can burnt forest lands be used for black truffle cultivation?
Baciarelli Falini L., Bencivenga M., Donnini D., Di Massimo G. – Research results in truffle beds of the Umbria Region
Gregori G., Ponzio G., Sbrissa F., Verlato G., Giomaro G., Sisti D. - Auxometry of the *Tuber aestivum/uncinatum* carpophore
Hall I.R., Zambonelli A. - A quarter century of truffle cultivation in New Zealand – successes and problems
Reyna S., Garcia-Barreda S. - Feasibility of truffle plantations in firebreaks

H 05.00 – 05.30 p.m. : Coffee break

H 17.30 – 19.00
Chairman Pierre Sourzat
Palazón C., Barriuso J. J., Sánchez-Durán S. - The project "Desarrollo integral de la truficultura de Teruel (Spain)"
Pardo C., Lafon S., Guillon A. - The territorial analysis of the truffle production:

a prerequisite in the biotechnical experimentation

Reyna S., Forcadell R., Martín-Santafé M., Garcia-Barreda S. - Trufficulture and forest management in Teruel

Morcillo M., Sánchez M., Vidal C., Mateu J., Gracia E. - Inoculation of adult hazelnut groves with *Tuber brumale* and *Tuber melanosporum*

Saenz W. - Impact of the cultural practices the tree first years of the plantation on the truffle-producing potential

28/11/2008
Cultivation session

H 9.00 – 9.20
Chairman Giuseppe Venturella
Reyna S. - Truffle cultivation in Spain

H 9.20 – 11.00

Ágreda T., Águeda B., Modrego M. P., Martinez-Peña F. - Ecology of truffle plantations in Teruel (Spain): sampling method for its characterization

Sourzat P. - The principle of precaution in truffle cultivation

Gregori G., Sisti D., Romagnoli E., Zambonelli A., Giomaro G. - A new perspective in controlling *Tuber* infected plants: the use of ROC curves make applicable a visual estimation

Williams K., Zahran A. - Keeping the earth eggs in different baskets: the future of desert truffles in Qatar

Vezzola V. - *Tuber macrosporum* Vittad. cultivation

Zengoni G., Piccioni M., Bernardini W. - Wireless sensors network and their application on truflle cultivation

H 11.00 – 11.30: Coffee break

H 11.30 – 13.00 - Discussione dei poster

H 15.00 – 17.00 Tavola rotonda: Esperienze e problematiche di coltivazione dei tartufi

Introduce: **Bencivenga M., Baciarelli Falini L., Di Massimo G., Donnini D.** - Truffle-cultivation: joy and pain

H 17.00 – 18.00
Cerimonia di chiusura del Congresso

a prerequisite in the biotechnical experimentation

Reyna S., Forcadell R., Martín-Santafé M., Garcia-Barreda S. - Trufficulture and forest management in Teruel

Morcillo M., Sánchez M., Vidal C., Mateu J., Gracia E. - Inoculation of adult hazelnut groves with *Tuber brumale* and *Tuber melanosporum*

Saenz W. - Impact of the cultural practices the tree first years of the plantation on the truffle-producing potential

11/28/2008
Cultivation session

H 09.00 – 9.20 a.m.
Chairman Giuseppe Venturella
Reyna S. - Truffle cultivation in Spain

H 9.20 – 11.00 a.m.

Ágreda T., Águeda B., Modrego M. P., Martinez-Peña F. - Ecology of truffle plantations in Teruel (Spain): sampling method for its characterization

Sourzat P. - The principle of precaution in truffle cultivation

Gregori G., Sisti D., Romagnoli E., Zambonelli A., Giomaro G. - A new perspective in controlling *Tuber* infected plants: the use of ROC curves make applicable a visual estimation

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Vezzola V. - *Tuber macrosporum* Vittad. cultivation

Zengoni G., Piccioni M., Bernardini W. - Wireless sensors network and their application on truflle cultivation

H 11.00 – 11.30 a.m. : Coffee break

H 11.30 – 01.00 p.m. - Discussion of the posters

H 03.00 – 05.00 p.m. Round table: experience of and problems with truffle cultivation

Introduces the work: **Bencivenga M., Baciarelli Falini L., Di Massimo G., Donnini D.** - Truffle-cultivation: joy and pain

H 05.00 – 06.00 p.m.
Closing ceremony of the Congress

Con estremo piacere presento gli Atti del 3° Congresso Internazionale di Spoleto sul Tartufo, tenutosi dal 25 al 28 novembre 2008.

Il Terzo Congresso Internazionale sul tartufo è un appuntamento molto importante per due ragioni: nell'ultimo decennio la ricerca scientifica sul tartufo e la sperimentazione sulla tartuficoltura hanno avuto importanti evoluzioni, coinvolgendo un numero sempre crescente di studiosi e ricercatori.
Grazie ai risultati raggiunti la coltivazione dei tartufi si è estesa interessando aree sempre più vaste. Questo sviluppo si deve soprattutto all'interesse mostrato dagli agricoltori che hanno intuito come la tartuficoltura possa essere un'interessante integrazione del reddito e consentire di utilizzare al meglio, in senso eco-sostenibile, anche aree marginali altrimenti svantaggiate.
In questa occasione, vorrei ricordare il legame che da sempre lega strettamente Spoleto e il suo comprensorio al tartufo e alla tartuficoltura; ricordo ai profani che la città ha segnato per sempre il nome di una specie.
Il 1°Congresso Internazionale di Spoleto sul Tartufo fu organizzato, nel 1968 e vide la partecipazione di un folto numero di ricercatori, raccoglitori, commercianti e agricoltori che desideravano avviare la coltivazione di questi pregiati funghi ipogei.
Alcuni anni dopo, il panorama amministrativo italiano si è arricchito di un nuovo soggetto istituzionale, le Comunità Montane costituite con l'obiettivo di assicurare la permanenza delle popolazioni nelle aree svantaggiate della montagna e favorirne lo sviluppo economico e sociale.
In tale contesto, nel 1988, a distanza di venti anni dal primo, fu proprio la Comunità Montana dei Monti Martani e del Serano a prendere in mano le redini dell'organizzazione, contribuendo in maniera determinante al successo del 2° Congresso Internazionale di Spoleto sul Tartufo: un appuntamento che si è rivelato una pietra miliare per tutti gli addetti ai lavori, segnando l'inizio della moderna tartuficoltura, tanto che gli Atti di quel Congresso sono ancora oggi richiesti da ricercatori e operatori del settore di tutto il mondo.
Rispettando la cadenza dei venti anni, in linea con il contributo forte e pregnante che gli Enti montani hanno sempre assicurato allo sviluppo del territorio e alla promozione dei prodotti tipici locali che lo caratterizzano, a novembre del 2008 la Comunità Montana ha organizzato anche il 3° Congresso Internazionale di Spoleto sul Tartufo.
Si è trattato di quattro giorni di incontri, dibattiti, tavole rotonde e visite sul territorio che hanno permesso il confronto tra gli esperti ed operatori dal quale è scaturito il punto sullo stato attuale della tartuficoltura a livello mondiale.
Come era già accaduto in occasione del secondo Congresso, l'Ente che oggi rappresento ha dimostrato ancora una volta il valore di una Istituzione che, pure dibattendosi tra mille difficoltà, ha saputo cogliere l'occasione di una data così importante per la città di Spoleto e per l'intera regione organizzando un evento di portata internazionale: un momento di incontro irrinunciabile per tutti gli addetti ai lavori, a partire dai ricercatori sino ai tartufai e ai tartuficoltori.
Per la Comunità Montana, il 3° Congresso Internazionale di Spoleto sul Tartufo ha poi rappresentato una tappa del proprio cammino ricca di tanti significati e sfumature.
Il 22 dicembre 2008, infatti, circa un mese dopo la conclusione del Congresso, per la riforma endoregionale che ha interessato in particolare gli Enti montani, la Comunità Montana dei Monti Martani e del Serano si è unita alla Comunità Montana Monte Subasio per divenire un unico soggetto istituzionale, oggi denominato Comunità Montana dei Monti Martani, Serano e Subasio.
Il 3° Congresso, dunque, ha segnato, in un certo qual modo, un passaggio di testimone nella storia dell'Ente ma, al contempo, con la pubblicazione di questi Atti sembra sancirne anche la continuità, sia amministrativa che lavorativa.
Gli Atti sono stati, infatti, fortemente voluti da questa Amministrazione come segno concreto dell'impegno che la nuova Comunità Montana continua a profondere sul territorio, non solo nel campo della tartuficoltura ma in generale per lo sviluppo eco- sostenibile di questi luoghi di particolare pregio ambientale, storico, artistico e culturale.

Colgo l'occasione per formulare alcuni ringraziamenti che nascono dalla consapevolezza dell'importanza di questa opera e dei contributi che ha raccolto: un grazie doveroso lo rivolgo alla passata Amministrazione che ha saputo raccogliere il testimone dei due Congressi precedenti e portare la città di Spoleto e tutto il territorio comunitario al centro di un evento che si è concluso con un successo straordinario; un ringraziamento particolare lo dedico alla struttura dell'Ente per l'encomiabile disponibilità e competenza che pone in tutte le sue azioni. Ringrazio, infine, il prof. Mattia Bencivenga e la dott.sa Domizia Donnini del Dipartimento di Botanica Applicata dell'Università degli Studi di Perugia, che hanno curato gli aspetti scientifici del Congresso e questa pubblicazione e hanno saputo sapientemente accompagnare la Comunità Montana lungo tutto il percorso di questo evento.

Agosto 2010

Giuliano Nalli
Presidente della Comunità Montana dei Monti Martani Serano e Subasio

It is with great pleasure that I present the Acts of this the 3rd International Congress on Truffles, which took place in Spoleto from the 25th November to the 28th November 2008.

The 3rd International Congress on Truffles was a very important event for two reasons: in the last decade scientific research on truffles and experimental truffle cultivation have made important headway, with an ever increasing number of investigators and researchers. Thanks to the results obtained truffle cultivation has been extended to a wider and wider area. This development has been brought about by the interest shown among farmers in particular, who have realised that truffle cultivation can provide added income and enable marginal areas, otherwise disadvantaged, to be put to an ideal, eco-friendly use. Here I would remind you of the historic link between Spoleto and the surrounding area, and truffles and truffle cultivation; some of you may even need reminding that the town of Spoleto actually figures in the name of one of the species. The 1st International Congress on Truffles in Spoleto was organised in 1968 and was attended by a great number of researchers, truffle gatherers, shopkeepers and farmers who wanted to start up the cultivation of these prized epigeal fungi. Some years later, the Italian government brought in a new institution responsible for the disadvantaged mountain areas, the 'Comunità Montane', or mountain development associations. The aim was to help the inhabitants to remain in their area and promote economic and social development. Thus in 1988, twenty years after the first congress, it was the Comunità Montana of the Martani and Serano mountains which took over the organization of Spoleto's 2nd International Congress on Truffles, contributing greatly to its success. It turned out to be a milestone for everyone involved in the field, marking the beginning of modern truffle cultivation. In fact the Acts of that Congress are still requested by researchers and others involved in the sector from all parts of the world. The 3rd International Congress on Truffles was organized by the Comunità Montana in November 2008, once again after a twenty year gap. It was typical of the substantial and significant contribution they have always made to developing the territory and promoting local produce. There were four days of meetings, debates, round tables and local trips, all of which enabled experts and all those involved in the sector to exchange ideas and information, and arrive at a precise assessment of the current state of the art in truffle cultivation around the world. As had already occurred at the 2nd congress, the Association which I represent today showed its worth as an institution capable, despite the many difficulties, of organizing an event of international scale on such an important date for the town of Spoleto and for the whole region. A meeting that couldn't be missed, for researchers, truffle gatherers and cultivators alike. For the Comunità Montana, the 3rd International Congress on Truffles in Spoleto represented another meaningful and complex stage in its progression,. On the 22nd December, about one month after the congress had ended, in the regional reforms which involved the mountain development associations, the Comunità Montana of the Martani and Serano mountains joined up with the Comunità Montana of Mount Subasio and became a single Institution, today called the Comunità Montana of the Martani, Serano and Subasio mountains. The 3rd congress therefore has been a sort of milestone in the history of the association, and the publication of these Acts seems to sanction its continuity too, both on an administrative and grass roots level. The Acts were in fact strongly desired by this Administration, as a concrete sign of the commitment which the Comunità Montana continuously maintains towards the area, not only in the field of truffle cultivation but more in general for the sustainable development of these places which have particular environmental, historic, artistic and cultural value. I would like to take the opportunity to express my gratitude, conscious as I am of the importance of this work and the contributions it has raised; a well deserved thank you goes to the past Administration, which bore witness to the two previous Congresses and managed to fully engage the town of Spoleto and surrounding areas in an event of extraordinary success ; special thanks go to the Association for the commendable helpfulness and skill evident in all its undertakings. Lastly I thank Professors Mattia Bencivenga and Domizia Donnini of the Department of Applied

Biology at Perugia University, responsible for scientific aspects of the Congress and for this publication, who wisely accompanied the Comunità Montana in their work from the start to the finish of this event.

August 2010

Giuliano Nalli
President of the Comunità Montana of the Martani, Serano and Subasio mountains

Con vero piacere porto i saluti del preside prof. Francesco Pennacchi e dell'intero Consiglio della Facoltà di Agraria dell'Università di Perugia a questo interessante Congresso che vede impegnati, in prima persona, colleghi della nostra Facoltà che da decenni si dedicano a ricerche sui tartufi e sulla tartuficoltura finalizzate all'incremento della produzione di questi particolari funghi.
Innanzitutto vorrei compiacermi per la cornice in cui si svolgeranno i lavori del Congresso, dotata di questa bellissima sala e di ampi ambienti per i lavori collaterali come l'esposizione dei poster, riunioni di gruppo, ecc.
Ritornando al tema del Congresso, la Facoltà di Agraria ha sostenuto e sostiene le ricerche sulla coltivazione dei tartufi che ritiene importante per il recupero produttivo ed ecologico soprattutto di quelle aree dove l'agricoltura tradizionale è di difficile attuazione e, come ho letto dai lavori degli organizzatori, anche per l'utilizzazione dei buoni terreni agrari dove il tartufo può fornire redditi molto interessanti nel rispetto dell'ambiente.
Per sostenere tali attività la Facoltà ha istituito un master post-laurea dal titolo "Biologia, Ecologia e Coltivazione dei Tartufi" tra i cui docenti compaiono i nomi di esperti di tartuficoltura provenienti dai paesi europei produttori di tartufi pregiati. Inoltre, nel proprio ordinamento didattico, ha inserito un insegnamento libero annuale di Micologia Applicata nel cui programma trova ampio spazio la coltivazione dei tartufi e dei funghi epigei.
Per non togliere altro tempo, porgo i miei migliori auguri per la riuscita scientifica del Congresso che certamente sarà di elevato valore come dimostra il numero degli iscritti, la loro provenienza che copre quasi tutti i Continenti, e il nutrito elenco delle relazioni, comunicazioni e poster.

25 Novembre 2008.

Francesco Tei
Vice Preside della Facoltà di Agraria dell'Università degli Studi di Perugia

I am here with great pleasure to pass on good wishes from Professor Francesco Pennacchi and the whole of Perugia University's Board of the Faculty of Agricultural Sciences to the participants in this interesting Congress. It is an event which directly involves colleagues from our Faculty who have dedicated decades of research to truffles and truffle cultivation with the aim of increasing the production of these particular fungi.
First of all I would like to express my satisfaction with the Congress venue, with this beautiful hall and spacious rooms for subsidiary activities such as the poster exhibitions, meetings etc.
To return to the Congress itself, the Faculty of Agricultural Studies has supported and continues to support research on truffle cultivation, considering it important for the productive and ecological recovery of those areas where traditional agricultural practices are difficult to put into action and also, as the organizers say, to put good agricultural land to use, land where truffles can provide a substantial source of income while still respecting the environment.
To support these activities, the Faculty has created a Masters called 'Biology, Ecology and Cultivation of Truffles'. Among the lecturers are experts in the field of truffle cultivation, originating from EU countries producing prized truffles. It has also introduced a free, annual Applied Mycology course, large part of which is dedicated to truffle cultivation and epigeal fungi.
Without taking up any more of your time, I wish all the best for the scientific success of the Congress, which can only be excellent if one considers the number of participants from all parts of the world, and the wealth of talks, information and posters on the programme.

25 November 2008.

Francesco Tei
Vice President of the Faculty of Agricultural Sciences, Perugia University

OPENING SESSION

LE PRINCIPALI LINEE DI RICERCA SUI TARTUFI DOPO IL CONGRESSO DI AIX-EN-PROVENCE

Granetti Bruno
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Abstract: The main areas of truffle research since the Congress in Aix-en-Provence
Research on truffles has progressed in numerous sectors since the congress in Aix-en-Provence (1999). In the field of truffle cultivation, work is being done by Henri Dessolas to develop a new methodology known as “Formula I.A.AD.” that will overcome some of the shortcomings of the Pallier and Tanguy methods. Attempts are also being made to improve the mycorrhized plants by using new mycorrhizing techniques. Particular attention has been given to the physiological relationship between the root apex and the truffle mycelium when the two come into contact with each other. The formation of ectomycorrhizae, an important phase in the truffle life cycle, has been analysed in-depth using molecular methods. Contrary to what has been previously hypothesized, the haploid mycelium of spore origin generates the mycorrhizae and the ascomes are made up primarily of haploid hyphae. Dikaryotization probably occurs inside the young ascocarps which contain the sporophytic apparatus. In the field of systematics, molecular analysis has been used to identify and characterize already known truffle species as well as to identify new ones. Molecular analyses has also been used to recognize mycorrhizae produced by different fungal species which is useful when evaluating *Tuber*-mycorrhized plants. Isoenzymes, monoclonal antibodies and aroma composition have also been used for this purpose. Aiso worthy of note is the work “Le specie europee del genere *Tuber* - Una revisione storica” by A. Ceruti, A. Fontana and C. Nosenzo (2003) which has put a number of synonyms into order. A number of studies have investigated the behaviour of the symbiotic plants and the evolution of the ectomycorrhizae in the root apparatus after being planted out in the field under very diverse soil and climatic conditions. Molecular analyses have shown various degrees of affinity between the European and Chinese truffle species which suggests a common ancestral origin. A study on the genetic variability of *T melanosporum* has allowed the path of recolonization in the French territory around the central massif after the last glaciations to be reconstructed. Some studies have been conducted on the intraspecific variability of economically important species and others have used molecular analyses to identify fraud in both fresh and preserved truffles. Several publications report the chemical composition of fruiting bodies, particularly the aromas. There has been a notable increase in the number of studies on truffle physiology (the reference species is usually *T. borchii*) in all of the life cycle phases (mycelium, mycorrhiza, young and mature ascocarp) including the identification, characterization and gene expression. Finally, the sequencing of the *Tuber* genome was recently completed. This complex work was the initiative of Dr F. Martin of INRA in Nancy who acted as the coordinator of several research groups.
Key words: *Tuber*, cultivation, systematic, biology.

Porgo il mio saluto cordiale al Presidente e a tutti i Congressisti e ringrazio sentitamente il Comitato scientifico per avermi onorato invitandomi a tenere la relazione introduttiva.

Premessa
La ricerca sui tartufi, dopo il Congresso di Aix-en-Provence (1999), che ha registrato un numero elevato di contributi di notevole interesse scientifico, è proseguita con lo scopo prevalente di incrementare la produzione di questi pregiati funghi attraverso l’approfondimento delle conoscenze della loro biologia e il miglioramento delle tecniche di coltivazione. Nonostante l’impegno profuso dai ricercatori, per quanto riguarda la tartuficoltura i problemi da risolvere sono ancora molti; dopo 35 anni dai suoi primi passi l’entrata in produzione delle tartufaie è aleatoria e nella maggior parte dei casi riguarda una percentuale limitata di piante per cui è

variabilissima l'entità del prodotto. Soddisfacenti risultati, comunque, sono stati ottenuti per il *T. melanosporum* e per il *T. aestivum*, mentre per il *T. magnatum* sono emerse serie difficoltà. Tutti gli studiosi hanno ravvisato la necessità e l'urgenza di sviluppare ulteriormente le ricerche in molteplici direzioni senza escludere quelle sul campo che, purtroppo, negli ultimi tempi hanno subito una certa flessione. L'urgenza è dovuta al fatto che la produzione complessiva di tartufi è in forte declino da oltre un secolo nonostante che venissero realizzate tartufaie coltivate per alcune migliaia di ettari a partire dal 1973. Nel frattempo sta crescendo il numero dei paesi che, oltre l'Italia, la Francia e la Spagna, sono sempre più interessati alla coltivazione dei tartufi o che la praticano già in modo efficiente; se ne contano una quindicina di tutti i continenti (Chevalier, 2008). Per meglio utilizzare le scarse risorse disponibili è necessario un coordinamento tra i vari ricercatori per conseguire risultati scientifici ed applicativi di alto livello e in tempi brevi.
Diamo ora un veloce sguardo alle principali linee di ricerca dell'ultimo decennio.

Sistematica
Nel campo della Sistematica si segnalano alcune importanti opere di recente pubblicazione utili per la determinazione delle specie di tartufo europee, dell'area mediterranea e di alcune cinesi.
"Funghi ipogei d'Europa" di Montecchi e Sarasini (2000), opera in cui le specie sono descritte in modo accurato e illustrate con materiale fotografico originale: carpofori interi, in sezione, aschi e ascospore.
"Truffes d'Europe et de Chine" di Riousset L. et G., Chevalier et Bardet (2000) in cui ogni specie è descritta in modo analitico, comparandola con specie affini; la documentazione fotografica è originale e ricca di particolari.
"Le specie europee del genere *Tuber* – Una revisione storica" di Ceruti, Fontana e Nosenzo (2003) in cui materiale d'erbario e materiale fresco sono stati esaminati comparativamente con l'ausilio del microscopio e la validità delle singole entità è stata confermata dall'analisi molecolare nei casi in cui era disponibile la sequenza ITS dell'rDNA in GenBank. Una vasta ricerca bibliografica ha, infine, consentito, insieme ai dati precedenti, di stabilire le sinonimie che sono in numero straordinariamente elevato.
Il volume è corredato di copie delle tavole di Ceruti (1960) e di Rebaudengo e di tavole originali a cura di Meotto illustranti sezioni del peridio, aschi e ascospore.

Identificazione dei tartufi
La identificazione delle specie tramite i caratteri morfologici e strutturali dell'ascocarpo e delle spore ha trovato negli ultimi anni un valido supporto da altre metodologie di analisi.

a) Analisi molecolare
Le analisi molecolari che fanno uso di primers specie specifici, entrate nell'uso corrente, consentono di distinguere con assoluta certezza le specie di tartufi bianchi e neri a livello di ascocarpi, di micorrize e di micelio (Amicucci *et al*., 1998 e 2000; Rubini *et al*., 1998; Bertini *et al*., 1999; Mello *et al*., 1998, 1999, 2001; Rossi *et al*., 2000; Halasz *et al*., 2005). Queste metodologie di ricerca e l'analisi strutturale del peridio hanno consentito di istituire i neotipi di *T. borchii* e di *T. maculatum* punto di riferimento per una sicura diagnosi di queste due specie (Mello *et al.,* 2000).
Anche per una specie con alta variabilità genetica come il *T. rufum* è stato possibile selezionare primers specifici per la sua identificazione in ogni stadio del suo ciclo vitale (Iotti *et al*., 2007).
Infine la identificazione delle sequenze dei motifs in *T. magnatum*, *T. melanosporum*, *T. indicum*, *T. aestivum* e *T. mesentericum*, oltre a rappresentare un primo passo verso la codificazione del loro DNA, è un promettente strumento per il riconoscimento delle specie (El Karkouri *et al*., 2007).

b) Analisi delle proteine e degli aromi.
L'analisi di particolari proteine può consentire il riconoscimento di alcune specie come evidenziato per una proteina omologa a TBF1 che è presente negli ascocarpi di *T. borchii* e di

T. dryophilum e assente in altre specie di *Tuber* (Agostini *et al*., 2000). La composizione della frazione volatile analizzata mediante gas-cromatografia e spettrometria di massa consente di identificare in modo rapido le differenti specie di tartufo (Gioacchini *et al*., 2005).

c) Utilizzazione degli anticorpi.
L'allestimento degli anticorpi specifici per il riconoscimento delle specie risale a molti anni fa; questa tecnica è stata ripresa di recente. Anticorpi sono stati preparati utilizzando il siero di giovani polli usando soluzioni concentrate di estratti di tartufo (Weng Fulai *et al*., 2000).
Sono stati preparati anticorpi policlonali capaci di diagnosticare il *T. borchii* e il *T. dryophilum* nel materiale vivo e nelle preparazioni alimentari (Palma *et al*., 2005).

d) Analisi spettroscopica all'infrarosso.
Mediante l'analisi spettroscopica all'infrarosso è possibile distinguere tra loro le specie presenti in una stessa località (Yunnan-Sud Ovest della Cina) e nell'ambito di una specie è possibile evidenziare differenze tra i tartufi provenienti da aree diverse usando semplicemente tartufi intatti (Zhao De-Zhang *et al*., 2006).

Ciclo ontogenetico
Da quasi quaranta anni si è cercato di ricostruire il ciclo vitale del tartufo ma a tutt'oggi alcune fasi non sono sufficientemente chiarite. Le ricerche più recenti sul *T. magnatum* e sul *T. melanosporum* apportano ulteriori e originali contributi alla comprensione del ciclo biologico. Con le conoscenze sin qui acquisite, vecchie e nuove, possiamo percorrere le principali tappe del ciclo ontogenetico. Le ascospore, germinando, producono ife aploidi che danno origine alle ectomicorrize, anch'esse aploidi, contrariamente a quanto è stato ritenuto fino ad ora. Il micelio delle fini radichette micorrizate dà origine al primordio dell'ascocarpo cui segue lo stadio apotecioide; il piccolo apotecio è costituito nella quasi totalità da ife aploidi (monocariotiche). Nell'intreccio ifale di questo minuscolo corpo fruttifero è stato individuato (già da molto tempo) l'apparato sporofitico così chiamato in quanto destinato a produrre gli aschi. Nell'interno degli aschi ha luogo la cariogamia con formazione di un nucleo diploide che subisce la meiosi generando nuclei aploidi; segue la sporogenesi con la differenziazione delle spore. A questo punto si richiama l'attenzione su fatti nuovi ben accertati: le micorrize sono formate da micelio aploide; la gleba dell'ascocarpo maturo risulta formata da ife aploidi (monocariotiche) con almeno un allele per locus; le ascospore di un ascoma esaminate nel loro insieme sono aploidi e presentano sempre gli alleli della gleba ed altri di provenienza da verificare; ne consegue che nel corso del ciclo del tartufo hanno luogo fenomeni di incrocio (fertilizzazione) (Paolocci *et al*., 2006). Ci poniamo ora due interrogativi: la fertilizzazione dove avviene? Con quale modalità si realizza? Attendiamo ulteriori ricerche su questo argomento.
Il tartufo si riproduce anche per via vegetativa attraverso la moltiplicazione delle micorrize e sembra ormai certo anche producendo esoconidi evidenziati in *T. borchii* e *T. oligospermum* mediante uno studio morfologico e molecolare (Urban *et al*., 2004).
Per quanto riguarda la germinazione delle ascospore di *T. melanosporum*, già ottenuta in passato, le ricerche sono proseguite con risultati positivi (Fischer *et al*., 2005).

Il micelio
Le ricerche per individuare substrati idonei alla coltivazione del micelio sono iniziate da alcuni decenni ma ancora non sono stati raggiunti risultati soddisfacenti, e cioè non vi è al momento attuale la possibilità di produrre quantità significative di micelio da impiegare nella inoculazione su larga scala di piantine da micorrizare. E' ormai assodato che le varie specie hanno esigenze nutrizionali diversificate. Un ulteriore problema è la variabilità intraspecifica nei confronti dell'utilizzazione degli zuccheri come evidenziata in *T. borchii*: un ceppo, ad esempio, dei quattro sperimentati è cresciuto bene in substrato liquido contenente glucosio (Saltarelli *et al*., 1999); il mannosio è stato utilizzato da tutti i ceppi, mentre il mannitolo soltanto da due (Ceccaroli *et al*., 2001). Di recente è stato messo a punto un metodo per coltivare il micelio di

T. sinense in tempo breve e a basso costo utilizzando un substrato liquido contenente solfato di magnesio, fosfato monopotassico, maltosio, lattosio, fruttosio o galattosio e sorgenti azotate (peptone, urea, estratto di lievito, estratto di carne, solfato di ammonio, cloruro di ammonio, nitrato di sodio, nitrato di potassio o nitrato di ammonio (Tang Yajie *et al*., 2007).
Accanto ai problemi sopra accennati vi è poi quello di caratterizzare i vari ceppi e le specie di appartenenza: con l'analisi morfologica riguardante il tipo di anastomosi, le vescicole e le aggregazioni ifali è stato possibile distinguere tra loro le seguenti specie: *T. maculatum*, *T. melanosporum*, *T. aestivum*, *T. macrosporum*, *T. rufum* e *T. brumale* (Iotti *et al*., 2002). Un fatto sorprendente è che i processi di anastomosi tra le ife di alcuni ceppi di *T. borchii* hanno luogo liberamente nell'ambito di uno stesso ceppo, mentre possono non verificarsi tra ceppi differenti o verificarsi con vari gradi di incompatibilità (Sbrana *et al*., 2007). Questi fenomeni potrebbero essere presenti anche in altre specie. A parte il riconoscimento su base morfologica che presenta serie difficoltà, l'analisi molecolare (DNA) effettuata parallelamente sul materiale studiato ne ha sempre consentito una sicura diagnosi (Iotti *et al.,* 2002; Sbrana *et al*., 2007). La crescita del micelio in vitro di *T. borchii* è inibita dagli inibitori della tirosinasi (Poma *et al*., 1999) e da un'alta concentrazione del glucosio (50g/l) in quanto si riduce l'attività del mannitolo deidrogenasi (Saltarelli *et al.,* 2003).

Struttura del peridio
Al fine di studiare i rapporti tra gli ascocarpi e il microambiente in cui si sviluppano è importante conoscere la fine struttura del peridio. Carpofori di *T. borchii*, *T. borchii* subsp. *sphaerosperma*, *T. oligospermum* (tartufi a peridio liscio) e *T. aestivum*, *T. melanosporum* e *T. mesentericum* (tartufi a peridio verrucoso), sono stati sottoposti ad una approfondita analisi del peridio studiando i vari strati cellulari, la presenza di filamenti miceliari esterni, il grado di ispessimento della parete cellulare e la cementazione o meno delle cellule del peridio, da cui sono emerse differenze notevoli tra i due gruppi (Pargney, 2006). A fini tassonomici sono stati comparati i cistidi dei carpofori di *T. borchii* con quelli delle micorrize della medesima specie rilevando la stessa morfologia (Giovagnotti *et al*., 2000).

Composizione chimica
Lo studio della composizione chimica dei tartufi, iniziata già ai tempi di Chatin, seconda metà dell'ottocento, è proseguita fino ad oggi, avvalendosi di apparecchiature di analisi sempre più complesse. In letteratura su questo argomento sono riportati molti lavori nei decenni precedenti. In questi ultimi anni, oggetto di ricerca sono state soprattutto sostanze particolari. Per esempio un nuovo acido grasso triidrossilato monoinsaturo è stato estratto dai carpofori di *T. indicum* nei quali gli acidi grassi oleico e linoleico sono circa il 68% del complesso lipidico (Gao Jin – Ming *et al*., 2001). Dai corpi fruttiferi della stessa specie *T. indicum* sono stati isolati altri numerosi composti organici, di cui è stata determinata la struttura chimica, tra i quali alcuni acidi grassi: docosanoico, esadecanoico (predominante), tricosanoico, ottadecanoico e nonadecanoico (Gao Jin – Ming *et al*., 2004). Per la prima volta dagli ascocarpi di *T. indicum* sono stati estratti degli sfingolipidi la cui struttura è stata stabilita con metodi chimici e spettroscopici (Gao Jin - Ming *et al.,* 2004).
In un brodo di fermentazione di *T. indicum* è stato evidenziato, tramite una nuova metodica di analisi, l'androstenolo, un composto steroideo che risulta assente in *T. aestivum* (Guan Wang *et al*., 2008). Il tartufo estivo (*T. aestivum*) del Molise è stato oggetto di analisi per la componente organica e oligominerale da cui risulta una concentrazione non patologica di alcuni metalli pesanti (Riccioni *et al*., 2004). Gli estratti acquosi organici di *T. aestivum* sono stati esaminati valutando zuccheri, alcoli, amminoacidi, acidi organici ecc; esaminati anche i componenti organici delle membrane cellulari quali lipidi, steroli e acidi grassi (Mannina *et al.,* 2004). Studiando l'evoluzione chimica dei carpofori di *T. melanosporum* nel corso del loro accrescimento è stato rilevato che in quelli maturi è presente un alto livello di carboidrati (30%) e di melanina (15%) in peso secco (Harki *et al*., 2006). La composizione proteica dei carpofori di *T. borchii* è stata analizzata da Pierleoni *et al*. (2004).

Gli aromi

Dopo la prima scoperta di una sostanza, il bismetiltiometano, importante componente dell'aroma di *T. magnatum* (Fiecchi *et al*., 1967) le ricerche sugli aromi dei tartufi sono proseguite con crescente impegno e interesse. Negli ultimi anni, grazie anche alle metodiche sempre più raffinate, i risultati in questo campo sono stati veramente straordinari, caratterizzati dalla identificazione di molte decine di sostanze organiche, alcune delle quali nuove per la scienza. E' emerso, tra l'altro, che nell'ambito di una stessa specie il complesso degli aromi varia con la provenienza geografica e nel corso del ciclo ontogenetico si rilevano importanti cambiamenti fra la fase vegetativa (micelio) incapace di produrre composti solforati e lo stadio riproduttivo in cui queste sostanze sono ben rappresentate. Si citano qui di seguito i maggiori contributi.

Nei carpofori di *T. melanosporum* raccolti a Norcia, Italia centrale, sono stati evidenziati i composti più importanti e precisamente il butan-2,3-dione e il 2 e 3-metilbutanale, il dimetil-disolfuro e il dimetiltrisolfuro (Bellesia *et al*., 1998). In carpofori freschi di *T.uncinatum* è stato trovato il 2-metilbutanolo ed altri composti (Bellesia *et al*., 1998). Lo stesso composto 2-metilbutanolo è stato evidenziato in *T. melanosporum* da Doumenc – Faure *et al*. (2001). Nel micelio coltivato in vitro di *T. borchii* sono stati individuati ventinove composti organici volatili (Tirillini *et al.,* 2000). In dodici specie eduli di tartufo dell'area europea sono stati trovati settanta composti complessivamente di cui alcuni sono comuni alle entità esaminate; è stato possibile perciò elaborare una chiave (Key flavor) per identificare le singole specie (Talou *et al*., 2001). Nei carpofori di *T. aestivum* mediante cromatografia e spettrometria di massa sono stati evidenziati trentasette composti responsabili dell'aroma, molti di più di quelli precedentemente descritti (Diaz *et al*., 2002). Nei carpofori di *T. indicum* il principale composto responsabile dell'aroma è il 3-metilbutanolo; a differenza di altri tartufi neri non sono stati trovati i solfuri (Bellesia *et al*., 2002).

Con l'ausilio della gas-cromatografia e dello spettrometro di massa sono stati identificati in *T. aestivum* e *T. melanosporum* ottantanove composti. Gli Autori spiegano la differente composizione dell'aroma dei vari carpofori esaminati con la loro diversa provenienza territoriale (Diaz *et al*., 2003). Individuati quarantanove composti organici volatili in varie fasi di sviluppo dell'ascocarpo di *T. borchii*; ognuna di queste sostanze è stata trovata soltanto in un particolare stadio di maturazione; si tratta di sostanze del gruppo dei terpenoidi. La produzione di aromadendrene da parte del corpo fruttifero completamente immaturo suggerisce l'esistenza di eventi di comunicazione fra il fungo e la pianta ospite nei primi stadi di formazione degli ascocarpi. Un'altra sostanza, l'alfa farnesene, rappresenterebbe lo stadio saprofitico e quello di maturazione delle spore e svolgerebbe un'attrazione chemiotattica verso gli organismi responsabili della dispersione delle spore (Zeppa *et al*., 2004). E' stato analizzato il mezzo di coltura in vitro di *Tilia americana – T. borchii* ove sono stati identificati settantatré composti organici volatili; ventinove di tali composti sono stati prodotti soltanto durante la fase di interazione tra i due partners, come se avessero una funzione di messaggero nella formazione delle ectomicorrize (Menotta *et al*., 2004). Sono stati esaminati mediante due diversi metodi di estrazione i costituenti dell'aroma dei carpofori di *T. melanosporum* raccolti in due aree distinte del Sud della Francia, Tricastin e Alpes de Haute Provence; individuate centotrentuno sostanze di cui una interessante frazione, costituita di composti eterociclici, è presente nei carpofori di Tricastin. La quantità e la composizione dell'aroma sono differenti in rapporto ai metodi di estrazione e alle zone di provenienza dei tartufi (Vernin *et al*., 2005). Da nove specie di tartufo e due forme sono state isolate e identificate settantacinque sostanze organiche volatili tra cui le più abbondanti risultano il dimetilsolfuro, il 2 e 3-metilbutanale, il 2-metilpropanolo e il butanone (Mauriello *et al.,* 2004). Poco è conosciuto in merito al contributo alla formazione dell'aroma di *T. melanosporum* del basso o alto punto di ebollizione delle suddette sostanze. Le aldeidi e i solfuri sono i più rappresentati e sono le sostanze con il minor punto di ebollizione (Jansen *et al*.,2005). Nel *T. magnatum* mediante gas-cromatografia e spettrometro di massa è stata confermata la presenza del bismetil-tiometano e la sua importanza dal punto di vista quantitativo e qualitativo nella formazione dell'aroma. Due sostanze sono state identificate per la prima volta, il dimetil-sulfone e il metil-sulfinil-metiltiometano (Piloni *et al*., 2005). Sono state esaminate le sostanze dell'aroma di sei specie di tartufo; dei trentasei composti volatili

identificati, quindici sono comuni a tutte le sei specie. Il dimetil solfuro è presente in cinque specie ma non in *T. brumale* (March *et al*., 2006). L'analisi delle sostanze dell'aroma dei corpi fruttiferi e del micelio di *T. borchii*, *T. indicum* e *T. melanosporum* ha evidenziato che alcune di esse sono presenti nel micelio e negli ascocarpi mentre i composti solforati sono assenti nel micelio e presenti nei corpi fruttiferi (Splivallo *et al.,* 2007). Negli ascocarpi delle suddette specie sono stati identificati centodiciannove composti organici volatili di cui settanta mai descritti nei tartufi e sessanta non segnalati nei funghi. Il profilo evidenzia un'alta variabilità intraspecifica e interspecifica. Ciò nonostante otto di queste sostanze, secondo gli Autori, potrebbero essere utilizzate come marcatori per distinguere le tre specie studiate. La totale assenza di alcune classi di composti (es. i solfuri) nel micelio, pone la questione dell'origine di questi composti nei corpi fruttiferi; si confermano così cambiamenti metabolici fra la fase vegetativa (micelio) e lo stadio riproduttivo (Splivallo *et al*., 2007). E' stato messo a punto un metodo di analisi dell'aroma di *T. magnatum*, rapido e non distruttivo basato su "Proton transfer reaction mass spectrometry" (PTR-MS) che consente valutazioni qualitative e quantitative (Aprea *et al*., 2007).

Fisiologia dei tartufi

Sono stati presi in esame i meccanismi fisiologici delle varie fasi del ciclo vitale dei tartufi cercando di individuare i geni e le proteine da essi codificate.

De Bellis *et al*., (1998) ha isolato il gene Tbf-1 dal *T. borchii* che codifica una proteina della parete cellulare, espressa nel corpo fruttifero. Nella fase di accrescimento del micelio e ancor più nello stadio di formazione e sviluppo del corpo fruttifero interviene l'enzima chitinsintasi regolato da geni evidenziati in *T. borchii* (Balestrini *et al*., 2000) e in *T. magnatum* (Garnero *et al*., 2000); l'enzima presiede alla sintesi della chitina che, come è noto, è un importante costituente della parete cellulare. L'enzima malato deidrogenasi (MDH) è stato individuato e caratterizzato in *T. melanosporum*, *T. brumale*, *T. aestivum*, *T. magnatum*, *T. rufum*. In *T. melanosporum* l'enzima è stato parzialmente purificato e utilizzato per produrre anticorpi capaci a localizzare MDH nelle micorrize e negli ascocarpi (Zarivi *et al*., 2000). E' stato clonato e caratterizzato tramite sequenziamento il gene della poliubiquitina in *T. borchii*, implicato in differenti processi vitali della cellula (Zeppa *et al*., 2001). Nei processi di sviluppo e differenziazione dei tartufi è coinvolto un altro enzima, la tirosinasi, estratto da *T. melanosporum*, purificato e caratterizzato, di cui è stata accertata una inibizione reversibile da parte del dimetilsolfuro e del bismetil-tiometano, composti dell'aroma di *T. melanosporum* e di *T. magnatum* (Zarivi *et al*., 2003).

Per quanto riguarda il metabolismo degli zuccheri è stato isolato dal micelio di *T. borchii*, il gene (hxk-1) clonato e caratterizzato che codifica la esochinasi, un enzima chiave nel processo glicolitico (Agostini *et al*., 2001). Il micelio dello stesso tartufo è stato oggetto di altre ricerche riguardanti l'utilizzazione del glucosio e degli amminoacidi (Ceccaroli *et al.,* 2003); da esso è stato estratto un enzima, l'enolasi, codificato dal gene eno-1, clonato e caratterizzato, di cui è stato confermato l'importante ruolo nella glicolisi (Polidori *et al*., 2004). Il processo di assorbimento degli zuccheri è regolato dal gene tbhxt 1 che codifica una proteina con funzione di trasportatore del D-glucosio in *T. borchii* (Polidori *et al*., 2006). In *T. borchii* tre frammenti cDNA (Tbf 12, Tbf 20 e Tbf 21) risultano espressi soltanto nella fase di fruttificazione mentre il Tbf 55 è espresso fortemente nel corpo fruttifero ed è presente anche nel micelio (Zeppa *et al*., 2000). E' stato studiato il meccanismo di controllo dello sviluppo dei corpi fruttiferi in *T. borchii* ed evidenziate differenze nella espressione dei geni tra la fase vegetativa del micelio e quella riproduttiva rispetto a due stadi di maturazione degli ascocarpi (Gabella *et al*., 2005). Nelle diverse fasi di maturazione del corpo fruttifero è stato chiarito il ruolo del ciclo gliossilato (Abba' *et al*., 2007). Per quanto riguarda il metabolismo azotato è stato isolato dal micelio di *T. borchii* e caratterizzato un trasportatore dell'ammonio (Montanini *et al*., 2002) e l'enzima glutamminasintetasi (Montanini *et al*., 2003). Nella fase dell'assunzione di Azoto intervengono geni coinvolti nella nutrizione azotata della pianta ospite a livello dell'associazione micorrizica (Guescini *et al.,* 2006). La carenza di Azoto nel substrato determina uno stress che induce una risposta nell'espressione genica e influenza i meccanismi fisiologici connessi al metabolismo degli zuccheri (Montanini *et al*., 2006). Altre ricerche riguardano la identificazione di nuove

deidrine (Ambra *et al*., 2006) e l'isolamento da *T. borchii* di un nuovo enzima, il D-mannitolo-deidrogenasi che appartiene ad un gruppo nuovo di proteine (Ceccaroli *et al.,* 2007). Interessante è la individuazione in *T.borchii* di una proteina codificata dal gene Tb fis-1, fortemente espresso nello stadio simbiotico e nei primi stadi di formazione degli ascocarpi. Questo gene è presente anche in altri numerosi gruppi di organismi: funghi filamentosi, lieviti, piante, vermi, mosche e mammiferi, e ciò indica che la proteina da esso codificata e la sua funzione sono state conservate durante l'evoluzione (Guidi *et al*., 2003). Si segnala l'isolamento di un fotorecettore dal *T. borchii* omologo al fotorecettore per la luce blu di *Neurospora crassa* (Abbà *et al*., 2004). Da *T. borchii* è stato isolato, identificato e sequenziato il gene Tb smt 3 che è espresso in tutte le fasi del ciclo vitale del tartufo: micelio, ectomicorrize e ascocarpo; tuttavia la sua fase attiva decresce durante la maturazione del corpo fruttifero (Zeppa *et al*., 2006). Queste ricerche di carattere fisiologico contribuiscono a chiarire alcuni importanti aspetti del metabolismo cellulare nella fase vegetativa e riproduttiva del tartufo.

Variabilità intraspecifica

Le metodologie di analisi molecolare hanno consentito di studiare la variabilità genetica delle specie più importanti nell'ambito del loro areale conseguendo importanti risultati dal punto di vista naturalistico ed applicativo. Un primo passo è stato la ricerca di marcatori molecolari selezionati per lo studio del polimorfismo intraspecifico (Amicucci *et al*., 2001). Il clonaggio e la caratterizzazione di due sequenze ripetute del DNA di *T. melanosporum* può consentire di studiare la variabilità intraspecifica di questa specie (Paolocci *et al.,* 2000). Per quanto riguarda il *T. magnatum* sono stati isolati e caratterizzati otto loci e studiata la loro variabilità in trecentosettanta esemplari provenienti da varie località del suo areale. Sono stati trovati da due a diciotto alleli per "locus"; non sono stati osservati eterozigoti individuali. La ricerca consente di valutare la struttura genetica e la dinamica della popolazione nell'ambito della specie (Rubini *et al*., 2004). Nella stessa specie *T. magnatum* l'analisi del polimorfismo dei microsatelliti applicato su trecentosedici campioni provenienti dall'intero areale della specie ha evidenziato che le popolazioni più a Sud e più a Nord-Ovest sono significativamente differenti rispetto alle altre popolazioni (Rubini *et al*., 2005). La variabilità intraspecifica di *T. magnatum* è stata ulteriormente accertata studiando i carpofori per cinque anni raccolti in una stessa tartufaia e comparandoli con quelli provenienti da altre regioni. Nella suddetta tartufaia sono stati individuati due genotipi mentre in altre zone è stato rilevato un ampio polimorfismo. E' evidente una differenziazione genetica in *T. magnatum* (Mello *et al*., 2005). La variabilità intraspecifica di *T. magnatum* è stata valutata anche mediante l'analisi degli isoenzimi in popolazioni italiane, da cui risulta che tutti i sistemi gene-enzima sono fissati in omozigosi. Questi risultati indicano un sistema autoriproduttivo e una bassa variabilità genetica in accordo con il limitato areale della specie (Frizzi *et al*., 2001). Tecniche di analisi similari applicate allo studio di carpofori di *T. uncinatum* di una stessa tartufaia e di siti italiani differenti hanno evidenziato un livello di polimorfismo genetico molto più elevato rispetto ad altre specie come ad esempio il *T. melanosporum* già studiato negli anni passati. Importante è la constatazione che l'analisi del rDNA mitocondriale di *T. uncinatum* ha dimostrato che morfotipi differenti possono appartenere ad uno stesso gruppo geneticamente definito (Mello *et al*., 2002). Il *T. aestivum* (= *T. uncinatum*) nell'isola Gotland presenta una bassa variabilità genetica in quanto costituisce un ecotipo ben definito (Weden *et al*., 2004). Un'altra specie, il *T. mesentericum*, ha mostrato un'ampia variabilità genetica nell'ambito del suo areale europeo e i campioni italiani risultano appartenere a un gruppo ben distinto (Sica *et al*., 2007). Per concludere questo argomento vorrei precisare che la conoscenza della struttura genetica dei tartufi è un primo passo per soddisfare una importante esigenza della tartuficoltura che è quella di individuare, caratterizzare, delimitare e selezionare gli ecotipi nell'ambito dell'areale di ogni specie; questi sono l'espressione di un lungo processo evolutivo, di adattamento a ben determinate condizioni pedologiche e climatiche; gli ecotipi sono dotati di una certa variabilità, hanno la peculiare caratteristica di trasmettere per via ereditaria un complesso di caratteri fortemente connessi all'ambiente in cui si sono differenziati. Gli ecotipi andrebbero studiati

sul posto per stabilire i loro rapporti con gli ecotipi delle piante simbionti e con l'ambiente pedoclimatico al fine di selezionare un materiale fungino e vegetale da utilizzare per produrre piante micorrizate di alta qualità.
Lo studio della variabilità genetica di *T. melanosporum*, tramite l'analisi del rDNA di carpofori raccolti in centoventi località francesi, ha consentito di individuare una decina di genotipi caratterizzati da una precisa posizione geografica del Sud della Francia e a Est e a Ovest del massiccio centrale. L'attuale distribuzione dei genotipi del *T. melanosporum* è spiegabile ipotizzando la ricolonizzazione della Francia a partire dall'Italia dopo l'ultima glaciazione seguendo due linee principali di migrazione da parte di due genotipi distinti più abbondanti rispetto agli altri.
Queste due linee di migrazione corrispondono perfettamente a quelle delle Querce che sono ospiti importanti del tartufo nero (Murat *et al*., 2004).

Variabilità interspecifica e filogenesi
Lo studio della variabilità interspecifica mediante l'analisi delle sequenze dell'ITS ha contribuito a evidenziare i rapporti di affinità e differenziali nell'ambito dei tartufi europei e di quelli cinesi, individuando alcune linee filetiche. Secondo Roux *et al*. (1999) la linea dei tartufi bianchi risulta polifiletica per cui il *T. magnatum* è stato raggruppato con i tartufi neri e non con le altre specie "bianche": *T. maculatum*, *T. borchii*, *T.dryophilum* e *T. puberulum*. I tartufi neri *T. brumale*, *T. melanosporum*, *T. indicum* e *T. himalayense* sono stati raggruppati in una clade indipendente da cui risulta una stretta vicinanza tra il *T. melanosporum* e il *T. indicum*, confermata da Rubini *et al*., 2001 e da Zhang Li-Fang *et al*. (2005). Questi ultimi Autori ritengono che il *T. pseudohimalayense* e il *T. sinense* possano essere considerati sinonimi di *T. indicum*. Secondo ulteriori ricerche sui campioni originali effettuate al microscopio elettronico a scansione e sulle sequenze di ITS e della β-tubulina di *T. indicum*, *T. himalayense*, *T. sinense* e *T. pseudohimalayense*, queste quattro entità sarebbero da ricondurre ad una sola specie: *T. indicum* nell'ambito della quale si distinguono due gruppi da considerarsi due ecotipi geografici cinesi (Wang *et al.,* 2006a). Lo stesso Autore (Wang *et al*., 2006b) analizzando le sequenze di quattro geni di tartufi neri cinesi ed europei ha evidenziato due subcladi di cui una comprende tartufi asiatici: *T. indicum*. *T. himalayense*, *T. sinense* e il *T. melanosporum*; la seconda comprende *T. pseudoexcavatum* e *T. brumale*. Le due subcladi si sarebbero diversificate relativamente presto. Si ritiene che esista una comune forma ancestrale localizzata tra Europa e Cina. La subclade *T. brumale/T. pseudoexcavatum* avrebbe iniziato a divergere per prima: il *T. brumale* sarebbe migrato verso l'Europa percorrendo una via settentrionale e il *T. pseudoexcavatum* verso la Cina. Seguendo lo stesso percorso il *T. melanosporum* dell'altra subclade sarebbe migrato verso l'Europa e il *T. indicum* verso la Cina accompagnato dalle specie vicarianti.
Un altro argomento da lungo tempo dibattuto, riguarda i rapporti filogenetici tra *T. aestivum* Vittadini e *T. uncinatum* Chatin. Paolocci *et al.* (2004) mediante l'analisi dell'ITS, dei geni della betatubulina e alfa E F-1, effettuata su un ampio numero di carpofori italiani ed europei di tartufi riferibili su basi morfologiche a *T. aestivum* e a *T. uncinatum*, ha evidenziato che non è possibile identificare un morfotipo con specifiche caratteristiche molecolari per cui le due entità sono riconducibili ad una sola specie, *T. aestivum* e le differenze tra le medesime sarebbero dovute a fattori ecologici. Questo risultato è stato confermato anche da Weden *et al*. (2005). Da ultimo si ricorda lo studio morfologico e molecolare effettuato da Laessoee *et al*. (2007) su carpofori ipogei di centotrentanove entità di Pezizali che ha consentito di individuare almeno quindici linee evolutive indipendenti dei tartufi considerati in senso lato. Il genere *Choiromyces* secondo l'Autore, dovrebbe essere inserito nella famiglia *Tuberaceae*, insieme al genere *Tuber*.

Genetica
Le ricerche nel campo della genetica dei tartufi si stanno sviluppando e contribuiscono a chiarire importanti processi fisiologici tra cui la proliferazione e l'accrescimento delle cellule tramite la

clonazione e la caratterizzazione dei geni PKC, omologhi in *T. borchii* e in *T. magnatum*, correlati alla Kinasi (Ambra *et al*., 2000). Nell'accrescimento del micelio di *T. borchii* sono espressi geni differenti da quelli implicati nella formazione del corpo fruttifero (Lacourt *et al*., 2002).
Nuove e interessanti ricerche riguardano la trasformazione genetica sperimentata in *T. borchii* ed effettuata mediante un ceppo ipervirulento di *Agrobacterium tumefaciens*; i geni della resistenza all'igromicina e quelli codificanti la proteina che aumenta il verde fluorescente (EGFP) sono stati impiegati come marcatori selettivi (Grimaldi *et al*., 2005). Le ricerche genetiche e le applicazioni biomolecolari in tartuficoltura sono state illustrate da Riccioni *et al.,* (2005).
Una linea di ricerca che meriterebbe di essere ulteriormente sviluppata è lo studio dei protoplasti, ottenuti di recente dalle ife di *T. borchii*; possono essere utilizzati per le ricerche di biochimica, di fusione e trasformazione genetica con importanti applicazioni nel campo delle biotecnologie (Poma *et al*., 2005). Di recente sono stati clonati e caratterizzati i geni codificanti gli isoprenoidi del *T. borchii* (Guidi *et al*., 2006). L'analisi genetica della variabilità intraspecifica di *T. melanosporum* è stata studiata da Riccioni *et al*., (2008a). Per lo studio genetico delle popolazioni sono stati isolati dalle ectomicorrize di *T. melanosporum* e caratterizzati i retrotransposoni che consentiranno di mettere a punto idonei marcatori molecolari (Riccioni *et al.,* 2008b). Infine, risultati eccellenti, sono stati conseguiti nello studio conoscitivo dell'intero genoma del tartufo nero attraverso il progetto innovativo "Genome sequencing of the black truffle *Tuber melanosporum*" che vede coinvolti alcuni gruppi di ricerca coordinati dal Dott. Martin F. dell'Istituto INRA di Nancy.

Piante simbionti
Il numero delle specie arboree simbionti cresce ogni anno. In Israele sono state micorrizate con *T. melanosporum* querce locali: *Quercus boissieri*, *Q. calliprinos*, *Q. ithaburensis*, *Q. libani* e specie introdotte quali *Q. hartwissiana* e *Q. pedunculiflora* (Pinkas *et al*., 2000). Con lo stesso *T. melanosporum* è stato micorrizato un nocciolo dell'Asia orientale, *Corylus heterophylla* Fisch. (Craddock, 2003). In Cile è stata realizzata la simbiosi micorrizica tra *T. melanosporum* e *Nothofagus obliqua* e *Nothofagus glauca* in vista dell'utilizzazione di piantine micorrizate nella riforestazione (Perez *et al*., 2007). Negli Stati Uniti (USA) è stato utilizzato come simbionte un ibrido tra *Quercus robur* e *Q. bicolor* e inoculato con *T. aestivum* raccolto nell'isola di Gotland, per produrre piantine resistenti all'oidio che è comune nella suddetta isola svedese come nel Missouri ove si intende realizzare tartufaie coltivate (Bruhn, 2007). In Nuova Zelanda il *Pinus radiata* e il *P. pinea* micorrizati con *T. borchii* hanno dato buoni risultati (Guerin-Laguette, 2007). Le ricerche degli anni precedenti hanno mostrato un'ampia variabilità intraspecifica delle stesse specie notoriamente tartuficole per cui è necessario individuare i biotipi o gli ecotipi meglio rispondenti ai fini della tartuficoltura. Zuccherelli *et al*., (2003) ha micorrizato in vitro con *T. magnatum* due varietà di nocciolo comune (*Corylus avellana*) la tonda romana e la tonda gentile delle Langhe ottenendo risultati positivi. A questo riguardo ci domandiamo quando le oltre 40 cultivar di Nocciolo verranno sperimentate ai fini della loro utilizzazione come piante tartufigene. La necessità di selezionare biotipi con particolari doti di affinità per le varie specie di *Tuber* e di produttività è sentita da oltre 20 anni; difatti sono state messe a punto in laboratorio tecniche di micropropagazione delle più comuni piante tartuficole (*Quercus* spp.) al fine di produrre piante omogenee, conservanti le caratteristiche prescelte. Ma, come fa osservare Averseng (2007) questa tecnica non è attualmente impiegata su larga scala; solo poche specie di Pioppo e di Tiglio sono riprodotte per via vegetativa. D'altra parte l'utilizzo di queste metologie di riproduzione, oltre al complesso lavoro di selezione che sta a monte, richiede una impegnativa organizzazione delle aziende vivaistiche che dovrebbero collezionare i cloni selezionati, replicarli e micorrizarli con ceppi selezionati di tartufo. Questa è comunque la strada da seguire per produrre piante micorrizate di alta qualità da destinare alle più diverse aree tartuficole.

La micorrizazione: metodologie ed aspetti fisiologici
Sono continuate le ricerche per ottenere piantine con abbondante apparato radicale; a tal fine

sono state sperimentate varie metodologie:

1. ricerca di substrati particolari;
2. taglio delle radici in due tempi (metodo brevettato);
3. trattamento delle radici con *Agrobacterium rizogenes*.

La provenienza geografica del tartufo influenza lo sviluppo dell'apparato radicale (Grechen *et al*., 2007). La scelta dell'ecotipo o biotipo di pianta simbionte e del tartufo da inoculare deve essere fatta in base all'affinità tra i due partners. Per ragioni di studio la tecnica di micorrizazione in vitro usando il micelio come inoculo si è notevolmente diffusa. Con questa metodologia sono state micorrizate *Q. pubescens* e *Tilia americana* con *T. brumale* evidenziando forti differenze tra le micorrize dei due ospiti tali da richiedere l'analisi molecolare per riconoscere la specie del tartufo utilizzato (Giomaro *et al*., 2002). Fenomeno analogo è stato ottenuto utilizzando ceppi diversi di *T. borchii* per produrre micorrize in *Tilia platyphyllos* che sono risultate tra loro differenti per la morfologia e l'anatomia (Sisti *et al*., 2003).Anche il *Cistus incanus* è stato micorrizato in vitro con il *T. melanosporum* utilizzando il micelio in coltura pura (Ventura *et al*., 2004). Nel processo di micorrizazione intervengono vari batteri alcune volte dannosi come lo *Staphylococcus pasteuri* che produce sessantacinque sostanze organiche volatili fortemente tossiche nei confronti del micelio di *T. borchii* (Barbieri *et al*., 2005). Accanto agli inoculi tradizionali ne viene consigliato uno, che mi pare nuovo, costituito da branche di radichette micorrizate in vitro che sarebbe più efficace del micelio. Da alcuni anni sono oggetto di studio i meccanismi fisiologici della instaurazione della simbiosi micorrizica (Stocchi *et al.,* 2000). Molti geni della pianta e del tartufo sono espressi ad alti livelli durante la fase simbiotica (Polidori *et al*., 2002); interviene anche un ferormone, il Germacrene D, evidenziato nel sistema di sintesi in vitro tra *T. borchii* e *Tilia americana* il quale potrebbe essere attivo nel primo stadio della formazione della micorriza essendo capace di stimolare recettori specifici nei confronti dei tartufi (Gioacchini *et al*., 2002). Nelle ectomicorrize di *Tilia platyphyllos* per *T. borchii* il metabolismo dell'ammonio è profondamente influenzato dai due partners (Pierleoni *et al*., 2001). Le ectomicorrize di *T. borchii* con *Tilia americana* ottenute in vitro producono composti organici volatili identificati mediante gas-cromatografia e spettrometro di massa; dei settantatré composti organici volatili evidenziati, ventinove sono prodotti durante la fase di interazione tra i due partners per cui si può fare l'ipotesi che queste molecole svolgano un ruolo di messaggero nella formazione delle ectomicorrize (Menotta *et al*., 2004). Sono stati studiati i sistemi ectomicorrizici di *Tilia platyphyllos* per *T. brumale* e *Tilia platyphyllos* per *T. borchii* inoculati in vitro con il micelio in coltura pura. In *T. platyphyllos* per *T. brumale* si rileva una più alta biomassa fungina e più alti livelli proteici rispetto alla pianta, il che significa che i geni del fungo e le proteine sono regolati in alto dopo la instaurazione della simbiosi. Nell'altra coppia (*T. platyphyllos* per *T. borchii*) i risultati sono completamente divergenti. Ciò evidenzia un ruolo diverso delle varie specie di *Tuber* nella simbiosi micorrizica (Zeppa *et al*., 2005). Di recente è stato isolato e caratterizzato il gene di *T. borchii* coinvolto nelle fasi di preinfezione di apici radicali di *Tilia americana* (Menotta *et al*., 2006) durante le quali raggiunge una forte espressione, in modo particolare dopo la stimolazione di essudati radicali (Menotta *et al*., 2007).
Ci aspettiamo ulteriori contributi altrettanto importanti in questo specifico settore.

Affinità tra le piante simbionti e i tartufi

E' di fondamentale importanza la conoscenza dell'affinità tra le piante simbionti e le varie specie di tartufo, che può emergere da un duplice controllo: in vivaio e in pieno campo.
L'esame degli apparati radicali delle piante di *Quercus petraea* e di *Pinus halepensis* di un anno di età micorrizate con *Tuber melanosporum* consente di valutare la varietà delle micorrize presenti e la loro percentuale relativa da cui emerge il grado di affinità tra il tartufo e la pianta simbionte (Nuñez Dominguez *et al*., 2007; Donnini *et al*., 2003). La verifica dello stato di micorrizazione nelle piante messe a dimora o delle tartufaie naturali è altrettanto importante e può riservare anche insospettati fenomeni. Mettendo a confronto la Roverella con il Carpino nero, specie tartuficola molto diffusa in Italia centro meridionale, risulta che la prima ha una affinità molto più forte della seconda rispetto al *T. melanosporum* (Donnini *et al*., 2003). Un'altra specie, il Castagno (*Castanea sativa*) in determinate condizioni pedologiche

si micorriza spontaneamente con il *T. aestivum* dando luogo a buone produzioni di carpofori (Donnini e Baciarelli Falini, 2006) evidenziando una buona affinità tra le due entità. Anche il Faggio mostra una forte affinità con il *T. aestivum* e il *T. melanosporum* e in alcune situazioni pedoclimatiche dell'Umbria è un ottimo produttore di questi tartufi (Granetti *et al.,* 2005).

Caratterizzazione delle micorrize

Alla descrizione morfologica delle micorrize è seguita, già da lungo tempo, la loro caratterizzazione tramite analisi molecolare che consente come ben sappiamo, il loro riconoscimento in qualunque stadio di sviluppo (Amicucci *et al.,* 1998). Le ricerche sono estese alla maggior parte dei tartufi.

Sin dal 1999 è stato messo a punto un protocollo per l'isolamento e l'amplificazione del DNA delle ectomicorrize usando primer ITS specie specifici in multiplex PCR con il quale è stato possibile identificare le micorrize di *T. melanosporum, T. indicum* e *T. brumale* in un singolo "step" di amplificazione (Paolocci *et al*., 1999). Con metodo molecolare veloce e sensibile sono state ulteriormente identificate le micorrize di *T. indicum* e differenziate da quelle di *T. melanosporum* (Mabru *et al*., 2001). L'analisi molecolare ha consentito di identificare le micorrize di *T. magnatum* e di poterle così anche descrivere morfologicamente ponendo fine a incertezze pluriennali sulla loro vera identità (Mello *et al*., 2001; Rubini *et al*., 2001b). Tramite primers specie-specifici e sequenziamento dell'ITS di *T. magnatum* e *T. borchii* è stato possibile distinguere le micorrize di questi due tartufi in piantine inoculate in condizioni controllate (Mello *et al*., 2003). Ricordiamo che il *T. borchii* compare negli apparati radicali anche quando questi vengono inoculati con sospensioni sporali di *T. magnatum*. Con tecniche analoghe sono state individuate le micorrize di *T. magnatum* in una tartufaia naturale ove sono risultate rare e indipendenti dalla produzione di ascocarpi (Murat *et al*., 2005). Altri ricercatori (Kovacs *et al.,* 2006) hanno contribuito ad approfondire questo importante argomento effettuando comparazioni morfologiche e molecolari delle ectomicorrize e dei carpofori di *T. rapaeodorum, T. rufum, T. puberulum* raccolti in una foresta dell'Ungheria. L'analisi ha consentito di individuare quattro gruppi ben distinti per cui si ravvisa la necessità di una revisione tassonomica delle tre specie studiate. Possiamo concludere che in questo settore le metodologie molecolari hanno dato un contributo determinante all'analisi delle micorrize e delle piante tartufigene poste in commercio e allo studio dell'evoluzione delle micorrize negli apparati radicali delle piante messe a dimora.

Controllo delle piante micorrizate

I primi metodi proposti per il controllo delle piante micorrizate in vivaio, oltre a indicare le procedure di prelievo delle piante e di analisi degli apparati radicali, erano basati sul riconoscimento delle micorrize di *Tuber* e di quelle di funghi estranei affidandosi alle sole caratteristiche morfologiche di queste strutture. Negli ultimi anni i suddetti metodi sono stati integrati dalle tecniche di analisi molecolare rese sempre più semplici e di facile applicazione con le quali è possibile riconoscere le micorrize anche se prive degli elementi peritrofici (Dominguez *et al*., 2007; Donnini, 2005; Donnini *et al.,* 2003; Fischer *et al*., 2003; Rubini *et al.,* 2005). Grazie alle metodologie attualmente disponibili, morfologiche e molecolari, il controllo delle piante micorrizate in vivaio è decisamente sicuro ma richiede un tempo relativamente troppo lungo per cui si auspica la messa a punto di metodi veloci e quindi anche meno costosi di quelli attuali.

Batteri e funghi associati agli ascocarpi, alle micorrize e al micelio

Da quasi settanta anni sono stati evidenziati batteri di varia natura all'interno della gleba (Sappa, 1940). Sono capaci di vivere tra le ife o al loro interno (Tocci *et al*., 1990; Pacioni,1992). L'isolamento in coltura pura da sette specie di tartufi ha consentito di individuare vari gruppi funzionali: batteri aerobi mesofili, azotobatteri aerobi (questi solo in *T. melanosporum*), batteri proteolitici, ammonizzanti, nitrosanti, nitratanti, denitrificanti. La popolazione batterica numericamente più povera è risultata in *T. indicum*. I batteri isolati (157 colture) hanno mostrato

una diffusa capacità ad utilizzare solfiti e composti organici solforati per produrre acido solfidrico (Angelini *et al*., 1998). Più di recente sono stati isolati in coltura pura dai carpofori di *T. borchii* numerosi ceppi batterici ed estratto l'intero DNA genomico rappresentativo della comunità batterica del tartufo. Individuati gamma-proteobatteri, principalmente *Pseudomonas* fluorescenti, alcuni batteri Gram-positivi e *Bacillaceae*; prevalgono gli alfa-proteobatteri che mostrano una somiglianza molto forte con i membri del gruppo *Sinorhizobium/Ensifer/Rhizobium* e *Bradyrhizobium* spp. non indicati in precedenza come batteri associati ai tartufi (Barbieri *et al*., 2005). Nei carpofori di *T. magnatum*, a vari stadi di maturazione sono stati evidenziati i suddetti gruppi batterici, sia pure con percentuali diverse. Gli alfa-proteobatteri sono risultati i maggiori costituenti della flora batterica associata ai carpofori (82%), indipendentemente dal loro grado di maturazione (Barbieri *et al.,* 2007). Oltre ai batteri, vivono associati con i carpofori di tartufo anche varie specie di lieviti. Difatti da ascocarpi di *T. melanosporum* e di *T. magnatum* sono stati isolati e identificati alcuni lieviti dei generi *Candida, Debaryomyces, Cryptococcus, Rhodotorula* e *Trichosporon* che si nutrono soltanto di metionina come fonte azotata. Questi lieviti producono in coltura pura numerose sostanze organiche volatili, tra cui il dimetilsolfuro, dimetildisolfuro, il dimetiltrisolfuro che notoriamente sono importanti componenti dell'aroma di varie specie di tartufo. Si ipotizza che detti lieviti svolgano un ruolo complementare nella sintesi degli aromi da parte dei tartufi (Buzzini *et al*., 2005).
Gli ascocarpi possono ospitare al loro interno anche funghi filamentosi evidenziati tramite analisi molecolare ma non ancora ben identificati, alcuni dei quali sono del tipo degli endofiti delle piante (Pacioni *et al*., 2007). In definitiva, gli ascocarpi sono da considerarsi come dei *microhabitat* capaci di ospitare una ricca e diversificata flora batterica e fungina di cui sono ancora poco note le interazioni fisiologiche con il tartufo. I tartufi stabiliscono rapporti con i batteri anche a livello delle ectomicorrize. Sono stati, infatti, isolati dalla superficie delle micorrize di *Quercus robur* con *T. borchii* numerosi ceppi batterici caratterizzati dal punto di vista fisiologico e tramite analisi molecolare. Molti sono Attinomiceti; ben rappresentati sono *Pseudomonas fluorescens* e *P. corrugata*. Numerosi ceppi hanno mostrato attività antifungina, mentre altri stimolano fortemente la crescita del micelio del tartufo (Sbrana *et al*., 2002). Numerose altre specie di batteri sono risultate associate al *T. borchii* durante l'instaurazione della simbiosi, molte di più di quelle finora identificate: *Pseudomonas* fluorescenti, gamma-proteobatteri, membri del gruppo *Cytophaga-Flexibacter.* Bacteroidi e Batteri Gram-Positivi. I batteri vivono anche associati al micelio coltivato in vitro come è il caso di *Staphylococcus pasteuri* individuato in una coltura pura di *T. borchii;* alcuni batteri potrebbero accompagnare il *T. borchii* nel suo intero ciclo biologico (Barbieri *et al.,* 2005).

Insetti
Un buon numero di ricerche sono state fatte nel recente passato sugli insetti che vivono nel tartufo, attratti spesso dal loro aroma e danneggiandolo in modo grave. Una specie di Coleottero molto comune in Europa è *Leiodes cinnamomea*, il cui maschio non è attratto dall'odore del tartufo maturo ma da un ferormone prodotto dalle femmine che hanno colonizzato in precedenza l'ascocarpo. Le femmine non sono attratte né dal tartufo integro maturo, né da quello infestato dalle larve di altri insetti. Tutto questo fa ipotizzare che l'attrazione dell'insetto sia dovuta a sostanze emesse dal tartufo prima dello stadio di maturazione (Hochberg *et al*., 2003).

Tartuficoltura
Al fine di costituire tartufaie coltivate altamente produttive, Dessolas (2007) ha ideato già da vari anni, in collaborazione con i ricercatori di INRA, un nuovo metodo colturale detto "Formula J.A.AD. che si colloca concettualmente tra il primo metodo di Pallier e il secondo di Tanguy. Questo metodo, descritto con dovizia di particolari nel "Nouveau Manuel de trufficulture" (Dessolas *et al.,* 2007) è in fase di sperimentazione. Esso si basa sull'utilizzazione di piantine ben micorrizate in vivai specializzati o micorrizate sul posto secondo il metodo Chevalier; sull'uso di piante comari quali la Lavanda, la *Festuca ovina*, il Ginepro, il Cisto, il Bosso, ecc, variamente

alternate alle piante simbionti; sulla stimolazione dell'attività biologica del suolo assicurata dalla microflora, microfauna, mesofauna e piante erbacee e sul sistema di allevamento delle piante secondo le indicazioni di De Bosredon (1887) e di Pradel (1914). In sostanza si tratta di costituire un ecosistema tartuficolo molto vicino a quello naturale e probabilmente più efficiente. Alla luce di questa nuova concezione naturalistica della coltivazione del tartufo sono consigliate da Dessolas, per il *T. melanosporum,* alcune semplici pratiche colturali ripartite in tre fasi: della giovinezza (J) dalla piantagione fino a tre anni; dell'adolescenza (A) da quattro a sei anni e della fase adulta (AD) oltre sei anni quando compaiono i primi tartufi; da qui la formula J.A.AD. Negli ultimi anni sono state pubblicate opere sulla tartuficoltura e argomenti ad essa connessi, frutto di pluriennali ricerche di laboratorio e sperimentazioni sul campo: Truffe e trufficulture di Olivier *et al.,* 2002; Atti del Seminario sullo stato attuale della tartuficoltura italiana a cura di Bencivenga *et al.,* 2005; Umbria, terra di tartufi di Granetti *et al.,* 2005; Appennino modenese, terre da tartufo di Zambonelli *et al.,* 2005; Truficultura-Fundamentos y Tecnicas – coordinatore Santiago Reyna, 2007; La trufa - Guida de truficultura di Sáez-Garcia-Falces *et al.,* 2008.

Accrescimento delle piante

Da lungo tempo si studiano gli effetti dei funghi simbionti sull'accrescimento delle piante ospiti; sono stati osservati vari comportamenti quali nessuno stimolo sulla crescita, forte stimolazione e rallentamento dell'accrescimento. Un esempio è quello del *T. borchii* che induce una maggiore crescita in altezza del fusto di *Pinus pinea* di cinque anni del 18% rispetto al *T. aestivum* (Angelini *et al*., 2004). Piante giovani di *Quercus petraea, Q. ilex, Q. faginea* e di *Pinus halepensis* micorrizate con *T. melanosporum* crescono meglio, in quanto assorbono bene l'acqua e più fosforo rispetto a quelle non micorrizate (Núñez Dominguez *et al*., 2003, 2006, 2007). Comunque piante di Roverella in fase produttiva si presentano alcune volte "sofferenti" come se il tartufo nero svolgesse nei loro confronti un'azione parassitaria, che sarebbe interessante studiare in modo approfondito.

La flora delle tartufaie e le aree bruciate

Lo studio della flora e della vegetazione delle tartufaie naturali delle principali specie di tartufo è iniziato da molto tempo (Atti del Congresso internazionale sul tartufo, 1968, 1988) ed è continuato negli ultimi anni (Actes du V° Congrés international, 2001) per il forte significato di bioindicatori delle condizioni pedoclimatiche di varie specie vegetali (Perez *et al.,* 2001). Le aree bruciate o "brulé" prodotte dal *T. melanosporum* e dal *T. aestivum* sono note da tempo indeterminato e oggetto di studio da alcuni decenni per individuarne le cause. Secondo Lulli *et al.* (1999) la differenziazione del suolo e le proprietà dello strato superficiale, particolarmente l'aerazione, incidono in modo chiaro sulla capacità del *T. melanosporum* di formare le aree bruciate. E' stato accertato che estratti acquosi e con DMSO dal terreno dove si è sviluppato il carpoforo di *T. aestivum* hanno attività fitotossica e genotossica sulle radici di *Vicia faba* (Lanza *et al*., 2004). Altre ricerche hanno evidenziato che sostanze volatili prodotte dagli ascocarpi di *T. melanosporum,T. indicum* e *T. borchii*, inibiscono lo sviluppo e modificano il metabolismo ossidativo di *Arabidopsis thaliana*; e questi sarebbero i motivi della scomparsa dell'erba nelle tartufaie (Splivallo *et al*., 2007).

Riforestazione

Quando si intende effettuare la riforestazione con piante micorrizate è necessario studiare bene la loro capacità di accrescimento e la potenzialità produttiva. Conviene affidarsi agli esperimenti effettuati in pieno campo. A questo riguardo si rivela interessante la caratterizzazione ecologica della massa forestale naturale che produce il *T. melanosporum* come è stato fatto nella provincia di Castellon (Spagna) da Dominguez Nuñez (2001). La riforestazione di aree bruciate con *Q. ilex* subsp. *ballota* micorrizato con *T. melanosporum*, dalle analisi fatte tre anni dopo la piantagione, ha dato risultati positivi in quanto le micorrize di *T. melanosporum* si mantengono bene nelle condizioni del campo e competono con quelle spontanee presenti nel terreno (De Miguel, *et al.,* 2005; De Roman *et al.,* 2005). In una foresta del centro della

Spagna, dopo sette anni di osservazioni è stato accertato che il *Pinus sylvestris* e il *Pinus nigra* subsp. *salzmannii* sono di scarso interesse quali produttori del tartufo nero (*T. melanosporum*) nonostante la rispondente qualità del terreno e la forte capacità di questi Pini di micorrizarsi bene sia in laboratorio sia in campo (Garcia Montero *et al*., 2007).

Biodiversità delle micorrize nelle tartufaie

Lo studio dell'evoluzione delle micorrize di *Tuber* negli apparati radicali delle piante messe a dimora è iniziato da qualche decennio con i primi lavori di Chevalier (1982); è diventato di uso corrente e indispensabile per giudicare la validità della scelta fatta del tartufo e della pianta simbionte; normalmente si effettua prima dell'inizio della produzione o per individuare le cause del declino di una tartufaia. E' importante descrivere tutti i morfotipi rinvenuti anche se non identificati, sia riferibili al genere *Tuber* che ad altre specie; di ciascun morfotipo si dovrà effettuare la caratterizzazione biomolecolare per consentire utili comparazioni tra le ricerche dei vari Autori.

E' ben noto che per il *T. melanosporum* le migliori piante simbionti sono la Roverella e il Leccio, mentre il Nocciolo e il Carpino nero perdono facilmente le micorrize di questo tartufo che vengono sostituite da quelle di funghi estranei (Baciarelli Falini *et al*., 2000). E' stata studiata la dinamica della colonizzazione del *T. melanosporum* attraverso l'analisi dei microsatelliti e dei marcatori RAPD in alberi di tre specie usando inoculo miceliare di tre differenti origini; il monitoraggio dopo tre anni dalla prima produzione di carpofori ha evidenziato un basso livello di diversità genetica (Bertault *et al*., 2001). In Israele sono state inoculate piantine di Nocciolo con *T. melanosporum* e piantate in terreno ritenuto idoneo. Dopo quattro anni le micorrize identificate tramite analisi molecolare sono risultate ancora presenti (Kagan-Zur *et al*., 2001). Nelle tartufaie naturali e coltivate a *T. melanosporum* dell'Umbria sono risultate molto frequenti le micorrize di *Sphaerosporella brunnea, Hymenogaster citrinus, Cenococcum geophilum, Scleroderma verrucosum, Pulvinula constellatio, Pisolithus arhizus,* di *T. aestivum* e di *T. brumale* (Baciarelli Falini *et al*., 2006a; Donnini *et al*., 2006); anche in giovani tartufaie di Spagna di soli tre anni di *T. melanosporum* con *Corylus avellana* e *Q. ilex* subsp. *ballota* compaiono numerose micorrize estranee (De Miguel *et al*., 2005). In tartufaie di *T. melanosporum* nel corso di dodici anni di studio sono stati evidenziati numerosi funghi micorrizici di Ascomiceti e Basidiomiceti, concorrenti nei confronti del tartufo nero pregiato. Potrebbero essere la causa dell'insuccesso di alcune tartufaie (De Miguel, 2005).

E' stata studiata la dinamica delle micorrize di *T. melanosporum* con *Q. ilex* subsp. *ballota* dopo l'incendio (De Roman Martinez *et al.,* 2004). Lo studio della biodiversità delle micorrize di una tartufaia coltivata di *T. melanosporum* dell'Umbria, ha consentito di individuare micorrize riferibili a *T. rufum, T. ferrugineum* e a *T. scruposum* e di evidenziare un fungo che colonizza le radici dell'orchidea *Epipactis*. Diagnosi queste impossibili da effettuare soltanto su basi morfologiche (Baciarelli-Falini *et al*., 2006b). In qualche fortunato caso le micorrize di *Laccaria bicolor, Hebeloma sinapizans* e di *Sphaerosporella brunnea*, dopo quattro o cinque anni dalla piantagione vengono sostituite da quelle di *T. borchii* (Zambonelli *et al*., 2000). In una tartufaia coltivata a *T. magnatum* micorrizato con varie piante simbionti (*Q. robur, Q. pubescens, Q. cerris, Corylus avellana, Ostrya carpinifolia, Populus nigra, P. alba, Salix caprea, S. alba, S. eleagnos, S. apennina*) dopo quindici anni dall'impianto sono stati trovati numerosi morfotipi di micorrize inquinanti di cui è stata fatta accurata descrizione e valutata la percentuale di presenza (Angelini *et al*., 2006). Dalle ricerche fin qui effettuate si conferma l'urgenza di risolvere il problema che assilla studiosi e tartuficoltori da molti anni che è quello di poter contenere lo sviluppo delle micorrize estranee e di favorire la crescita di quelle del tartufo prescelto. E' ben noto che la natura del suolo e le cure colturali possono svolgere un ruolo importante nella lotta contro il *T. brumale* e altre specie inquinanti. La ricerca di sostanze antifungine ad azione selettiva potrebbe fornire fruttuosi risultati.

A questo riguardo è stato accertato che il fungicida oxicarbossina sopprime *in vitro* il micelio di *Hebeloma sinapizans* senza danneggiare il *T. borchii* (Zambonelli *et al.,* 2001).

Questo settore di ricerca merita ulteriori approfondimenti.

Miglioramento delle tartufaie

E' questo un tema vecchio ma ancora di attualità dato che molte tartufaie sia naturali, sia coltivate, presentano un progressivo declino produttivo dovuto a molteplici fattori.

Prima di effettuare gli interventi colturali per ripristinare un soddisfacente livello di produzione è assolutamente necessario studiare in modo approfondito le cause dell'invecchiamento della tartufaia. Ben sappiamo che può dipendere dall'eccessivo ombreggiamento del suolo, soprattutto per il *T. melanosporum*, dalla insufficiente aerazione del terreno, dalla carenza di calcare attivo, di nutrienti, di acqua nei periodi critici, da irrigazioni male applicate, dall'insorgenza di una forte competizione di funghi estranei nei confronti dei tartufi pregiati, ecc.

Non potendo scendere nei particolari si rimanda ai lavori in questo settore (Tanfulli *et al*., 2001; Baciarelli Falini *et al*., 2002; Granetti *et al*., 2005; Dessolas *et al*., 2007; Reyna, 2007).

E' stato accertato che il carbonato di calcio attivo e il calcio scambiabile partecipano al mantenimento della struttura del suolo e alla nutrizione del tartufo; la produzione dei carpofori di *T. melanosporum* è in forte correlazione con la concentrazione di questi elementi (Garcia-Montero *et al*., 2007). Naturalmente ogni specie di pianta simbionte ha le sue esigenze in fatto di carbonati come ad esempio il *Cistus laurifolius* che vive bene nell'area bruciata ove è presente una bassissima percentuale di carbonato attivo (Garcia Montero *et al*., 2007). Gli effetti degli interventi agronomici possono essere visibili sin dall'anno successivo della loro applicazione.

Il suolo

Il suolo è stato oggetto di studio sin dai tempi di Chatin (1892) e negli ultimi decenni vi è stata una vasta mole di dati sulla composizione chimica e fisica del suolo idoneo alla crescita dei tartufi.

L'attenzione prevalente è stata rivolta allo studio dello spessore del suolo ove maggiormente si sviluppano gli apparati radicali micorrizati, e cioè da 0 a circa 25-30 cm di profondità. Questi dati non sono agevolmente comparabili tra loro data la forte soggettività dell'operatore addetto al prelievo dei campioni di terra. Una nota di chiarezza è fornita da Callot (1999) che dopo molti anni di ricerche sui suoli tartufigeni raccomanda di analizzare l'intero profilo del suolo e di effettuare le analisi sui vari orizzonti, compresi quelli più profondi che influenzano senza alcun dubbio la crescita dei tartufi anche se localizzati molto più in alto. Un ulteriore fattore da tenere in attenta considerazione è il grado di porosità del suolo la cui importanza non era sfuggita ai tartuficoltori del 1800; oggi questo parametro è misurabile e le ricerche effettuate fino ad ora hanno evidenziato che esso condiziona fortemente la crescita dei tartufi. Lo studio dei suoli tartufigeni registra attualmente una certa flessione. In alcune regioni, comunque, vi è la necessità di caratterizzare i suoli e le aree tartuficole e ciò viene fatto con una metodologia aggiornata che tiene conto del profilo del suolo, delle sue proprietà chimiche e fisiche e di tutto l'ambiente esterno quali altitudine, esposizione, ecc. (Casermeiro *et al*., 2002). Nell'ambito dell'areale di una specie è altrettanto importante individuare le tipologie di suolo in cui essa si riproduce, comparandole con metodologie statistiche come è stato fatto di recente per il *T. magnatum* in alcuni siti italiani (Rondelli, 2003). L'interesse prevalente nello studio del suolo è rivolto negli ultimi anni ad alcuni aspetti particolari, anche se di sicura importanza. Si è cercato di individuare una correlazione tra la presenza e la produzione di carpofori di *T. melanosporum* e vari parametri, chimici e fisici del suolo tramite elaborazioni statistiche (Castrignanò *et al*., 2000). L'analisi del suolo assume un importante significato ai fini della tartuficoltura se rapportata alla flora locale e al clima come è stato fatto in molteplici casi particolarmente in una zona di Valladolid (Spagna) da Blanco Ferrero *et al*. (2001). Poche specie, come il *T. magnatum*, sono fortemente condizionate nel loro accrescimento dalla natura del suolo (Lulli *et al.,* 1999), tale da limitarne l'areale. L'importanza del suolo sulla crescita dei tartufi è evidenziata anche da ricerche recenti (Raglione *et al.,* 2005). Lo studio del suolo a stretto contatto con l'ascocarpo che costituisce il microambiente denso di attività biologiche, riveste una importanza particolare. L'analisi del suolo aderente al corpo fruttifero di *T. mesentericum* ha mostrato un intenso scambio di cationi tra il tartufo e il terreno (Boumaza *et al*., 2001).

Attraverso lo studio di sottili lamine di suolo aderente al corpo fruttifero di *T. mesentericum* è stato possibile evidenziare il ruolo della micro e mesofauna nel processo di nutrizione ed accrescimento del tartufo (Boumaza *et al*., 2002). I rapporti tra il suolo e il tartufo si realizzano mediante le ife peritrofiche delle micorrize (ife nutrizionali e cistidi), le ife emergenti degli stromi (Pargney *et al.,* 2001) e i ciuffi di ife prodotti dal peridio (Pargney *et al.,* 2006); è importante che questi rapporti possano realizzarsi in modo ottimale (Pargney, 2007). Giustamente Pargney (2007) afferma che "Il suolo non deve essere considerato come un semplice supporto in cui si sviluppa il tartufo, ma come una struttura vivente".
Finalmente al suolo è attribuito un ruolo fondamentale nella tartuficoltura.

I tartufi del deserto
I tartufi del deserto che normalmente vivono nei paesi del Medio Oriente e del Nord Africa sono talora presenti anche nelle frange meridionali dell'Europa. Pur avendo un interesse economico locale sono oggetto da lungo tempo di ricerche accurate al pari di quelle effettuate per i tartufi pregiati neri e bianchi. Si citano alcuni lavori di maggiore rilievo.
Sono stati studiati in riferimento alla loro capacità di concentrare i radionuclidi, particolarmente il Cesio 137 in modo vario a seconda delle Regioni (Al-Azmi *et al*., 1999). La possibilità di *Terfezia pfeilii* di unirsi in simbiosi con il cocomero (*Citrullus vulgaris)* formando endomicorrize rilevate tramite analisi molecolare è stata evidenziata da Kagan-Zur *et al*. (1999). E' stato studiato l'effetto benefico dello stress idrico applicato a piantine micorrizate e non di *Helianthemum almeriense* rilevando che esso agevola l'assorbimento di Azoto, Fosforo e Potassio e predispone le piante micorrizate ad un migliore adattamento alla carenza idrica (Morte *et al*., 2000). A differenza di quanto si verifica in altre specie del genere *Terfezia* è stata evidenziata una omogeneità intraspecifica della regione ITS in *Terfezia terfezioides* (Kovacs *et al*., 2001). Dai carpofori di *Terfezia claveryi*, per la prima volta, sono state estratte, purificate e caratterizzate la polifenolossidasi (Perez-Gilabert *et al.,* 2001a) e la monofenolasi (Perez-Gilabert *et al.,* 2001b) e individuata la loro localizzazione istochimica. La composizione chimica e gli enzimi sono stati studiati in tartufi del genere *Terfezia* raccolti nel Sinai (Egitto) (Mohawed, 2002). Dagli estratti acquosi e metanolici di ascocarpi di *Terfezia claveryi* è stata isolata una proteina che ha le caratteristiche di un potente agente antimicrobico e che potrebbe essere usato nel trattamento delle infezioni dell'occhio causate da *Pseudomonas aeruginosa* (Janakat Sana *et al*., 2005). Per la prima volta è stata descritta e localizzata una esterasi non specifica da ascocarpi di *Terfezia claveryi* e valutata la sua attività (Perez – Gilabert *et al*., 2005). Con il metodo elettroforetico sono state analizzate le proteine di *Terfezia claveryi* dell'Iran (Ammarellou, 2007). I rapporti tra *Terfezia terfezioides* e *Robinia pseudoacacia* si concretizzano con la formazione di endomicorrize. Lo stesso tartufo con *Helianthemum ovatum* produce micorrize con ife intracellulari e intercellulari (reticolo di Hartig) (Kovacs *et al*., 2002). Sono state ottenute e studiate dal punto di vista morfologico e anatomico le micorrize di *Helianthemum almeriense* con *Terfezia claveryi* e *Picoa lefebvrei*, evidenziando che detta pianta forma endomicorrize in campo e ectoendomicorrize e ectomicorrize senza micoclena in vasetto, oppure ectomicorrize con micoclena e reticolo di Hartig *in vitro* (Gutierrez *et al*., 2003). Lo studio filogenetico tramite analisi molecolare di *Terfezia* e *Tirmania* ha evidenziato una stretta relazione tra i due generi; si fa l'ipotesi che derivino da una unica linea evolutiva nell'ambito delle *Pezizaceae* (Diez *et al*., 2002). Studi molecolari su *Terfezia pfeilii* e *Choiromyces echinulatus* hanno indotto gli Autori a istituire due nuovi generi: *Kalaharituber pfeilii* (sin.: *Terfezia pfeilii*) e *Eremiomyces echinulatus* (sin.:*Choiromyces echinulatus*). I nuovi generi sono della famiglia *Pezizaceae* (Ferdman *et al*., 2005).

Conservazione dei tartufi
I comuni metodi di conservazione dei tartufi, sterilizzazione, surgelazione ed essiccamento, inducono modificazioni più o meno profonde delle qualità organolettiche soprattutto per ciò che riguarda l'aroma. Come già evidenziato da Bellesia *et al*. (1997) e Tirillini *et al*. (2000) durante la conservazione fatta anche in un frigorifero da cucina le sostanze costituenti

l'aroma si modificano, diminuiscono in percentuale e si formano nuove sostanze con odore poco gradevole. Sono state studiate le variazioni nella composizione chimica dell'aroma di *T. uncinatum* dell'Italia centrale durante la conservazione dei carpofori a varie temperature (Bellesia *et al*., 1998). La quantità e la qualità dei composti organici volatili di *T. borchii* è influenzata dalla temperatura di conservazione. A temperatura ambiente la degradazione è molto forte rispetto alla bassa temperatura (Bellesia *et al*., 2001). Sono state sperimentate metodologie di conservazione a carattere familiare capaci di preservare il meglio possibile l'aroma dei tartufi *Terfezia claveryi, Terfezia hafizi* e *Tirmania nivea* (Al-Ruqaie, 2002, 2006). I processi industriali di conservazione producono forti cambiamenti nella composizione chimica degli ascocarpi di *Terfezia claveryi* e *Picoa juniperi* (Murcia *et al*., 2003). I carpofori freschi di *Terfezia claveryi* si conservano con difficoltà dato che i lipidi irrancidiscono a causa dell'attività di un enzima, *lipoxigenasi*, estratto e purificato di cui sono state studiate le proprietà cinetiche (Perez – Gilabert *et al*., 2005a; Perez – Gilabert *et al*., 2005b). Una ricetta nuova per i tartufi del deserto è quella di far bollire i tartufi in una soluzione di Na Cl al 4% per quattro minuti. Anche il trattamento a bassa temperatura (-18°C) mantiene abbastanza bene i tartufi. La disidratazione viene posta dagli Autori al 3° posto (Al-Ruqaie, 2006). Di recente sono stati analizzati gli effetti di differenti trattamenti di conservazione sui tartufi *T. magnatum, T. borchii, T. melanosporum* e *T. aestivum* monitorando il profilo biochimico e microbiologico. I tartufi freschi sono conservati a +4°C per trenta giorni, altri a -20°C per un mese e altri ancora autoclavati. La conservazione a +4°C preserva meglio le caratteristiche biochimiche e microbiologiche dei tartufi freschi. Il *T. melanosporum* e altri tartufi neri sono risultati più resistenti alla alterazione biochimica e il *T. magnatum* più resistente al deterioramento batterico (Saltarelli *et al*., 2007). Un'altra importante sperimentazione sui tartufi neri riguarda il trattamento con bassa e alta dose di raggi gamma di tartufi freschi e dopo trenta giorni di conservazione a 4°C; l'analisi per via elettroforetica e cromatografia di proteine, peptidi, polifenoli, perossidi e lo studio del profilo microbiologico hanno consentito di valutare l'efficacia del trattamento. Trattando con 1,5 KGY di irradiazione si preservano in modo migliore le caratteristiche del prodotto fresco (Nazzaro *et al*., 2007).

Le frodi

Sono perpetrate dalla notte dei tempi e da quelle tradizionali consistenti nella mescolanza di tartufi di scarso valore gastronomico e commerciale con tartufi di alto pregio si è passati all'aggiunta non dichiarata di altri funghi come ad esempio il Prataiolo (*Agaricus bisporus*). Da alcuni anni sono state poste in commercio creme al tartufo bianco nelle quali non è riscontrabile nemmeno una spora o un filamento miceliare. Il riconoscimento dei tartufi freschi è generalmente agevole con l'ausilio del microscopio. I tartufi conservati interi o a pezzi sono ancora identificabili soprattutto se utilizzati maturi. Il tritume e le creme al tartufo richiedono l'analisi delle spore che non sempre sono presenti. Le metodologie di analisi molecolare consentono di identificare i tartufi indipendentemente dai trattamenti termici adottati per la loro conservazione. Il *T. melanosporum* è identificabile rispetto ad altre specie con mezzi molecolari messi a punto da Sejalon – Delmas *et al*. (2000). Anche piccole quantità di tartufi sono svelabili nei prodotti alimentari soggetti ai più diversi trattamenti drastici, sempre con metodologie molecolari (Amicucci *et al*., 2002). Dopo l'introduzione in Europa del *T. indicum* sono state messe a punto metodologie molecolari per distinguere questa specie dal *T. melanosporum* nei prodotti trattati termicamente (Ministère de l'Économie des Finances et de l'Industrie, 2003). Ulteriori miglioramenti tecnici di estrazione e purificazione del DNA e relativa analisi molecolare, hanno consentito di individuare il *T. melanosporum, T. brumale* e *T. indicum*, specie a spore aculeate, nei prodotti conservati (Douet *et al*., 2004). La medesima tecnica è stata adottata per distinguere nei prodotti conservati le tre specie suddette e il *T. aestivum*, quest'ultimo a spore alveolate (Mabru *et al*., 2004). Di recente è stata identificata una proteina specifica nei corpi fruttiferi di *T. borchii* (TBF-1) che non è presente in altre specie di *Tuber* ad eccezione di *Tuber dryophilum* dove è evidenziata una proteina simile. Sono stati allestiti anticorpi policlonali specifici contro la proteina TBF-1, che potrebbero essere utilizzati per svelare la presenza delle suddette specie in campioni naturali e in prodotti alimentari (Palma

et al., 2005). Le metodologie di analisi macroscopica, microscopica, biochimica e molecolare consentono di identificare le specie di tartufo in tutte le tipologie di conservazione e di rilevare la più piccola frode che si possa immaginare.

Conclusioni

I contributi scientifici nell'ultimo decennio sono numerosi e riguardano oltre trenta linee di ricerca.

La ricerca di base è in forte sviluppo mentre le sperimentazioni sul campo segnano un declino probabilmente per la difficoltà di organizzare le ricerche sul territorio ove gravitano gli interessi di più soggetti: proprietari dei terreni, tartufai, normative regionali, ecc.

Nonostante l'impegno profuso dai Centri di ricerca ove operano molti giovani animati da un lodevole entusiasmo, rimangono ancora insoluti alcuni problemi di basilare importanza:

- conoscenza di tutte le fasi del ciclo vitale del tartufo;
- formulazione di substrati nutritivi per la crescita vigorosa del micelio da utilizzare per la micorrizazione delle piante su larga scala;
- individuazione dei fattori che inducono la fruttificazione;
- approfondimento delle conoscenze delle esigenze ecologiche dei tartufi nel corso del lungo ciclo vitale;
- miglioramento delle tecniche colturali in vivaio e sul campo al fine di conseguire elevate produzioni e di accorciare il periodo improduttivo che intercorre tra la messa a dimora delle piante e l'inizio della produzione;
- allestimento di piante micorrizate ottenute con ecotipi di specie boschive e biotipi di tartufo, selezionati in rapporto alle caratteristiche pedoclimatiche delle diverse aree tartuficole;
- ricerche di tecniche di conservazione che garantiscano la più alta qualità del tartufo posto in commercio.

Concludendo, si può affermare che le conoscenze scientifiche sui tartufi, nel loro complesso sono cresciute nell'ultimo decennio in modo sorprendente e mi auguro che questo Congresso possa dare risposte concrete ai problemi ancora non risolti.

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TRUFFLE RESEARCH AND CULTIVATION IN CHINA

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Abstract
Truffle research and cultivation in China. Research on truffles and their cultivation has expanded rapidly in China since the 2nd International Congress of Truffles in Spoleto, 1988. Before then, scant information on truffles was available in China - truffles were not recorded in ancient Chinese books and only 8 *Tuber* species were recorded before 1989. Chinese black truffles, part of the *T. indicum* complex, were scientifically described for the first time in 1989 and have been exploited commercially since the 1990s. Two genera: *Paradoxa* and *Tuber*, have been found in China. Within those, 25 species of *Tuber* and 1 in *Paradoxa* have been identified in China in the last two decades, half of which are new species. A few desert truffle species have also been recorded. These results suggest that China has a rich diversity of truffles. The nature of China's truffle flora also suggests that close biogeographic relationships exist between south-west China and the Mediterranean. South-west China might be a key centre of origin in terms of truffle phylogeny and distribution. While Chinese black truffles have been collected for consumption and trade locally for many years, they were virtually unknown internationally until the early 1990s when exports to international markets commenced. This has created concern about the risk of biopollution of European endemic truffle populations by Chinese endemics and aroused great interest in the study of Chinese black truffles. Research results over the last 20 years on Chinese black truffles has confirmed that the *T. indicum* species is genetically and morphologically very diverse and might be better called "the *T. indicum* complex", which would include *T. indicum, T. sinense* and *T. himalayensis*. Harvesting and trading in Chinese black truffles is a multi-million dollar industry that has created considerable income for small rural communities and farmers. In 2006 China exported 800 tonnes of Chinese black truffles worth over US$20 million. Unfortunately, forests in which truffles grow naturally have been damaged following unregulated harvesting. Careful management, regulation and conservation of truffles in China are urgently needed. The first truffle plantation was established in Taiwan in 1989 and produced truffles in 1996. Truffle plantations have been established in Hunan, Guizhou and Yunnan Province recently. The first group of ascocarps of *T. indicum* and *T. melanosporum* were produced at the Guizhou plantation in 2008 but the other plantations have not produced truffles yet. The production of truffle-infected trees and establishment of truffle plantations are underway and have considerable potential in China. China is still well behind the Western world in terms of both research on and cultivation of truffles.
Key words: China, truffles, research, cultivation.

RESEARCH

Taxonomy

Truffles are not recorded in ancient Chinese books and only 8 *Tuber* species were recorded before 1989. The first species of *Tuber* to be recorded was *Tuber taiyuanense,* which was described by B. Liu in 1985. The holotype was destroyed by a fire and the neotype was chosen by Z. Wang in 2001 (HMAS 75888) and deposited in HMAS. At the 2nd Spoleto meeting Y. Wang reported 7 *Tuber* species found in China (Wang, 1989).

Research on truffle has expanded rapidly in the last 20 years, particular on their taxonomy (Wang *et al*. 2008). Currently 2 genera: *Paradoxa* and *Tuber*, have been recorded with 25 species and 1 variety of *Tuber* and 1 in *Paradoxa* in China. Among them the 21 *Tuber* species and 1 variety are "non-black coloured" and 3-4 are "Black coloured" truffle species (kown as

"Chinese back truffles"), eleven of those species are new to science, and 7 are similar but not identical to known species and so could be new species or subspecies (Zhang, 1990; Ren 2003; Song, 2005; Wang, *et al*. 2006; Chen, 2007).
Chinese black truffles have been collected for consumption and trade for many years in Sichuan and Yunnan, China (Zhang & Wang, 1990). However, they were not formally described or well-known in China until 1989 when they were named as new species of *Tuber sinense* by Tao *et al*. (1989). They then became known around the world in the early 1990s when increasing quantities were exported (Wang *et al.* 2008). This has aroused interest in the study of these Chinese *Tuber* species (Fourré pers. comm. 1995; Wang and Hall, 2001). They were identified by European mycologists as *T. indicum*, *T. himalayense* (Zhang & Minter, 1988; Paolocci *et al*., 1997), *T. pseudohimalayense* (Majon, 1988) and *T. pseudoexcavatum* (Wang *et al*., 1998). Results of morphological and molecular studies on Chinese truffle species in the last 15 years have revealed that *T. indicum, T. sinense* and *T. himalayense* are almost indistinguishable morphologically. It was then suggested that *T. sinense* and *T. himalayense* were synonyms of *T. indicum* (Paolocci *et al*., 1997; Mabru *et al*. 2001; Song, 1995; Zhang *et al.* 2005; Wang, *et al*. 2006; Chen, 2007). Also, their molecular analyses indicated that the *T. indicum* is genetically and morphologically very diverse. Therefore, it would be better to call them "the *T. indicum* complex" which would include *T. indicum, T. sinense* and *T. himalayensis*. Most commercialised Chinese black truffles belong to the *T. indicum* complex. *T. formosanum* (nom. invalid) was described as a new species by Hu (1992) based on a collection from Taiwan but is, in fact, not a valid species. *T. pseudohimalayense* is a co-species of *T. pseudoexcavatum* (Chen, 2007). Recently *T. aestivum* (= *T. uncinatum*) was found on the Chinese black truffle market in Sichuan and confirmed to be identical to the European collections by morphological and molecular methods (Song, 1995; Chen *et al*., 1995).
A new combination of *Parodoxa gigantospora* (Y. Wang & Z.P. Li) Wang was published in 2008 based on *Tuber* species, *Tuber gigantosporum* Y. Wang & Z.P. Li described in 1991 because its asci invariably contain only 1 spore. The first time of the genus of *Paradoxa* has been found in China and only the 2nd time that the genus has been found since 1935 when it was described by Mattirolo in Italy.
Additionally, over 50 non-tubers truffle species belonging to 28 genera have been found in China in the last 20 years (Tao, 1988; Wang unpublished data).

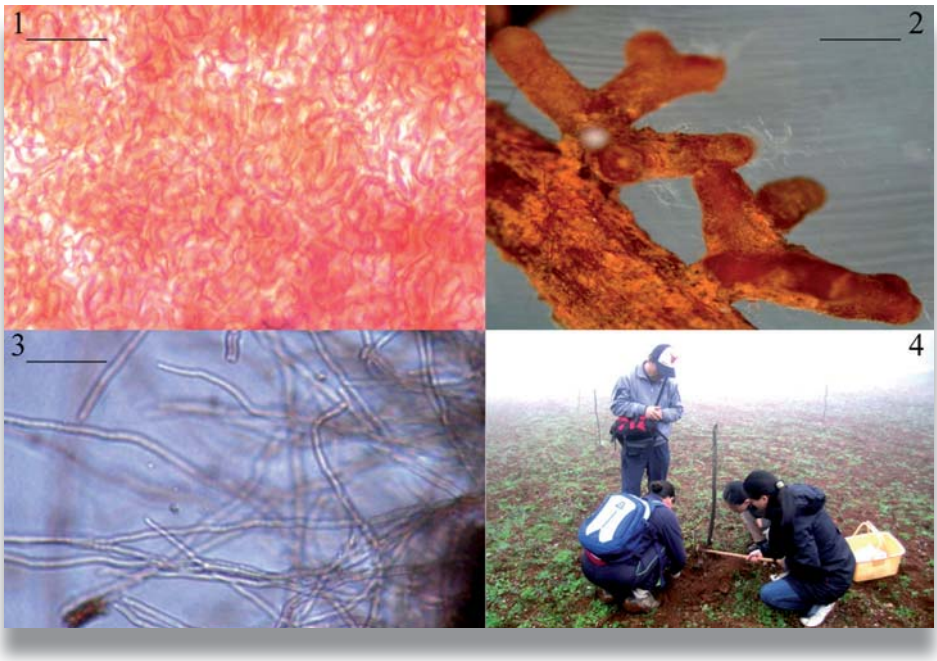
Natural truffle habitats
Chinese black truffles grow predominantly in south-west and south China (Yunnan, Sichuan, Tibet and Taiwan). The non-black truffles have been found in both south China (Yunnan, Sichuan, Tibet, Hubei, Hunan and Taiwan) and north China (Jilin, Liaoning, Inner Mongolia, Beijing, Hebei, Shanxi, Gansu and Xinjiang). Only small areas in the south-west China so the north-east and central regions of China have been searched for truffles and so truffle distribution in China has been found to be very spotty (Chen, 2007; Wang *et al*., 2008).
In northern China truffle species have been found in temperate forests, such as in coniferous forests of *Pinus, Larix, Abies* and *Picea* and in deciduous broad-leaved forests of *Quercus, Carpinus, Tilia* and *Betula*, as well as in mixed forests of pine and deciduous broad-leaved trees. Soils are usually acid or calcareous. In southern China, truffle species are found in subtropical forests, such as coniferous forests of *Pinus, Keteleeria* and *Tsuga,* in deciduous broad-leaved forests of *Quercus, Castanea* and *Carpinus,* in evergreen broad-leaved forests of *Lithocarpus, Castanopsis* and *Quercus,* and in mixed forests of pine and broad-leaved trees. Soils are usually calcareous or acid soils. Rainfall ranges from 300 to 600 mm annually.

Habitats of Chinese black truffles
Most of Chinese black truffles are produced from coniferous forests (10-40 years old) of *Pinus yunnanensis, P. armandii* and *Keteleeria evelyniana,* which are the second growth of evergreen broad-leaved forests. They have also been found in evergreen broadleaved forests of *Castanopsis, Lithocarpus*, *Cyclobalanopsis* and *Quercus* (1400-2600m). Soils are

calcareous with a pH of 7.2 (5.3-7.9), have a high content of Ca, Mg and Fe, and high ratio of Carbon to N (Chen, 2007). Annual rainfall ranges from 600 to 1000 mm with more than 2200 annual sunny hours. They produce brûlés inside grass, herbaceous plants and small shrubs died. Ascocarps form at 0-3 cm soils.

Mycorrhizas

The *T. indicum* complex can form ectomycorrhizal associations with both broad-leaved and coniferous trees. However, only a few ectomycorrhizal associations have been studied and confirmed by morphological and molecular methods, such as with *Pinus yunnanensis, P. armandii, Keteleeria evelyniana, Quercus franchetii* and *Castanea mollissima*. They are very similar to the ones produced by *Tuber melanosporum* in their vertical branching emanating hyphae and puzzling-like mantle structure (Figs 1, 2 and 3)



Figs: 1-4. Fig.1. Puzzling-like mantle structure of ectomycorrhiza of *Tuber indicum* with *Pinus yunnanensis* (bar = 30 µm). Fig.2. Mycorrhizal roots of *T. indicum* with *P. yunnanensis* (bar = 3 mm). Fig.3. Emanating hyphae on surface of ectomycorrhiza of *T. indicum* with *P. yunnanensis* (bar = 20 µm). Fig.4. A plantation established with *T. indicum* infected trees of *P. armadii* and *Castanea mollissima* at Kunming suburb, 2008.

CULTIVATION

Truffle resources in danger

Since the 1990's increasing amounts of Chinese black truffles have been exported. In 2006, 800 tonnes of Chinese black truffles worth over US$ 20 million were exported. Unfortunately, forests in which truffles grow naturally have been damaged from the plundering harvesting driven by greed. The total production of Chinese black truffles is still increasing because new regions of production are continually being found and exploited. However, local production is declining sharply due to the widespread use of destructive harvesting methods. Careful management and conservation of truffles in China are urgently needed. Cultivation of truffles to protect and expand these resources has just stated in China.

Truffle plantation establishment

The first truffle plantation was established in a 25-years-old cut-over area of *Cunnighamia lanceolata* in Taiwan in 1989 (Hu & Wang, 2004). It was located at a gentle slope at 1.200 ml. The mean annual temperature ranges between 15 and 18°C and the mean annual precipitation

varies from 1.128 to 2.272 mm. The natural soil was acid and limed with quick lime (5.0 t/ha in 1987 and 1.7 t/ha in 1988 and 1989 respectively) and the soil pH rose from 4.5 to 7.0. Two-years-old seedlings of *Cyclobalanopsis glauca* infected by *Tuber formosanum* were planted at a spacing of 6 x 6 m. Weeding was carried out three times a year. The truffle trees were pruned when they were four years old. No irrigation was applied. The first group of ascocarps was found in 1996 and 10 kg of truffles were harvested from 30 truffle trees in the following year. Most of the ascocarps were found in the soil at 1-15 cm. The largest one was 9-10 cm in diameter weighing 350 g. Brûlés formed around the trees where the ascocarps were found. Contaminated ectomycorrhizas have developed since 2.000 and fruiting bodies of *Scleroderma areolatum* and *Rhizopogon* sp. have been found since.

The second truffle plantation was established with *Quercus aliena* infected by *T. indicum*, *T. melanosporum* and *T. magnatum* at calcareous soils in Guizhou, south-west China, in 2004 and produced its first group of ascocarps of *T. indicum* and *T. melanosporum* in December 2008 (Hu pers. comm.). Four more truffle plantations have been established since 2002 but have not produced yet. One of them was established in Cili County, Hunan, China, in 2002, with *T. melanosporum*-infected hazel (*Corylus avellana*) and chestnut trees (*Castanea mollissima*). The hazel trees were obtained from France and chestnut trees were from the Institute of Forest Ecology and Environment, Hunan Academy of Forestry, China. Tree were spaced at 3 x 3 m. The soil was acid and had been used to cultivate rice. It was limed to raise the pH from 4.5 to over 7.0. Recently the roots were checked and heavy contaminations were detected. The other three plantations were established in Yunnan, in spring, 2008 (Fig. 4). One of them was in Lufeng County with *T. indicum* infected seedlings of *Quercus franchetii* and *Castanopsis delavayi* on acid red soil. The trees were produced by the Chuxiong Forest Research Institute, Yunnan in 2006. The soil was limed with quick lime only around the trees. The seedlings grew well but no new mycorrhizas had developed in November 2008.

The other two plantations were in the Kunming suburb and were planted with *T. indicum*-infected *Pinus armandii* and *Castanea mollissima* from Kunming Institute of Botany, Chinese Academy of Sciences. The soils were acid red soil and limed with limestone (around trees, 80 x 80 cm) to raise the pH to around 7.5. Tree spacing was 4 x 5 m. The trees grew well and had produced new mycorrhizas when checked in November 2008.

Production of truffle seedlings

Technologies for producing truffle-infected seedlings for establishing plantations have been either introduced from overseas (e.g. from Italy) or developed by Chinese scientists. Seedlings of *Pinus yunnanensis, P. armandii*, *Castanea mollissima*, *Quercus franchetii*, *Q. alien* and *Cyclobalanopsis glauca* have been successfully mycorrhized with *T. indicum* under greenhouse conditions. *Q. aliena* and other Chinese tree species have been successfully mycorrhized with *T. melanosporum* under greenhouse conditions. Research on technology for inoculating other Chinese oak species with *T. melanosporum* and *T. aestivum* is underway.

China is still well behind the Western world in terms of both research on and cultivation of truffles. However, south-west China has large areas of lime-stone soils and suitable climate for truffle growth where cultivation of truffles has considerable potential in China.

Discussion

The results of research on truffles in the last 20 years have indicated that China, in particular south-west China has a hunge diversity of truffle populations, genetics and habitats in what represents a key centre of truffle origin, differentiation and distribution. Around 30 species in the *Tuberaceae* and more than 50 other non-*Tuber* species have been recorded in China despite only small and limited regions of China having been searched for truffles. Compared with European truffle species Chinese truffles are genetically very diverse. For example, members of the *T. indicum* complex are very diverse genetically and morphologically, making discening their taxonomy a challenge. Three or four names have been proposed for this complex. DNA sequencings consistently divides them into two subgroups, but these two subgroups can not be

distinguished morphologically. Biodiversity is driven by habitat diversity. In China, in particular in south-west China, the Himalayas and Hengduan Mountains have formed since the Cenozoic Era, creating a complete climatic conditions from tropical, subtropical, temperate to alpine cold-temperate (including elevation from 1.000 to 7.700 m) and a complete vegetation belts from tropical rain forests to cold-temperate coniferous forests. These areas were somehow protected from the impact of the last ice age, creating a ideal refuge for living creatures, including truffles. These natural conditions have made this region one of the key centres of origin, differentiation and distribution of *Fagaceae*, *Betulaceae*, *Pinaceae* and *Salicaceae* (Lu, 1999) and, possibly, truffles.

The results of research on truffles in the last 20 years in China suggested that there are close biogeographic relationships between south-west China and the Mediterranean in truffle flora. For example, the some truffle species have been found in both regions: *T. aestivum, T. excavatum, T. borchii* var. *spherosperma* (Chen *et al*., 2008). Likewise, Chinese black truffles, the *Tuber indicum* complex in south-western China, resemble the European Périgord black truffle (*T. melanosporum*) (Yamanaka *et al*., 2001, Jeandroz *et al*., 2008). *T. pseudoexcavatum* from south-western China is closely related to the European species *T. brumale* (Jeandroz *et al*., 2008). *P. monospora* is known only from Italy and *P. gigantospora* only from south-western China. Chinese black truffles (The *T. indicum* complex) resemble Périgorde black truffle (*T. melanosporum*) not only in the morphology of their ascocarps but also their mycorrhizas. They may have the same ancestors (Jeandroz *et al*., 2008).

Harvesting and trading in Chinese black truffles has expanded rapidly in the last 20 years and brought good income to the local economy and farmers. Unfortunately, the destruction and deterioration of truffle habitats caused by commercial harvesting continue. Careful management, regulation and conservation of truffles in China are urgently needed. Fortunately, recognition that truffle habitats need to be protected is emerging. For example, the first group of truffle hunting dogs has been trained and used to detect truffles.

Acknowledgement

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TRUFFLES AND TRUFFLE CULTIVATION IN NORTH AMERICA

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Abstract
North America is home to considerable diversity of hypogeous ascomycetes, including three species celebrated in gastronomy. These include *Tuber oregonense*, the Oregon winter white truffle, *T. gibbosum*, the Oregon spring white truffle, and *Leucangium carthusianum*, known locally as the Oregon black truffle. Other common species have culinary potential, most notably *T. lyonii*, the pecan truffle. New species of truffles continue to be discovered in North America, such as the recently described black truffle from Mexico, *T. regimontanum*, which resembles *T. melanosporum* and *T. indicum*. In the genus *Tuber* alone, it is estimated that over twenty North American species still await description. The geographic range of the gastronomically important Oregon truffles largely corresponds with that of their principal host, *Pseudotsuga menziesii* var. *menziesii*, paralleling the Pacific Ocean from San Francisco, California to Vancouver, British Columbia. Unlike other ectomycorrhizal mushrooms commercially harvested throughout the region, the primary habitat of these three truffle species is typically near populated areas. While they do exist in natural forests, they are most abundant in afforested *P. menziesii* var *menziesii* plantations 15 to 30 years old established on former pasture or farm land. As early successional colonizers of anthropogenic habitats, these species would appear to be good candidates for agricultural domestication, although to-date, there have been no serious attempts. This is due, in part, to the fact that the Oregon truffles are harvested by raking, which produces predominantly immature truffles that lack aroma and have little culinary or commercial value. However, like other truffle species, when allowed to mature, the Oregon truffles develop powerful, attractive aromas. A switch from rakes to trained dogs may therefore significantly improve the general perception of Oregon's native truffles.The first *T. melanosporum* fruiting outside of Europe occurred in 1991 in California, but despite nearly continuous orchard establishment across the U.S. since that time, fewer than ten of these orchards have begun production. Nevertheless, orchard establishment has increased rapidly with the addition of a second seedling producer in 2001, and a current total of seven North American truffle tree nurseries. However, annual *T. melanosporum* yields to-date likely remain below 40 kg, most of which is from one young orchard in Tennessee and the original orchard in California. Success is likely to improve with recent increases in English language truffle cultivation literature, and with educational workshops offered through the Oregon Truffle Festival. Other European truffle species introduced in North America include *T. aestivum*, *T. borchii* and *T. magnatum*, although none have fruited to-date.
Key words: Oregon truffles, *Tuber gibbosum*, *T. oregonensis*, *Leucangium carthusianum*, *T. melanosporum*, Cultivation.

TRUFFES ET TRUFFICULTURE EN EUROPE

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Résumé
En Europe, la culture de *Tuber melanosporum* («truffe dite du Périgord», «truffe de Norcia et de Spoleto») est actuellement limitée, au nord, au 50ème parallèle (région d'Amiens, en France), au sud, à l'Espagne et à l'Italie. *T. uncinatum* (syn. *T. aestivum*?) («truffe dite de Bourgogne - Lorraine – Champagne - Franche-Comté», «truffe de Fragno») a une distribution géographique européenne. Il est probable qu'elle est indigène dans 26 des 27 pays de l'Europe, excepté la Finlande. Hors d'Europe, elle est indigène en Turquie et au Maroc. Sa large distribution est due à plusieurs facteurs d'ordre climatique (elle est beaucoup moins thermophile que *T. melanosporum*, mais plus exigeante en eau) et édaphique (elle supporte des sols de texture très variable, de sableux à très argileux, pauvres en calcaires ou même non calcaires, riches en matières organiques). Grâce au plant mycorhizé et éventuellement à l'apport d'amendements calcaires, l'aire de production de *T. melanosporum* a été considérablement agrandie: Australie, Israël, Nouvelle-Zélande, U.S.A. et récemment Maroc. En Europe, l'aire potentielle de culture de *T. uncinatum* est très importante. Le nombre d'essences-hôtes est élevé. Outre les chênes et les noisetiers communs, le noisetier turc (*Corylus colurna*), les charmes commun (*Carpinus betulus*) et -houblon (*Ostrya carpinifolia*), le pin noir d'Autriche (*Pinus nigra austriaca*), le cèdre de l'Atlas (*Cedrus atlantica*) ont donné, en France, de bons résultats. En Europe, beaucoup de sols sont propices, sans modification ou après apport d'un amendement calcaire. Le facteur limitant le plus important est le climat (froid). La culture de *T. uncinatum* a été introduite en Hongrie, en Nouvelle-Zélande et en Suède. Des essais sont en cours en Allemagne, Autriche, Grande-Bretagne, Finlande, Slovaquie, Slovénie, Serbie, Suisse et aux U.S.A.. La culture de *T. uncinatum* est différente de celle de *T. melanosporum*. Les points-clés sont: plantation à forte densité (1000 plants/hectare); taille de la partie aérienne minimale; création rapide d'un milieu ombragé, éventuellement en intercalant des feuillus précieux (alisiers, merisiers, cormier) ou des arbustes et des arbrisseaux à endomycorhizes. Comme pour *T. melanosporum*, il faut décompacter les sols et effectuer une «taille racinaire», avec des outils adaptés, mais à une époque différente de l'année. L'irrigation est encore plus importante que pour *T. melanosporum*: fréquence et importance des irrigations doivent être affinées.

Abstract: Truffle and truffle cultivation in Europe.
In Europe the cultivation of *Tuber melanosporum* ("Périgord truffle, "truffle of Norcia and Spoleto") is now limited to the 50th parallel in the North (Amiens area in France) and to Spain and Italy in the South. *T. uncinatum* (syn. *aestivum* ?) ("truffle of Burgundy – Lorraine – Champagne – French-County", "truffle of Fragno") has an European geographic distribution. It is probably native in 26 of the 27 countries of Europe (apart from Finland). Outside of Europe it is native in Turkey and in Morocco. Its wide distribution is due to several climatic factors (it is very less thermophilous than *T. melanosporum* but more water requiring) and edaphic ones (it endures very variable texture soils, from sandy to very clayey, little calcareous or even not calcareous, organic matter rich. Thanks to the mycorrhizal seedlings and eventually to the provision of calcareous enrichment, the area of production of *T. melanosporum* was considerably enlarged: Australia, Israel, New-Zealand, U.S.A. and recently Morocco. In Europe the potential area of cultivation of *T. uncinatum* is very important. The number of host-trees is high. Apart the oaks and the common hazels, Turkish hazel (*Corylus colurna*), common (*Carpinus betulus*) and –hop (*Ostrya carpinifolia*) hornbeams, Austrian black pine (*Pinus nigra austriaca*), Atlas cedar (*Cedrus atlantica*) have given good results in France. In Europe many soils are favourable without modification or after calcareous enrichment. The most important limiting factor is the

climate (cold). The cultivation of *T. uncinatum* has been introduced in Austria, Hungary, New-Zealand, Sweden. Experimentations are being carried out in Germany, Great-Britain, Finland, Slovakia, Slovenia, Serbia, Switzerland, U.S.A. The cultivation of *T. uncinatum* is different from the one of *T. melanosporum*. The keypoints are: high density of plantation (1000 plants/ha); minimal pruning of the aerial part; fast creation of shaded environment, eventually plantation of "precious" deciduous trees (wildservice trees, wildcherry trees, service trees) between the truffle mycorrhizal trees or of endomycorrhizal shrubs. As for *T. melanosporum*, it is necessary to uncompact the soil and to carry out a "root" pruning but at a different period the year. Irrigation is still more important than for *T. melanosporum*: frequency and importance of the irrigation have to be improved.

Key words: Europe, truffle, *T. melanosporum*, *T. uncinatum*, cultivation.

La truffe du Périgord et la truffe de Bourgogne en Europe

La truffe dite «de Périgord» en France ou «de Norcia et de Spoleto» en Italie (*Tuber melanosporum* Vittad.) est indigène dans le sud de l'Europe: France, Italie, Espagne, Suisse, ex-Yougoslavie. La truffe dite «de Bourgogne» en France, ou encore «de Bourgogne, Lorraine, Champagne, Franche-Comté», «truffe de Fragno» en Italie (*Tuber uncinatum* Chatin, *T. aestivum* Vittad.?) se rencontre pratiquement dans toute l'Europe. C'est la «truffe de l'Europe». Il est probable qu'elle est indigène dans 26 des 27 pays de l'Europe (seule la Finlande faisant exception). Elle est également indigène en Turquie et au Maroc. Il est vraisembable qu'elle existe en Ukraine. Elle a même été trouvée en Chine!

Pays européens supposés producteurs de *T. uncinatum*

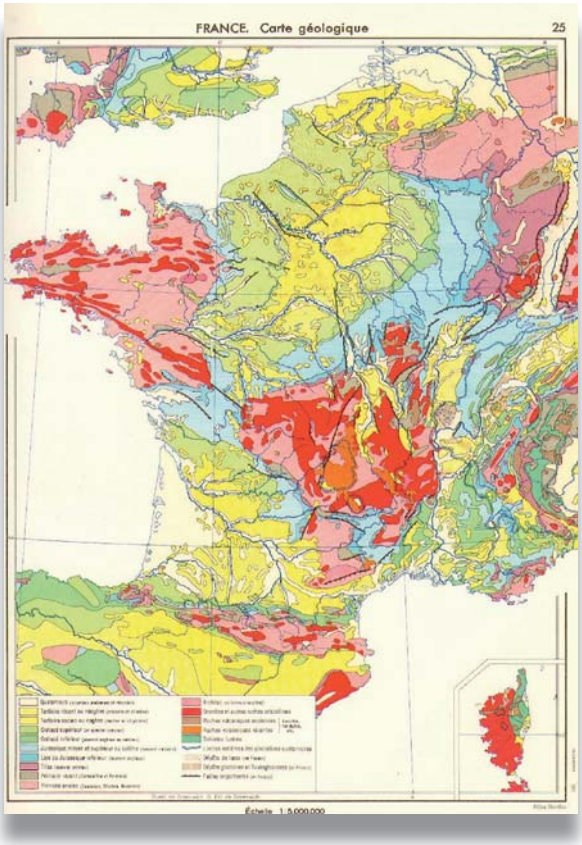
Les principaux caractères écologiques distinctifs entre la truffe du Périgord et la truffe de Bourgogne

La différence de distribution géographique entre les deux espèces est due essentiellement à deux facteurs: le sol et le climat.

Ecologie de *T. melanosporum*

Du point de vue climatique, *T. melanosporum* est une espèce xéro-thermophile, qui se développe dans les endroits ensoleillés (tendance héliophile); elle est cependant tolérante à la sécheresse, si elle n'est pas trop prolongée (tendance mésophile). C'est ce qui explique

qu'elle est plus répandue dans la partie sud de la France. Du point de vue édaphique, *T. melanosporum* craint les sols lourds, est exigeante en calcium, tolère des taux relativement élevés de matière organique. Les roches-mères sédimentaires donnant naissance à des sols aptes au développement de *T. melanosporum* couvrent d'importantes surfaces en France. Elles sont abondantes dans l'auréole calcaire du Bassin parisien, le sud-ouest (Quercy - Périgord, Charentes, Poitou), le sud–est (Rhône Alpes, Provence, Côte d' Azur, Languedoc, Roussillon). Elles correspondent aux zones en bleu, vert et jaune sur la carte géologique. Les zones en rouge et violet correspondent à des granites, des gneiss, des micaschistes et donnent des sols acides mais, par l'apport d'amendement calcaires, il est possible de les rendre aptes à la trufficulture.



Carte géologique de la France

Du point de vue phytosociologique, le champignon se développe sous des arbres isolés, des haies, des bois clairs, des friches. Les hôtes sont les chênes (*Quercus spp.*, dont les chêne pubescent, *Quercus pubescens*, et vert, *Quercus ilex*; plus rarement le chêne kermès, *Q. coccifera)*, les noisetiers (*Corylus avellana*), les tilleuls (*Tilia spp.*), les pins (*Pinus spp*.), dont le pin d' Alep (*Pinus halepensis)*.

Ecologie de *T. uncinatum* (Chevalier & Frochot, 1999)
Du point de vue climatique, la truffe de Bourgogne est beaucoup moins thermophile, ce qui explique sa distribution dans toute l'Europe, de l'Espagne à la Suède et de l'Irlande à l'Ukraine, ainsi qu'en altitude, en France (jusqu'à 1300 mètres) et sur les hauts plateaux du Maroc.
Du point de vue édaphique, *T. uncinatum* est capable de se développer dans une grande variété de sols, des plus sableux aux plus argileux. Beaucoup moins exigeante en calcium, elle peut fructifier dans des sols non calcaires, à pH légèrement inférieur à 7. Elle supporte des taux très élevés de matière organique. Le diagramme de texture ci-dessous montre que les sols suédois producteurs de *T. uncinatum* diffèrent profondément des sols français, les sols suédois étant sableux, les sols français argileux, à tendance plutôt «lourde».

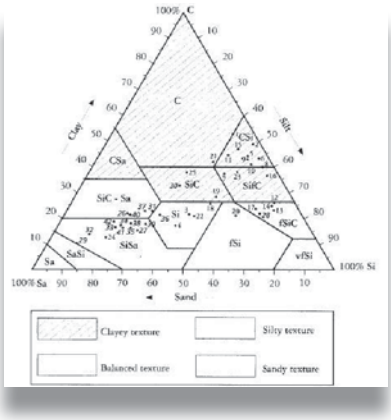
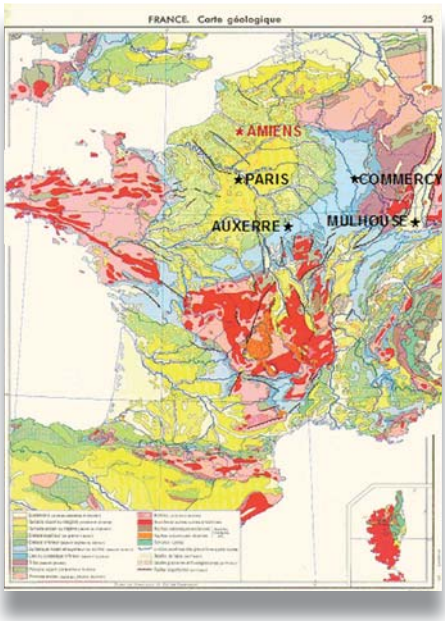


Diagramme comparatif de texture des sols français et suédois produisant *T. uncinatum* (sols suédois en bas, à gauche, numérotés en italique).

Du point de vue phytosociologique, *T. uncinatum* prospère sous des arbres isolés, des haies, des friches, dans les bois. Les plantes-hôtes sont, soit des feuillus: chênes chevelu (*Quercus cerris*), pédonculé (*Q. robur*), sessile (*Q. petraea*), pubescent (*Q. pubescens*), charmes commun (*Carpinus betulus*) et -houblon (*Ostrya carpinifolia*), noisetiers commun (*Corylus avellana*) et –de Byzance (*C. colurna*), à un degré moindre bouleau (*Betula spp*), tilleul (*Tilia spp.*), hêtre (*Fagus sylvatica*) et des résineux: pins (*Pinus spp*, dont le pin noir d' Autriche, *Pinus nigra austriaca*), cèdres (*Cedrus spp.*, dont le cèdre de l'Atlas *Cedrus atlantica*), sapins (*Abies spp*.), épicéas (*Picea spp*.).

L'aire potentielle de culture de *T. melanosporum*

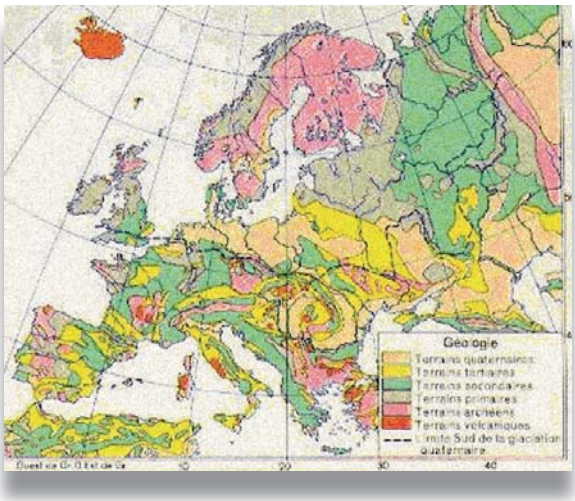
Le développement de *T. melanosporum* en France n'est pas limité par le sol mais par le climat. Il existe, dans le nord de la France, de nombreux sols aussi aptes à la trufficulture que ceux du sud-est et du sud-ouest. Par ailleurs, des sols acides peuvent devenir favorables après amendement calcaires. Les potentialités de culture sont donc énormes. Il est vraisemblable que, dans quelques décades, avec le réchauffement climatique, *T. melanosporum* sera cultivé dans la banlieue de Londres.



La culture de *T. melanosporum* dans le nord de la France

L'aire potentielle de culture de *T. uncinatum.*

Etant donné la plasticité de *T. uncinatum*, les aires potentielles de culture en Europe sont énormes. Du point de vue climatique, les zones de montagne et les zones trop nordiques sont à exclure. Du point de vue édaphique, sur la carte géologique ci-dessous, les aires en gris (terrains primaires), en vert (terrains secondaires), en jaune (terrains tertiaires) et en beige (terrains quaternaires) comprennent de nombreuses zones favorables. Les aires en rouge (terrains volcaniques) et violet (terrains archéens) sont a priori défavorables, mais peuvent être amendées par des apports massifs de calcaire. La culture de *T. uncinatum* pourrait ainsi être introduite en Finlande. Un autre problème important est le climat mais, la truffe de Bourgogne étant «remontante», c'est-à-dire capable de fructifier pratiquement toute l'année, il est probable que même un climat rude pourra permettre à la poussée estivale du champignon d'arriver à son terme. Enfin, du point de vue phytosociologique, de nombreuses essences forestières hôtes de T. *uncinatum* existent dans toute l' Europe.



La carte géologique de l'Europe

La culture de *T. uncinatum*

La première truffière à *T. uncinatum* a été plantée en France au printemps 1974, la deuxième en 1976. Dans la première, les premières truffes sont apparues 5 ans après plantation. (Chevalier, 1983). Depuis, il existe en France de nombreuses truffières âgées maintenant de plus de 20 ans. Elles ont permis de mettre au point un itinéraire cultural pour cette espèce. Il est loin d'être parfait et demande à être beaucoup amélioré.



De gauche à droite: truffières très productives de 20 ans sous noisetier commun (*Corylus avellana*), 15 ans sous charme-houblon (*Ostrya carpinifolia*), de 15 ans sous cèdre de l'Atlas (*Cedrus atlantica*)

Les points clefs sont la plantation à forte densité et une taille minimale des arbres, l'objectif étant d'obtenir de l'ombre le plus rapidement possible. L'ombre peut être obtenue plus rapidement en plantant sous couvert, entre des lignes d'arbres plantées (feuillus «précieux», arbres fruitiers, espèces d'accompagnement) ou dans des bandes défrichées (Chevalier *et al.*, 2007).

Plantation traditionnelle (noisetiers) de 13 ans ayant commençé à produire quelques truffes

Plantation contigue à la plantation ci-dessus, de même âge, entre des lignes de pins noirs d'Autriche, produisant abondamment.

Jeune plantation de plants mycorhizés par *T. uncinatum* dans des bandes forestières déboisées.

Comme pour *T. melanosporum*, il est indispensable de remettre à l'honneur le travail du sol, avec des outils adaptés, pour maintenir la pérennité de la production de truffes ou la rétablir, dans les vieilles truffières (Chevalier *et al*., 2008).
Une constante des plantations françaises, qui ne sont pas cultivées, est l'existence d'une poussée importante de truffes en été, truffes de qualité médiocre (morphotype *aestivum*), qui arrivent rarement à mûrir, et une poussée plus faible ou nulle de truffes de bonne qualité (morphotype *uncinatum*). Les conditions climatiques en été sont en effet impropres à la

maturation de *T. uncinatum* (trop chaud, trop sec). Des essais de travail du sol, avec des outils différents et à des périodes de l'année différentes, sont en cours pour essayer de remédier à ce phénomène. Une hypothèse de travail est que la suppression de la poussée estivale par un travail du sol adapté pourrait permettre à la truffe de consacrer toute son énergie à la production automnale qui seule donne des produits de qualité.

Corps fructifères superficiels dans la truffière sous charme-houblon présentée plus haut (août 2008)

Conclusion

Le facteur limitant principal à l'extension de *T. melanosporum* vers le nord de l'Europe est le climat. Le réchauffement climatique va contribuer à faire remonter cette espèce vers le nord. *T. uncinatum* est moins sensible que *T. melanosporum* au froid. Cependant, le réchauffement climatique risque également d' entraîner un déplacement de cette espèce encore plus au nord.

Les potentialités de culture de *T. uncinatum* en Europe sont énormes. En effet, non seulement tous les pays européens possèdent des essences forestières hôtes de cette espèce, mais de nombreux sols présentent naturellement des caractéristiques favorables au développement de cette truffe. Des caractéristiques chimiques défavorables peuvent être modifiées par des apports massifs d'amendements calcaires.

La récolte et/ou la culture de *T. uncinatum* peuvent constituer un trait d'union culturel, agricole et gastronomique entre Européens, bien plus étroit que ne le sont par exemple ceux de la défense et de la monnaie qui n'ont jamais réussi à rassembler l'ensemble des Européens.

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CHIC-CHAC

GENOMIC AND CELL BIOLOGY SESSION

THE GENOME OF THE PERIGORD TRUFFLE (*TUBER MELANOSPORUM*) REVEALS EVOLUTIONARY INSIGHTS INTO MYCORRHIZAL SYMBIOSIS

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Abstract
The Perigord truffle (*Tuber melanosporum* Vittad.) is a 'national, cult-food', one of the worldwide recognized icone of the European gastronomy and culture, for which genomic information could act as a knowledge platform to improve its production and environmental persistence. The fruiting body of *T. melanosporum* is an edible truffle, which is a highly appreciated delicacy for its delicate taste and perfumes. There is a pressing need to develop a thorough understanding of *Tuber* genetics so that new controlled mycorrhization procedures can be established and a better understanding of the population biology of this species acquired. Here, I will present the main features of the genome sequence from *T. melanosporum* and I will highlight gene sets involved in symbiosis and fruit body formation. This 120-million-base genome assembly contains ~10,000 predicted protein-encoding genes, only 50% showing significant similarity to sequences in protein databases, particularly those from other *Ascomycetes*. We detected unexpected genomic features most notably a very large number of transposons and repeated sequences, and a restricted number of gene families. In contrast to the ectomycorrhizal basidiomycete *L. bicolor*, the evolution toward mycorrhizal symbiosis in *T. melanosporum* led to the loss of gene families as it is often observed in obligatory symbiosis. Given the ability of *T. melanosporum* to efficiently colonize root tissues and the long standing belief that its fruiting body is able to survive without plant links, this truffle unexpectedly showed a very limited repertoire of hydrolytic enzymes involved in degradation of plant cell wall polysaccharides. Thus, *T. melanosporum* seems to be poorly adapted for efficient degradation of carbon-rich lignocellulose, which may reflect a reliance on host-supplied sucrose. The mating type genes have been identified, confirming that *T. melanosporum* is heterothallic. The analysis of these genes will allow a better understanding of the *T. melanosporum* lifecycle and the formation of ascocarps. A genome survey identified a large set of polymorphic microsatellite repeats providing new molecular markers to analyse the natural and inoculated populations. In addition, these polymorphic molecular markers will contribute to the development of forensics to control serious frauds caused by less valuable truffles.
The predicted gene inventory of *T. melanosporum* genome, therefore, points to previously unknown mechanisms of symbiosis operating in biotrophic mycorrhizal fungi.
Acknowledgements. We would like to thank the Génoscope teams for their outstanding efforts in sequencing, assembling and annotating the T. melanosporum genome. We would also like to acknowledge the members of the Tuber Genome Consortium for their dedication.

AN INTEGRATED CELL AND MOLECULAR VIEW POINT OF TRUFFLE BIOLOGY

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Abstract

Specific experiments aimed to assign a biological function to the DNA sequences found in the genome are crucial in a post-annotation phase. In order to archive this aim, mRNAs and gene products localization can be very informative and can confirm the putative function deduced by bioinformatics tools. In the past we have used several cell biology approaches, i.e. in situ hybridization and immunolabelling (immunofluorescence and immunogold) on truffles. These approaches have already permitted the localization of transcripts and several proteins in *Tuber borchii* hyphae during various step of its life cycle. Having the full genome, we suggest that these approaches would be useful to identify the specific roles for genes belonging to a complex gene family. The use of antibodies and the localization of antigens may surely suggest functions when other approaches (i.e. transformants, RNA interference, etc…) cannot be faced. LMD (Laser MicroDissection) is a powerful tool for isolating specific tissues and cell types from sectioned biological specimens, allowing a cell specific extraction of RNA, DNA, or proteins. Preliminary experiments performed on ectomycorrhizae will be presented. We propose the LMD technology to identify genes specifically activated in each of the two fungal compartments (mantle and Hartig net) forming an ectomycorhiza. We have already been applied this technique for gene expression studies in arbuscular mycorrhizae, where the cell specificity for plant and fungal genes involved in phosphate transport has been successfully demonstrated (Balestrini *et al*., 2007 – MPMI 20: 1055–1062). In addition, an accurate analysis at transmission electron microscopy could be also considered a valid support to integrate the molecular analyses, providing information about the presence of specific organelles/structures in fungal hyphae. We are using plunge-freezing procedure followed by freeze substitution to examine and describe the hyphal tip in *Tuber* species. These observations, together with the sequences annotated in the genome and the results derived from the other cell and molecular approaches, could highlight the mechanism at the basis of the hyphal growth in this fungus.

Key words: genomics and cell biology.

TRUFFLE LIFE CYCLE AND REPRODUCTIVE MODE: NEW INSIGHTS FOR TRANSLATING BASIC RESEARCH TO CULTIVATION

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Abstract
Many uncertainties still surround *Tuber* spp biology. Their reproductive mode, the ploidy level and nuclear configuration of hyphae that form their mycorrhizas and fruit bodies are still key, unsolved issues, with possible major practical implications. Molecular data coupled to microscopic observations have led mycologists to hypothesize that these fungi strictly self and that their life cycle is prevalently diploid/dikaryiotic. To gain more insights into these basic aspects of truffle life cycle, we performed molecular screenings of *T. melanosporum* and *T. magnatum* samples using co-dominant markers. These tools coupled to appropriate strategies to isolate and analyze DNA contributed by different structures that form the truffle fruit bodies allowed us to obtain data supporting the hypothesis that *Tuber* spp are prevalently outcrossing species. Furthermore, the investigation of the nuclear configuration of mycorrhizas obtained from host plant inoculation with genotyped *T. magnatum* fruit bodies entitled us to conclude that the truffle life is prevalently haploid.
The impact of these findings on truffle cultivation is discussed.
Key words: *Tuber* spp, life cycle, SSR markers, reproductive mode, SNP.

The difficulties to grow and impossibility to mate *Tuber* spp. under controlled conditions have precluded gaining direct insight into the reproductive mode of these fungi. Until now, however, the dogma was that these ascomycetes strict self because co-dominant markers never detected heterozygotic profiles in the putative diploid/dikaryiotc hyphae of ascocarps. *T. melanosporum* and *T. magnatum* fruitbodies screened with polymorphic simple sequence repeat (SSR) markers in fact always showed a single allele per locus (Bertault *et al*., 1998, 2001; Rubini *et al*., 2004). Similarly, despite the fact that extensive sequence analysis revealed the presence of single nucleotide polymorphisms (SNP) in the nuclear ITS region among *T. melanosporum* and *T. magnatum* ascocarps of different or, even, same geographical provenance, each single sample always displayed the ITS spacer without sequence ambiguities. Thus, the lack of any heterozygotic SSR and/or ITS patterns in hyphae of the truffle gleba, thought to be derived by the synthesis of two haploid primary mycelia, have been widely interpreted to mean that these fungi self (Bertault *et al*., 1998; Mello *et al*., 2005; Murat *et al*., 2004).
However, more recently, by analyzing the distribution of alleles at different SSR loci among samples collected within and among local populations of *T. magnatum* we provided genetic evidence for the occurrence of an extensive gene flow. More specifically, the data suggested the possibility of cross-fertilization between individuals belonging to the same natural populations or even belonging to geographically close *T. magnatum* populations (Rubini *et al*., 2005).
To reconcile the aforementioned evidence of gene flow among individuals with the lack of any heterozygous patterns exhibited by each given truffle, we hypothesized that the gleba that is the prevalent structure within the fruit body was indeed formed by primary, and hence, haploid mycelia. Along the same line, we also postulated that the only source of DNA highly recoverable from fruit bodies is that provided by the gleba. Indeed, the majority of the asci is largely unaffected by the grinding procedures used when standard DNA isolation protocols are applied.

To test this hypothesis, we developed a new strategy aiming at separating the asci from the surrounding gleba within each ascocarp, to isolate and differentially analyze the DNA contributed by these two structures. With this protocol in hand, we were able to fingerprint, via co-dominant SSR and/or ITS/SNP markers, both the hyphae that form the gleba and the asci that contain the meiotic spores within each given truffle. Interestingly, the genetic patterns exhibited by the asci were in many cases more complex than those exhibited by the corresponding gleba. Out of the 10 *T. magnatum* fruit bodies screened according to this protocol, seven showed asci with additional alleles as compared to those exhibited by the gleba (Paolocci *et al*., 2006). When SSR and ITS/SNP markers were used to type the asci and gleba from *T. melanosporum* ascocarps we obtained similar results: 24% of the truffle analyzed showed asci with additional alleles to those present in the gleba that always showed a single allele per locus and none ITS/SNPs (Riccioni *et al*., 2008). Noteworthy, the SSR allele present in the gleba was always detected in the asci from the same truffles. Further to this, the SSR alleles or ITS/SNPs detected in the asci but not in the gleba were generally displayed by the gleba of other fruit bodies belonging to the same populations.

All in all, these data proved our hypotheses to be correct. Gleba is formed by primary, uniparental mycelia whereas DNA from asci is recoverable only following a specific procedure. Further to this, these analyses showed that the alleles in the spores can be of different parental origin.

With this model in mind, we have reconciled the absence of heterozygous patterns in the gleba with the presence of additional alleles in the asci, reaching the conclusion that the truffle life cycle is prevalently haploid and a certain level of outcrossing can take place both in *T. melanosporum* and *T. magnatum* (Paolocci *et al.,* 2006; Rubini *et al.,* 2007; Riccioni *et al.,* 2008.). Under this scenario, we also predicted that in the truffle life cycle the diploid/dikaryiotic phase must be confined in a narrow stage during truffle development and that the fertilization step likely precedes and promotes the maternally-driven development of the gleba.

A definitive evidence that the truffle life cycle is prevalently haploid has been provided by the analysis of the SSR patterns displayed by single mycorrhizal root tips resulting from inoculation of *Quercus pubescens* seedlings with a *T. magnatum* ascocarp harboring two different alleles at two SSR loci in the asci (Paolocci *et al.,* 2006). Indeed, all the mycorrhizas showed just a single allele per locus. They could be sorted into 4 genetic classes as expected by the meiotic recombination between two unlinked loci exhibiting two alleles each. These results support the notion that mycorrhizas originate from primary and not secondary mycelia, differently from what has been thought until now (Lanfranco *et al.,* 1995).

In conclusion, our data shows that *T. magnatum* and *T. melanosporum* life cycle is prevalently haploid and these species can outcross. However, it remains to be addressed whether these fungi are obligate or even facultative outcrossing species that may switch from one reproductive mode to another according to environmental stimuli. The ongoing genome sequencing efforts will enable us to disclose the organization of the mating type genes in *T. melanosporum* and possibly in other *Tuber* spp, eventually unraveling whether these species are heterothallic or homothallic. At the moment, in the asci additional alleles on the top of those shown by the gleba were detected only in few truffles examined. Yet, this could be simply due to the relatively low number of polymorphic single locus markers isolated so far both in *T. magnatum* and *T. melanosporum*.

It will be also interesting to investigate if phylogenetically close species can intercross and, more importantly, if *T. indicum*, the Asiatic black truffle recently introduced in Europe, can interbreed with its relative *T. melanosporum* for which it represents an ecological threat (Murat *et al.,* 2008).

Our data substantially advance our understanding of the biology of *Tuber* spp., calling for a deep reconsideration of agronomic practices for truffle cultivation. Present evidence demonstrating that the two most prestigious truffle species outcross highlights the need for the development of new strategies to sustain a sufficient level of fungal biodiversity allowing strains to mate and fructify. New methodological approaches should be devised for host plant inoculation as well as for a more appropriate management of man-made truffle plantations.

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TRACKING THE ORIGIN OF *TUBER MAGNATUM* AND *T. MELANOSPORUM* TRUFFLES BY MOLECULAR MARKERS

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Abstract
The quality and market price of truffles depend upon the species and, traditionally, the place of origin. The premium species *Tuber magnatum* Pico and *T. melanosporum* Vittad. have restricted geographic distribution and show an apparent low level of intraspecific genetic polymorphism. Likely, these features stem from a population bottleneck suffered by these two species during the last glacial age and a restricted gene flow.
In this study we evaluated the genetic variability of natural populations of *T. magnatum* and *T. melanosporum* by using highly informative molecular markers and an extensive sampling representative of species distributional ranges. Our investigation proved that truffle populations are genetically differentiated and allowed us to make inference about glacial refugia and post-glacial expansion patterns of these highly valuable *Tuber* species.
Key words: SSR, AFLP, ITS, population biology, phylogeography.

Tuber magnatum Pico and *T. melanosporum* Vittad. are the two most prestigious truffle species. Both have a restricted geographic distributional range and are regarded as species with a very limited genetic polymorphism (Bertault *et al.,* 1998, 2001). Consequently, in these species the differences in phenotypic and organoleptic traits exhibited by truffles of different geographical origin have been almost exclusively imputed to environmental and ecological factors (Bertault *et al.,* 1998). The low level of genetic variability of the premium truffle species was basically explained as the results of two phenomena i) a very closed reproductive system based on self-fertilization ii) a strong population bottleneck suffered by these two species during the last glaciation. Indeed, recent studies, have revealed that truffle species are not obligate selfing organisms (Rubini *et al*., 2005, Paolocci *et al*., 2006, Riccioni *et al.,* 2008). This new evidence calls therefore for new sampling strategies (i.e. extensive sampling of natural populations and collection of multiple samples per population) and different interpretation of molecular data to carefully model and quantify genetic polymorphism within and among natural truffle populations.
To this end, highly informative microsatellites markers (Simple Sequence Repeats – SSR) developed in *T. magnatum* and *T. melanosporum* (Bertault *et al*., 2001, Rubini *et al*., 2004, Riccioni *et al*., 2008) have been here used to genotype truffles harvested throughout the distributional range of these two species.
In *T. magnatum* seven SSR markers have been used to evaluate more than 300 specimens (ascocarps) grouped into 26 populations. The SSR analyses revealed an high level of polymorphism with up to 18 alleles per locus. Considering all the loci, a total 263 different genotypes were detected within the 316 samples analyzed. The analysis of molecular variance (AMOVA) indicated the existence of a genetic structure of *T. magnatum* populations with an F*st* of 0.15. Furthermore, Mantel test and spatial autocorrelation analysis showed a significant correlation between genetic and geographic distances. Finally the spatial analysis of molecular variance (SAMOVA), clearly showed that *T. magnatum* populations are both genetically and phylogeographically structured, with the southernmost (Basilicata) and the north-westernmost (Piemonte) populations significantly differentiated from all the others.
In *T. melanosporum* SSR, ITS-SNP (Internal Transcribed Spacer, Single Nucleotide Polymorphism) and AFLP (Amplified Fragment Length Polymorphism) markers were employed to fingerprint 206 specimens grouped into 13 geographical populations.

All molecular marker used indicate that *T. melanosporum* populations are genetically structured (SSR: F*st* = 0.215, p < 0.01*;* AFLP*:* F*st* = 0.153, P < 0.01; ITS-SNP: F*st* = 0.12, p = 0.001). Moreover, the southernmost populations (Spanish populations and Italian population from Sardinia and Abruzzo) showed the highest values of SSR allelic richness and the highest level of AFLP diversity (measured using the Shannon index), providing the first genetic evidence in support of the hypothesis that the potential *T. melanosporum* refugia were located in the Italian and Spanish peninsulas and after the glaciation the species range expanded northward.
In conclusion, our study has revealed the presence of a genetic structure in *T. magnatum* and *T. melanosporum.* This new finding paves the way for tracking local populations according to molecular markers. Our findings are therefore of major relevance not only for the development of marketing strategies but also to preserve the ecological biodiversity within naturally productive truffle areas.

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THE MICROBIAL COMPLEXITY OF *TUBER MELANOSPORUM* TRUFFLEGROUNDS

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Abstract

Over the last years environmental genomics has been developed as an effective tool for the discovery of microbial communities, and for a better understanding of their interactions in complex habitats, like soil. We applied such approaches to characterize the microbial populations occurring in the rhizosphere of plants mycorrhized by *Tuber melanosporum*. In particular, we refer to a peculiar phenomenon that is closely related to this truffle species, and is called brulé (burnt area). It consists in a zone around or near the host mycorrhizal tree, where plant cover is very scarce and inside which truffles are usually collected. The reasons of such a phenomenon are still unclear, likely involving phytotoxic effects of truffle's metabolites, direct competition for nutrients and water, and parasitism on the roots of the herbaceous plants. We hypothesized an active direct or indirect role of *T. melanosporum* on the microbial populations and a relationship between the scanty plant cover and changes in biodiversity of microbial communities: with this goal, we compared fungal and bacterial microorganisms living inside and outside the burnt area. Soil samples were collected at Cahors, France, in natural and inoculated *T. melanosporum* truffle-grounds showing the peculiar burnt areas. Denaturing gradient gel electrophoresis (DGGE) was used to produce ribosomal rDNA fingerprintings of the fungal and bacterial organisms. As a complementary approach, some samples were also processed by molecular cloning of the PCR products in order to compare the efficiency of the two techniques and to identify the fungal taxa. DGGE and cloning results showed the same dynamics in fungal populations: increase of *T. melanosporum*, reduction of ectomycorrhizal *Basidiomycota* and increase of saprotrophic *Zygomycota* within the burnt area. From molecular cloning, AM fungi seemed not to be affected by the presence of *T. melanosporum* fruitbodies. At the mean time, bacterial DGGE showed that the principal populations remained unchanged inside and outside the burnt area. Taken in their whole, the results suggest that *T. melanosporum* may act as a powerful driver of microbial biodiversity in a truffe ground.

Key words: ecology and population biology, environmental genomics, truffles.

IDENTIFICATION OF MICROSATELLITES IN THE *TUBER MELANOSPORUM* VITTAD. GENOME

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Abstract

Since several years, we investigated the genetic diversity of *T. melanosporum* by sequencing some genomic regions (ITS, SCARs), analyzing few microsatellites (SSR) or applying multi-loci approaches (RAPD, AFLP). However, due to the apparent low level of *T. melanosporum* genetic diversity it was difficult to isolate polymorphic markers and all the analysis have been realized with a limited number of markers. Thanks to the genoscope and the *Tuber* Consortium, the genome of *T. melanosporum* is now available. A first screening indicated that a large number of repeat sequences, such as microsatellites, are presents in this genome. The aim of this study was (1) to identify the SSRs in the first *T. melanosporum* assembly and (2) to characterize the local populations of this truffle using these polymorphic markers.

In the *T. melanosporum* genome 22,425 SSRs (mono-, di-, tri-, tetra-, penta- hexa-nucleotides) have been identified. A subset of around 200 SSRs (di-, tri-, tetra-, penta- hexa-nucleotides) was selected for further investigations. The first results suggested that at least 50 % of the SSRs tested were polymorphs with a high numbers of alleles for some of them. This important level of genetic diversity was unexpected and is in contradiction with previous analyses. On the other hand these polymorphic SSRs have been analyzed for a large number of *T. melanosporum* ascocarps coming from Italy, France and Spain. This analysis allowed us to characterize the genetic structure and the gene flow through the natural populations of this species.

Key words: Ecology and population biology, genome, *Tuber melanosporum*, microsatellites.

We would like to thank the sequencing team of the genoscope and all colleagues from Italy, France and Spain that provided us *T. melanosporum* ascocarps.

INFLUENCE OF HOST PLANT AND DIFFERENT NUTRITIONAL SUPPLIES ON MORPHOLOGY AND GENE EXPRESSION IN *TUBER BORCHII*

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Abstract

During the different phases of mycorrhizal fungi life cycle, several morphological, genetic and metabolic modifications are induced for both symbiotic partners. These changes are influenced by multiple environmental factors: light, gravity, oxygen, temperature, soil typology, nutrient availability, root exudates and the presence of particular bacterial populations in the mycorrhizosphere. In the present study we focused on the influence of the host plant and of different nutritional substrates. A suppressive subtractive library has been constructed to find out genes differentially expressed in the pre-symbiotic phase using an *in vitro* model in which the plant *Tilia americana* and the fungus *Tuber borchii* were closed one each other, but physically separated by a membrane. The results obtained highlighted that in the presence of the plant, the fungus shows a major expression of genes involved in hyphal growth, detoxification processes and general metabolism. The same fungal response was obtained when we tested the effect of different carbon sources on the morphology and gene expression of *T. borchii* mycelia, suggesting that some sugars, in particular glucose, could act as molecular signals to prepare the fungus to the mycorrhizal symbiosis instauration.

Key words: truffle life cycle, environmental factors, morphological changes, gene expression**.**

Introduction

Truffle life cycle is highly complex and consists in the succession of three distinct phases: the saprobic, symbiotic and fructification phases (Harley and Smith, 1993). During the different phases of mycorrhizal fungi life cycle, several morphological, genetic and metabolic modifications occur (Podila *et al.,* 2002; Bue *et al.,* 2000; Nehls *et al.,* 1998). Little is known regarding these molecular processes in fungi belonging to the genus *Tuber*. It is well-known that morphological modifications of some fungal species are determined by deep cytoskeleton network modifications. The cytoskeleton structure is involved in cytoplasm distribution and reorganisation, contributes to the cell shape definition and plays a key role in cell motility and mitosis in several organisms. In filamentous fungi, the cytoskeleton reorganisation controls the continuous deposition of glico-proteic and lipid material assigned to the membrane and cell wall synthesis, making polarised apical growth possible (Lengeler *et al.,* 2000; Lee and Kronstad, 2000; Thomas *et al.,* 2003; Hazan and Liu, 2002; Bassilana *et al.,* 2003; Boyce *et al.,* 2005; Mahlert *et al.,* 2006). Changes in cytoskeleton reorganisation have been observed in symbiosis between different fungal species and specific host plant species (e.g. *Suillus bovinus* vs *Pinus sylvestris*, *Ceratobasidium cornigerum* vs. *Spiranthes sinensis*) (Gorfer *et al.,* 2001; Uetake *et al.,* 1997). In the last decade, the *Tuber* spp. have been investigated in order to gain insight into the morpho-functional changes occurring during its life cycle. These investigations have led to the identification of several genes and proteins mainly involved in the hyphal membrane and cell wall development, such as chitin synthase, protein kinase C, cell cycle regulator p21 protein and phospholipase A2 (Balestrini *et al.,* 2000; Ambra and Macino, 2000; Lacourt *et al.,* 2002; Soragni *et al.,* 2001). However, very few information is available on the cytoskeletal reorganisation during the truffle life cycle.

The elements that constitute microtubules and microfilaments, such as actin and tubulin, are highly conserved protein that interact with numerous modulator protids, regulating their

assembly, organisation and directionality in response to external factors. The molecular response is induced by extracellular signal transduction, involving signal perception, cascade amplification of the message and generation of a peculiar cell response (cell signaling). These events are often brought about by sensorial and regulatory proteins, whose activity induces specific effector genes. The complex biological mechanisms that control the succession of the various phases in the ontogenetic cycle of the ectomycorrhizal fungi are influenced by multiple environmental factors: light, gravity, oxygen, temperature, soil typology, nutrients availability, such as carbohydrates (Jejelowo and Trinci, 1998; Ceccaroli *et al.,* 2001; Saltarelli *et al.,* 2003) root exudates (Barker *et al.,* 1998) and the presence of particular bacterial populations in the mycorrhizosphere (Sbrana *et al.,* 2002; Barbieri *et al.,* 2005).
Very little information is available on the mechanisms of cell signaling induced by environmental factors and the consequent modifications both in the cellular cytoskeleton and in mitochondrial and general metabolism. In order to broaden our knowledge of these areas, our research have been focused on the identification and characterisation in the pre-symbiotic phase of genes influenced by the host plant and by different carbon sources.

Influence of the host plant
In order to gain insight into the genetic reorganization in *T. borchii* during its interaction with the symbiotic partner *T. americana*, we focused our research in the identification of differentially regulated genes during the early interaction between plant and fungus. An *in vitro* plate culture system in which the two symbionts (*T. borchii* and *T. americana*) were separated by a nylon membrane was set up and compared with a control sample represented by the same *in vitro* system containing only the fungus (Figure 1) (Menotta *et al.,* 2004b).
The strategy selected to detect the differentially expressed genes was the Suppressive Subtractive Hybridisation (SSH) technique followed by Reverse Northern and sequence analyses. The SSH technique allowed us to construct a high quality subtracted library: 115 clones were selected and 58 analysed. A total of 26 clones showed high similarity to known genes (E-value <1x10-4), 24 clones had no significant homology with known genes (E-value >1x10-4), and 8 clones represented unknown genes (Table 1).

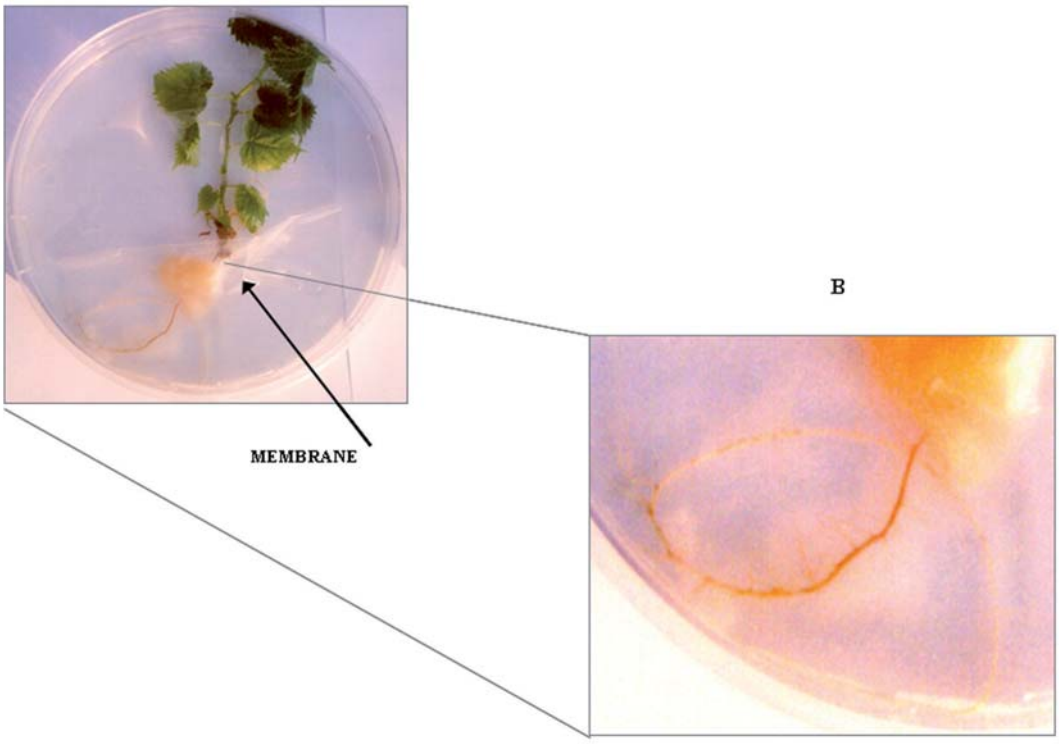

Figure 1 Culture system used in the present study. The enlarged image shows *T. borchii* mycelia growing around *Tilia* roots

The sequenced clones showed the highest homology with known proteins involved in 4 groups of cell processes: 1- cellular organelle dynamics and cell wall construction, 2- mitochondral/ microsomal metabolism and cellular detoxification processes, 3- cellular signaling and 4- cell cycle accomplishment and general metabolism.
These results lead us to hypothesise that in the early phase of the symbiosis instauration, before an actual physical contact between the two partners, a series of complex molecular mechanisms take place.

Table 1 *T. borchii* genes differentially expressed during the pre-symbiotic interaction between *T. borchii* and *T. americana.* The genes are sorted by E-values.

cDNA clone	Best BLASTX database match	E value
S103	Ribonucleotide reductase [*E. nidulans*]	5.00E-66
S102	GAS-2 homologue [*C. glabrata*]	9.00E-43
S93	26s proteasome regulatory subunit mts4 [*S. pombe*]	2.00E-41
S59	Histone H4 [*N. crassa*]	1.00E-39
S100	Putative GDP-mannose pyrophosphorylase [*C. albicans*]	4.00E-35
S35	Inorganic pyrophosphatase [*P. pastoris*]	2.00E-33
S76	COX1-i1 protein [*Y. lipolytica*]	3.00E-26
S4	Nuclear transport factor 2 [*A. nidulans*]	8.00E-25
S56/2	60S RIBOSOMAL PROTEIN L6 (YL16-LIKE) [*S. cerevisiae*]	7.00E-24
S28	Probable mRNA maturase aI5-alpha [*S. cerevisiae*]	9.00E-23
S67	Related to trichodiene oxygenase cytochrome P450 [*N. crassa*]	2.00E-19
S22	Aspartic protease [*A. oryzae*]	5.00E-19
S38	Glyoxal oxidase (glx1)	2.00E-17
S71	COI intron 9 protein [*P. anserina*]	6.00E-17
S41	Cytochrome P450 [*F. sporotrichioides*]	3.00E-16
S91	Hypothetical protein [*S. pombe*]	1.00E-15
S50	Hypothetical protein [*S. pombe*]	2.00E-13
S53/2	Putative secreted protein [*S. coelicolor* A3(2)]	3.00E-13
S43	Rho gdp dissociation inhibitor [*S. pombe*]	5.00E-13
S97	Syntaxin binding protein 1,sec1 family [*S. pombe*]	3.00E-12
S81	Related to fluconazole resistance protein (FLU1) [*N. crassa*]	2.00E-09
S29	DNA-binding protein amdA [*E. nidulans*]	6.00E-07
S27	Alpha-L-rhamnosidase A precursor [*A. aculeatus*]	1.00E-06
S42	Histidine-rich protein [*P. lophurae*]	1.00E-04
S11	Possible nuclear pore complex associated [*S. pombe*]	3.00E-04
S31	RNA-dependent RNA polymerase [*O. mitovirus 3a*]	6.00E-04

In fact during the pre-infection stage some salient events occur: in particular, a more rapid mycelial growth as the hypha gets close to the host roots is observed (Figure 2). These events depend on secretory processes, in fact the filamentous fungi grow by the continuous deposition of material for the cell wall construction at apical level. These morphological observations are consistent with the identification of the genes belonging to the first group of cell process mentioned above, such as the S97 clone which encodes for the protein SEC1, a molecule responsible for binding syntaxin and involved in vesicle membrane fusion (Brummer *et al.,* 2001; Peng and Gallwitz, 2002) and the clone S102, coding for an analogous to proteins

(GASP1p, PHR1, PHR2) that in other organisms codify a 1,3-β-glucanosil transferase and consequently have an important role in cell wall assembly (Mouyana *et al.,* 2000).

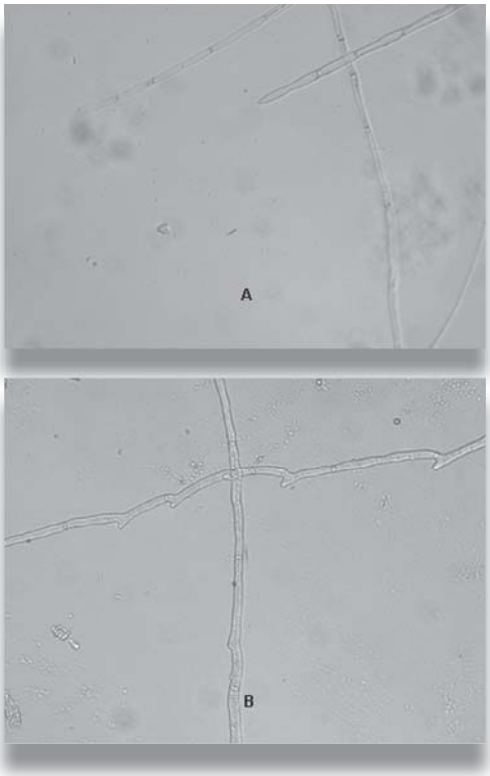

Figure 2 Representative microscopic images of a Driver sample (A) and Tester sample (B).

Among the genes involved in the detoxification processes (the second in the previous mentioned list) we found the clones S41 and S81, respectively. The first one encodes for the mitochondrial protein cytocrome P450, result which is in accordance with the detection of the VOC benzothiazole (Menotta *et al.,* 2004a) secreted by the plant during the pre-infection phase; in fact this molecule has been reported to induce strongly the cytocrome P450 expression (Suwanchaichinda and Brattsten 2002). The clone S81 codifies for an ABC transporter protein that provides azole resistance, as reported in *Neurospora crassa* and *Candida albicans* (protein FLU 1) (Calabrese *et al.,* 2000).

The activation of detoxification events is consistent with the detection of azole molecules and other volatile organic compounds in the *in vitro* ectomycorrhizal system (*T. borchii* / *T. americana*), as described in Menotta *et al.,* (2004a). Genes involved in the general cell metabolism are represented by the clones: S59, S71, S76, S87, S103 and S27. In particular the higher expression of S76 and S71 clones, which encode respectively for a COX1 protein and a COI intron 9 protein, is linked to an increased mitochondrial activity in the pre-infection stage. The expression of these genes suggests that the presence of the plant induces an increase of the fungus metabolism, probably as a response activated by mitogenic processes. From the analyses of the genes that switch on before the symbiosis instauration it is possible to highlight that a complex and tightly regulated network of molecular events happens. Interestingly, the results obtained are consistent with previous data obtained from *Tuber* or other filamentous fungi. For example the morphological evidence of an increased apical growth of *T. borchii* towards the roots of its symbiotic plant is absolutely in accordance with a higher expression of genes belonging of group one. Furthermore the genes 60S ribosomal protein and cytochrome P450 (corresponding to the clones S56/2 and S41) are known from literature to be directly or indirectly involved in fungus-plant signaling in arbuscolar symbiosis (Gianinazzi-Pearson *et al.,* 2002); an example is an improved mitochondria metabolism observed also in *Gigaspora rosea* (Tamasloukht *et al.,* 2002).

These evidences lead us to conclude that analogous molecular mechanisms take place in several endo- and ectomycorrhizal fungal species, in the preliminar step of symbiosis, before the actual contact between the two symbionts. The picture came out from the analysis of gene expression in *T. borchii* pre-infection stage, represents a first attempt to gain insight and better understand the molecular mechanisms that prime the symbiotic processes.
Moreover the differentially expressed genes are demonstrated to be involved in several molecular mechanisms, such as those aimed to recognise and attack plant roots and defend the fungus from substances secreted by the plant, which are accompanied by tightly regulated cytomorphological modifications (higher branching of hyphae that surround the root tips forming a pseudohyphal tissue and developing the Harting net).

Cdc42 and RhoGdi, key modulators of hyphal apical growth

The Rho GTP binding proteins (such as Cdc42) function as tightly regulated molecular switches that govern a variety of important cellular functions in a multitude of organisms (i.e. cytokinesis, cell motility, vacuole traffiking, secretion, apoptosis) (Hall, 1998). In particular Cdc42 is upstream a signaling cascade that, activated by a GTPase module, lead to the actin polarization and cytoskeleton organization. Rho GTPases are modulated by Guanine Nucleotide Exchange factors (GEFs), GTPase-activated proteins (GAPs) and Rho-GDI proteins. The Rho GDP-dissociation inhibitors (GDIs) serve as key multifunctional regulators of Rho family GTP-binding proteins, in that they modulate the cycling of Rho GTPases between active GTP-bound and inactive GDP-bound states and sequester the small GTP-binding proteins from the plasma membrane, inserting their C-terminally attached geranyl-geranyl moiety inside its hydrophobic pocket (Hoffman *et al.,* 2000, Rivero *et al.,* 2002).
The molecular characterisation of the *rhogdi* and *cdc42* genes was performed from a *T. borchii* cDNA library. The predicted amino acid sequences were very similar to the orthologue proteins and the similar domain structures suggested similar functions (Menotta *et al.*, 2007; Menotta *et al.,* 2008). Expression analyses performed by Real time PCR revealed an increased expression of *Tbgdi* and *TbCdc42* during the phase preparative to the symbiosis instauration, in particular after stimulation with root exudates extracts (Figure 3). In the presence of the plant, both genes show a two-fold higher expression, while the root exudates lead to four-fold major expression. This record is in accordance with the morphological evidence that during the phase which precedes the symbiosis, the mycelia grow and branch more, and confirming the role of these proteins in the apical growth.

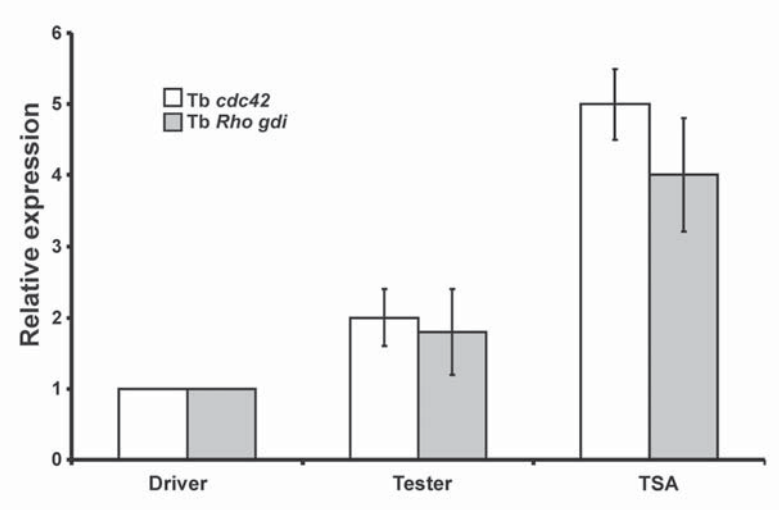

Figure 3 Real time PCR quantification of Tb*gdi* and of Tb*cdc42* in *Tuber borchii* mycelia grown in the presence of the host plant (Tester) or root exudates (TSA) compared to untreated mycelia (Driver). The ΔΔCT method was used as described in Material and Methods. Data are average ± standard deviation of at least four independent experiments each performed in triplicate.

In order to gain more information about the role of Cdc42 and since truffle transformation is still difficult, we used the yeast as a model for our studies. Primers modified (L63Q) have been used to synthesise a *Cdc42* mutated gene able to express a Cdc42 mutated protein unable to dissociate from GTP, consequently always in the active form. The mutated *Cdc42* was ligated into a pYES vector, and then transformed into yeast cells; yeast cells containing Cdc42 mutated over-expressed were compared to yeast cells containing Cdc42 wild type. Yeasts containing Cdc42 wild type didn't show any significant variation, while yeasts containing Cdc42 mutated show morphological changes characteristic of pseudo-hyphal growth, clear budding sites, giants cells and concatenated cells (Figure 4).

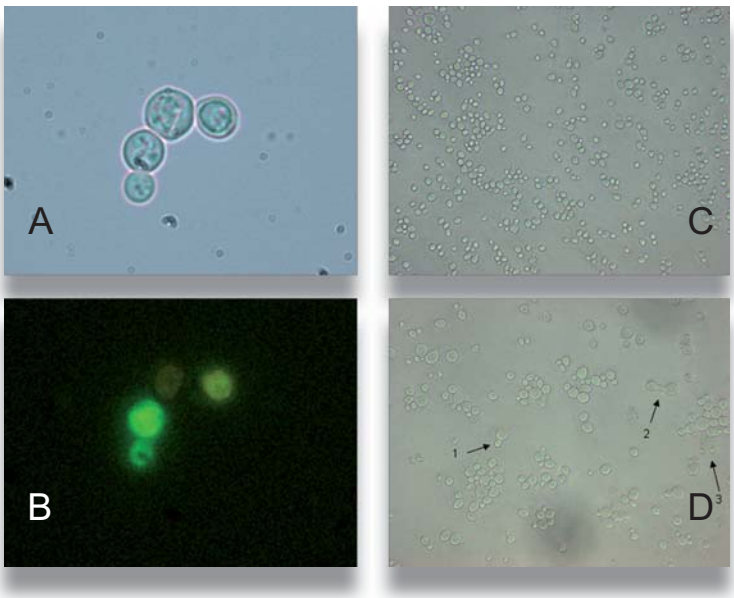

Figure 4 A, B: GFP -TbCDC42 expression in yeast. C, D: expression of Tb*Cdc42* in *S. cerevisiae* cells. C, Morphology of pYES2-Tb*Cdc42* WT (Wild Type) *S. cerevisiae* transformed cells; D, Morphology of pYES-Tb*Cdc42* Q63L *S. cerevisiae*. In D elongated budding sites (marked by arrow 1) and pseudo-hyphal growing cells (arrow 2) can be seen. Pseudo-hyphal growth and cell aggregates (arrow 3) are visible.

By the analyses of the 3D-structures obtained from comparison with known cristallographic structures available in SwissProt bank, it was possible to obtain a virtual model of TbCdc42-RhoGdi interaction structure, in which all the characteristic domains were detected, in particular the hydrophobic pocket and the Cdc42 interaction sites (data not shown).
The interaction between TbCdc42 and TbRho-Gdi has been proved also experimentally, through the realization of a yeast two hybrid system (Figure 5A) in which bait and pray were TbCdc42 and TbRho-Gdi, respectively (Figure 5B).

Figure 5 Tb CDC42 and TbGDI interaction by Yeast 2 Hybrid system. The presence of a blue colony proves the interaction of the two proteins in study, because each gene is coupled with a domain of the LacZ gene trascription factor. Surprisingly TbGdi interacts also with human Cdc42.

Influence of carbohydrate sources
Among the several environmental factors that affect the cytoskeleton modifications and

morphology one is represented by the nutrient availability. Recent studies performed on various mycelial strains of the *T. borchii* species highlighted a different infectious ability towards the plant *Tilia platyphyllos*, beyond to differences in the morphology of the mycelium, during the vegetative and symbiotic phases (Sisti *et al.,* 2003). Moreover, the same mycelial strains have shown a different capability to assimilate and metabolise sugars (Ceccaroli *et al.,* 2001). In fact, the nature and the composition of the substrate, in particular the presence and concentration of specific carbohydrates could directly influence the fungal morphology (Moore, 1998). For example, some sugars, such us L-sorbose and 2-deoxy-D-glucose can strongly affect hyphal morphology (Jejelowo and Trinci, 1998), whereas glucose can inhibit the development of the mycelium when present in moderately high concentrations (Saltarelli *et al.,* 2003), or the formation of fruit bodies in small quantities (Moor-Landeker, 1987). The substrate composition is known to influence also the infectivity of fungal isolates and their ability to form ectomycorrhizae as well as switching from ectomycorrhizal to ectendomycorrhizal (Gutierrez *et al.,* 2003).

The knowledge regarding the molecular and biochemical processes determining the marked morpho-functional modifications for both symbiotic partners induced by nutritional factors, is still limited in fungi belonging to the genus *Tuber* (Buee *et al.,* 2000; Podila *et al.,* 2002).

For these reasons, the fungal morphology of *T. borchii* mycelial culture cultivated in glucose, maltose and sucrose was evaluated to identify possible modifications related to the carbohydrate substrates used in the culture media. Furthermore, in order to gain insight the molecular mechanisms that lead to hyphal growth and change of cell morphology, a preliminary approach, through macroarray technique, was applied on genes involved in hyphal growth, detoxification processes and general metabolism. The morphological analyses were performed following the parameters described in Iotti *et al.*, 2002 and based on the measurement of: hyphal growth unit (= total length of mycelium: total number of tips), hyphal branching angle, distance between septa, hyphal diameter.

By morphological analyses the greater degree of hyphal branching was observed in mycelia grown in glucose, while in maltose, sucrose and without sugar a lower branching was observed (Figure 6).

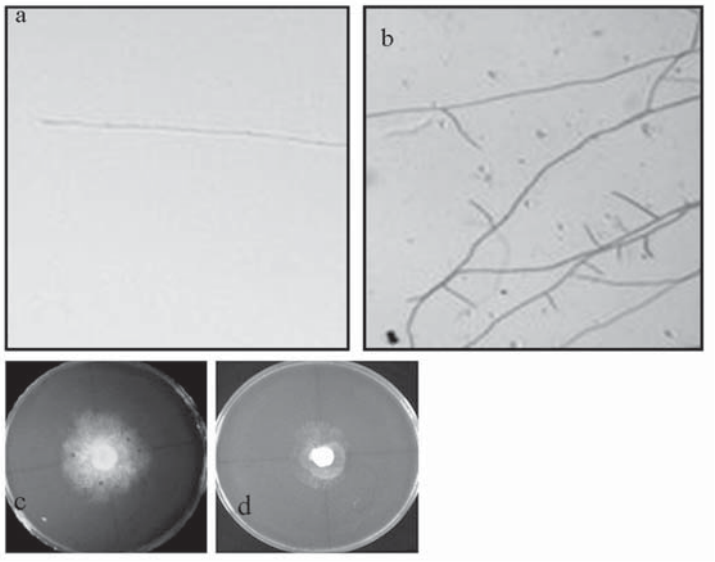

Figure 6 Poorly branched hyphae in sucrose (a); Highly branched in glucose (b); Dense mycelium in glucose (c), Rare in sucrose (d)

The molecular analyses were performed using the cDNA clones involved in hyphal growth, detoxification processes and general metabolism (shown in Table 1). The RNA isolated by mycelia grown in the different sugars, both in liquid and solid media, (in the presence of a nylon

membrane), was retro-transcribed using the SMART technique, radio-labelled and used as probes in experiment of Reverse Northern filters containing the described cDNAs.
The results obtained, as shown in Figure 7 highlighted that all the genes analyzed show a major expression in the presence of glucose.

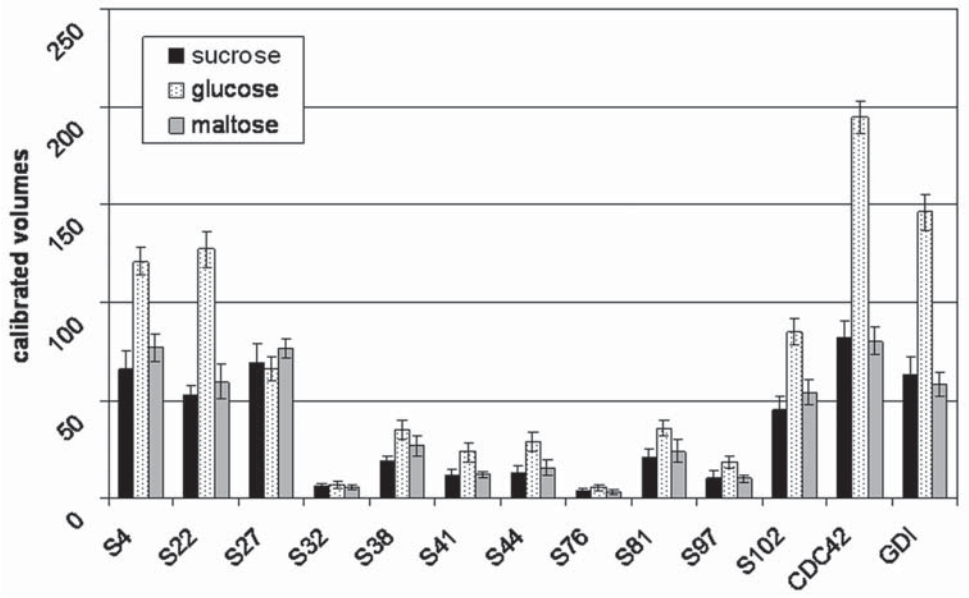

Figure 7 Expression levels calculated by reverse Northern experiments. The values are expressed in arbitrary units.

Conclusions

The results herein described, attempted to highlight the early signal exchanges that occur between the two symbionts prior to actual contact and allowed us to better understand the molecular mechanisms at work in the initial phases of symbiosis in *Tuber*. Several genes expressed in the presence of the plant and involved in various biochemical mechanisms, not yet cloned in the genus *Tuber*, were isolated. Although further studies are required, these results together with those reported in the literature, led us to assert that, in the presence of the host plant, the fungus switches on a series of mechanisms, to recognise and attack plant roots and defend the fungus from substances secreted by the plant. All of these mechanisms are accompanied by highly regulated metabolic modifications. Studies on the molecular mechanisms at work in the pre-infection phase in *T. borchii–T. americana* are an important part of research on symbiosis. In particular most of the present literature is mainly focused on endo-mycorrhizal fungi or ectomycorrhizal basidiomycete fungi, while the present study concerns with an ascomycetous fungus.
Moreover the data herein reported highlight that different nutritional substrates, in particular carbohydrates, can greatly effect the morphology and the whole life of fungal mycelia. The presence of glucose lead to an increased hyphal growth and branching, while sucrose almost inhibits the growth, due to the absence in *Tuber borchii* of the enzyme invertase, able to catabolise sucrose into glucose and fructose (Saltarelli *et al*., 1998).
Furthermore the knowledge of the molecular mechanisms at the bases of morphogenesis might provide new tools to develop new biotechnological strategies useful in ecology and forestry, in order to optimise *in vivo* mycorrhization processes with prized truffles, in particular with *Tuber magnatum*, the most precious among truffles, yet nobody has been able to cultivate.

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USE OF BETA-TUBULINE AS A DNA BARCODE TO IDENTIFY *TUBER* SP.

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Abstract

The DNA barcoding is a technique that uses short DNA sequences from a standardized region of the genome as a diagnostic "biomarker" for species identification. The Internal Transcribed Spacer of the rDNA (ITS) is the most exploited genomic region to design specific primers/ oligonucleotide barcodes when a single truffle species has to be detected. By contrast, primers which amplify exclusively all the *Tuber* species are not yet available. Since β-tubulin gene has been employed as an interspecific marker in different phylogenetic *Tuber* analyses, we wanted to check the efficiency of the β-tubulin gene for a quick and reliable identification of the *Tuber* genus.

To reach the aim, we designed two new primers, which amplified genomic DNA from 14 *Tuber* species and not that from fungi, which were phylogenetically related and not to *Tuber*. The primers were successfully used on soil DNA and mycorrhizas. As a second step we wanted to check the gene diagnostic power to amplify *Tuber magnatum* directly in the soil. A new forward primer was designed to amplify only DNA from *T. magnatum* and a nested protocol was then set up. Using serial dilution of a *T. magnatum* genomic DNA, we evaluated that 2-4 fg can be amplified with this approach. This result allowed us to follow the distribution of *T. magnatum* in the soil of a selected truffle-ground. Ten soil samples out of 13 gave a positive signal which sequence confirmed belonging to the expected fungus.

In conclusion, this is the first report of primers which are specific to *Tuber* genus. On the other hand, we set up a powerful protocol to detect 2-4 fg of *T. magnatum* DNA and we monitored its distribution in a truffle-ground. The β-tubulin gene is therefore a good candidate to be used as a DNA barcode for truffles.

Key words: *Tuber* genus, β-tubulin, specific primers, *T. magnatum*, nested PCR, soil, barcode, ecology and population biology.

THE ISOPRENOID BIOSYNTHESIS DURING *TUBER BORCHII* FRUIT BODY MATURATION

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Abstract

Truffles are hypogeous ectomycorrhizal fungi belonging to the *Ascomycetes*, within the species of the genera *Tuber* which have a great economical importance for the organoleptic properties of their fruit bodies. Some truffle species, including *Tuber borchii*, can be grown in pure cultures even if they grow slowly. Symbiotic relationships with specific host plants are required for the development of the truffle fruit bodies, which cannot be produced "in vitro". Furthermore, the formation of truffle fruit body depends on nutritional and environmental status**.** Some biochemical and molecular studies regarding *T. borchii* fruit body development and maturation suggest that the isoprenoid biosynthesis is regulated during fruit body morphogenesis.

Our studies, give the first insight into some aspect of *T. borchii* isoprenoid pathway, also known as mevalonic acids pathway (MVA). Three genes coding for the most important enzymes of the isoprenoid biosynthesis were characterised, and the activity of the major regulatory enzyme of the MVA was determined in particular during the ascomata maturation. Furthermore several isoprenoids were identified among the volatile organic compounds produced by ripening *T. borchii* ascomata.

Key words: Ectomycorrhizal fungi, *Tuber borchii*, fruit body maturation, isoprenoid pathway, 3-hydroxy-3-metilglutaryl CoA reductase, farnesyl-diphosphate synthase, squalene synthase.

Introduction

Isoprenoids, also known as terpenoids, are a large family of compounds involved in several physiological, metabolic and structural roles: hormones and intracellular messengers, photosynthetic pigments, electron carriers protein glycosylation and sterols. Other isoprenoids, classified as secondary metabolites (monoterpenes, diterpenes, sesquiterpenes, etc.) can be found in plants and fungi, and provide a wide range of commercially useful products, including solvents, flavouring and fragrances, adhesives, industrially useful polymers and a number of pharmaceuticals and agrochemicals. Because of the multiple roles played by isoprenoids, the level of their synthesis must be strictly controlled (Barkovich and Liao, 2001).

The isoprenoid biosynthesis pathway is also known as the mevalonic acid (MVA) pathway.

The 3-hydroxy-3-metilglutaryl CoA reductase (HMGR), the enzyme synthesising MVA, is the key regulatory enzyme of the MVA. HMGR is the most tightly regulated enzyme in nature. However, it is also accepted that mevalonic acid synthesis is not the only limiting step in isoprenoids biosynthesis, and that additional key enzymes are involved in the control of the flux through the pathway to maintain the appropriate cellular balance of isoprenoids under different physiological conditions (Cunillera *et al.,* 1996). In fact, there is increasing data showing that biosynthesis of dolichols, ubiquinones and isoprenilated proteins is regulated by enzymes distal to HMG-CoA reductase, in particular the pathway branch point enzyme farnesyl-diphosphate synthase (FPPS) and the first committed enzyme in the sterol biosynthesis, squalene synthase (SQS) (Szkopinska *et al*. 2000; Karst *et al*. 2004). The SQS catalyses the first reaction of the sterol biosynthesis, leading to the main end-product of the MVA pathway, cholesterol in mammals and ergosterol in fungi. These compounds are essential component of plasma membranes, affecting fluidity, permeability and the activity of membrane-bound enzymes.

Our studies, here summarised and discussed, represent the first step toward the understanding of some aspects of the isoprenoid pathway (MVA) in the ectomycorrhizal fungus *T. borchii.*

Characterisation of the *tbhmgr*, *tbfpps* and *tbsqs* genes encoding for the major regulatory enzymes of MVA pathway

The characterisation of three *T. borchii* genes, involved in an important metabolic pathway, allowed to widen the information regarding the genomics of this organism and as well as for the further advancement of *Tuber* functional genomics (Guidi *et al*. 2006).

The 3-hydroxy-3-metilglutaryl CoA reductase gene of *T. borchii* and its promoter region have been characterised using different molecular strategies. The identification of the *tbhmgr* promoter region will represent the basis for future analyses to identify the transcription regulatory elements which lead to the high expression of the gene in the mature ascomata. In addition, the knowledge of the complete sequences of *tbhmgr*, *tbfpps* and *tbsqs*, allowed us to analyse and compare their putative deduced amino acid sequences with other homologous proteins present in databases.

The *tbhmgr* deduced amino acid sequence, like all the known HMG-CoA reductase, from bacteria to eukaryotes, contain two domains: the NH2-terminal region encompassing the membrane-bound domain, whereas the COOH-terminal region contains the biologically active domain.

The N-terminal membrane anchor and the C-terminal catalytic region are separated by a non-homologous linker region. Whereas the catalytic domain is highly conserved, the hydrophobic one presents a variable number of transmembrane segments: in particular, *tbhgmr* deduced amino acid sequence presents 8 transmembrane helices, like other fungi, such as *Phycomyces blakesleeanus*, *Ustilago maydis*, *Schizosaccharomyces pombe*. It is known that HMGR is among the most tightly regulated enzymes in nature (Goldenstain and Brown 1990); also the *T. borchii* HMG-CoA reductase may be regulated at a post-translational level: it contains a Sterol Sensing Domain, which is conserved across phyla and confers sensitivity to regulation by sterols. The very high similarity of TBHMGR catalytic domain with HMG-CoA reductase from a wide range of organisms indicates the high sequence conservation of this enzyme during evolution, which may be associated with substrate and/or co-factor binding sites or catalytic activity.

The enzyme farnesyl-diphosphate synthase catalyses the sequential condensation of two molecules of isopentenyl diphosphate with both dimethylallil diphosphate and the resultant 10-carbon compound geranyl diphosphate to produce the 15-carbon compound farnesyl diphosphate. Because of the central branch point location of farnesyl diphosphate in the isoprenoid pathway, FPS is considered to play a key role in the regulation of the isoprenoid biosynthesis. In fact, FPP is the starting point of a variety of isoprenoid end-products (dolichols, ubiquinone, heme a, prenylated proteins).

Squalene synthase catalyses the first reaction of the sterol biosynthesis, in an unusual two-step reaction in which two molecules of FPP are condensed heat-to-head. Furthermore, the main end-product of the mevalonic acid pathway, cholesterol in mammals and ergosterol in fungi, is an essential component of plasma membranes, affecting fluidity, permeability and the activity of membrane-bound enzymes. The disruption of the yeast squalene synthase gene resulted in cuncurrently dimished activities of both FPP synthase and HMG-CoA reductase, indicating a regulatory role of this enzyme.

To isolate *T. borchii* farnesyl diphosphate synthase and squalene synthase genes, we designed two sets of degenerated primers, corresponding to the amino acid sequences of two highly conserved domains known for fungal FPPS (domains II and III) and SQS (domains IV and V). Following PCR reactions and cDNA library screenings we identified and cloned the cDNA sequences of the two genes (Guidi *et al*. 2006).

Also the *tbfpps* and *tbsqs* deduced amino acid sequences show the specific domains and structures of the known farnesyl-diphosphate synthase and squalene synthase respectively, confirming their highly conserved character already shown in other ascomycetes fungi (Homann *et al.* 1996). *Tbfpps* contains also the lysine 192, like the ascomycetes *Gibberella fujikuroi* and *Neurospora crassa*, which is thought to fix the pyrophosphate moiety of GPP.

These three genes are in single copy and are not clustered in *T. borchii* genome.

Gene expression analyses and TBHMGR enzymatic activity during *T. borchii* ascoma ripening

A quantitative real-time assays is set up to analyse *tbhmgr*, *tbfpps* and *tbsqs* genes expression during the complex process of *T. borchii* ascoma ripening.

The real-time PCR results show that all the three genes are over-expressed in mature fruit bodies, respect to the immature ones (Fig. 1).

In particular, *tbhmgr*, *tbfpps* and *tbsqs* gene expression was about four fold higher, ten fold higher and four fold higher respectively, in mature ascomata respect to the immature ones.

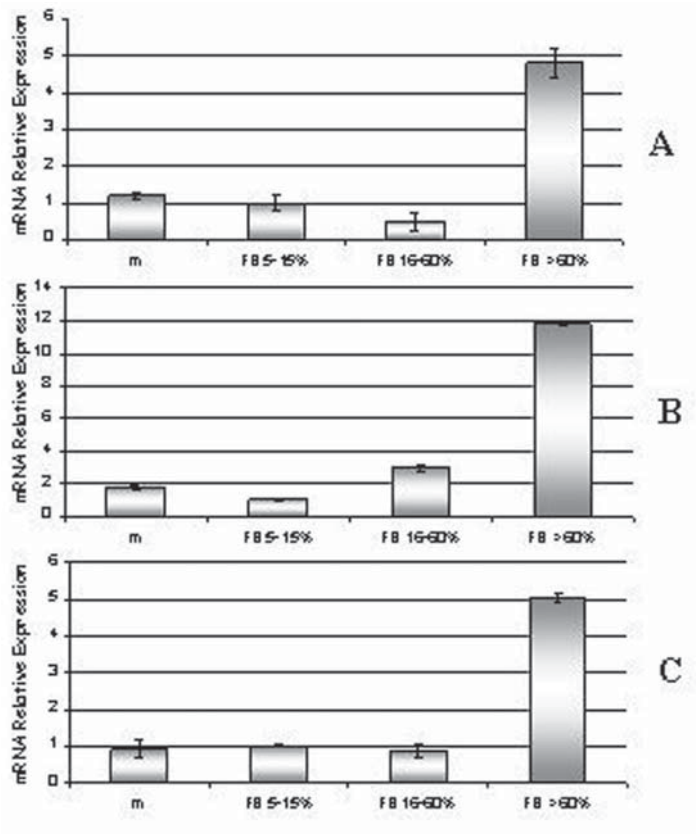

Fig. 1: Expression level of *tbhmgr* (A), *tbfpps* (B) and *tbsqs* (C) during ascoma maturation. The three different ascoma maturation stages are based on the asci containing mature spores (m: mycelia; FB 5-15%: immature ascoma; FB 16-60%: intermediate ascoma; FB >60%: mature ascoma).

By using the method of Buffalini *et al*., (2005) the enzymatic activity of TBHMG-CoA reductase was evaluated in the ascomata during their maturation process. The *T. borchii* HMG-CoA reductase enzyme activity shows the following specific values: 960 pmol mg^{-1} of protein min^{-1} (unripe ascomata); 400 pmol mg^{-1} of protein min^{-1} (intermediate ascomata); $3.3x10^3$ pmol mg^{-1} of protein min^{-1} (mature ascomata) (Guidi *et al*. 2006).

In particular, a lower value is found in the intermediate stage respect to the unripe ones, whereas a significant increase of the enzymatic activity occurred in the final phase of the maturation process. This trend may be explained as follow: the initial phases of fructification are strictly associated with high metabolic activity due to the fact that spores form and differentiate thus requiring the synthesis of cell walls, membranes and energy supply. Then, in the central phases of the maturation process, the HMG-CoA reductase enzyme activity markedly decreased as the major part of spores are already formed. Finally, in the last phase of fructification the enzyme activity reached a value 3-fold higher respect to that found in the initial phases of the process, confirming the importance of terpenic compounds, and thus of HMG-CoA reductase enzyme, in mature fruit bodies.

These findings imply that isoprenoids play a fundamental role in *Tuber* ascocarps, particularly

in the last phases of their maturation, when they could be involved in antifungal or/and antimicrobial processes and contribute to the famous flavour of the truffle ascomata.

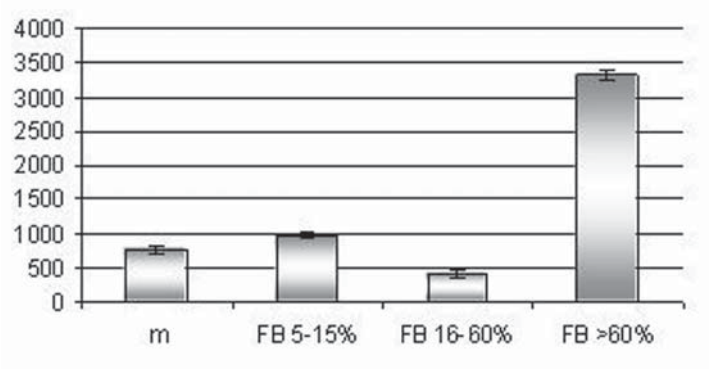

Fig. 2: TBHMG-CoA reductase activity during ascomata maturation. m: mycelia; FB 5-15%: immature ascoma; FB 16-60%: intermediate ascoma; FB >60%: mature ascoma.

Determination of isoprenoid compounds during *T. borchii* fruit body development

Tuber borchii fruit bodies of different maturation stage have been analysed by solid phase microextraction and gas chromatography/mass spectrometry (SPME/GC-MS) and comparing the spectra obtained, specific compounds for each stage have been detected (Zeppa *et al*. 2004). Several isoprenoid compounds were identified in our analyses (Table 1).

Table 1: Isoprenoid compounds identified in different stage of *T. borchii* fruit body maturation by SPME and GC/MS

***T. borchii* SAMPLES**	**ISOPRENOID COMPOUNDS**
Stage 0: 0-5% of mature spores	α-patchoulene cedrene aromadendrene valencene
Stage 1: 6-30% of mature spores	D-longifolene β-cedrene
Stage 2: 31-70% of mature spores	isopinocamphone 3-thujene
Stage 3: 71-90% of mature spores	D-limonene α-pinene α-farnesene trans-ocimene
pre-symbiotic stage (*T. borchii* mycelium grown in the presence of *T. americana* host plant)	aromadendrene bromocholestan3βol germacrene D longiciclene

Fungal isoprenoids comprise a structurally diverse group of primary and secondary metabolites, such as sterols, quinones and carotenoids which are essential for growth and development. Some of secondary metabolites produced by the isoprenoids biosynthetic pathway are fungal plant hormones (gibberellins, abscisic acid) and plant growth regulator (fusicoccin) (Homan *et al*. 1996).

The isoprenoids that we have identified were terpenic compounds (sesquiterpenes, monoterpenes,

trichothecenes) which belong to a large family of natural products that are best known as constituents of the essential oils and defensive oleoresins of aromatic plants (Table 1).
Aromadendrene has only been detected in the completely immature fruit body and it has been also identified in *T. borchii* mycelium grown in presence of the host plant, *Tilia platyphyllos*, but not in *T. borchii* free-living mycelium (Menotta *et al*. 2004). This evidence led us to hypothesise that this terpenoid is a signal molecule released during plant-fungus interaction. The presence of this compound also in completely unripe ascomata could indicate that the information exchange between the two partners exist also in the first step of fruit body formation. Other terpenic compounds, detected in stage 0, 1 and 2 fruit bodies, such as α-patchoulene, (M) cedrene, β-cedrene, D-longifolene and selinene, showed antimicrobial activity against bacteria, yeast and opportunistic fungi (Demetzos *et al*. 1997). The cedrene is also efficacy against termite tunnelling (Maistrello *et al.* 2001).
A number of investigators have reported a correlation between the induction of isoprenoid byosinthesis and the activity of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase.
This enzyme catalyses the reductive deacylation of HMG-CoA to mevalonate and provides an important control mechanism for the flow of metabolites into mevalonate, and especially, into steroid biosynthesis.
T. borchii HMG-CoA gene is highly expressed in ripe fruit body whereas it presents a lower expression in the 30-day-old mycelium and unripe fruit body (Zeppa *et al*. 2002).
A high expression of HMGR in the ripe fruit body could be explained by an increasing synthesis of the terpenic compounds that could contribute to the flavour of the truffle ascomata and have antimicrobial and antifungal activity.
In fact 1R-alpha-pinene and D-limonene, terpenic compounds identified in stage 3, are constituents of many aromatic plants. Furthermore, 1R-alpha-pinene shows antifungal and antibacterial activity (Cobos *et al.,* 2001; Alvarez-Castellanos *et al.,* 2001), while D-limonene shows an insecticidal activities (Chang *et al.* 2009).
α-farnesene, another terpenic compounds specific of mature fruit body, has been well studied in some plants but little is known about its biosynthesis and regulation in fungi. It has been studied in apples, because it accumulates in the skin of apple fruit after harvests and it is implicated as the causal agents of superficial scald (Rupasinghe *et al.* 2001). α-farnesene presence in *T. borchii* mature fruit body could be explained as an involvement of such terpenic compound in fruit body "breaking up".
Furthermore, α-farnesene is a component of sex pheromones (Lu *et al.* 2001) and an alarm pheromone of the termite (Sabotník *et al*. 2008). Signalling by means of pheromones plays an important role in mating in *Ascomycetes*, and probably also in post-fertilisation events (Coppin *et al.* 1997), which occur in mature fruit body. This could explain the presence of this compound in stage 3 fruit body.

Conclusion

Studies on truffle biology are difficult because, unlike other filamentous fungi such as *Pisolithus tinctorius*, their mycelia grow very slowly *in vitro* and it is difficult (or impossible for some species) to obtain mycorrhizae or fruit bodies under axenic and controlled conditions. Mycelial *T. borchii* strains, the species addressed in this study, can be propagated *in vitro* (Saltarelli *et al*. 1998); furthermore, *in vitro T. borchii* ectomycorrhizae (Sisti *et al*. 2003) and a productive truffle orchard where the ascomata can be collected (Zambonelli *et al.* 2000) are available. For these reasons, *T. borchii* life cycle have started to be analysed by molecular tools in the last years; nevertheless, the molecular mechanisms controlling its complex ontogenetic cycle remain still to elucidate.
The isoprenoid pathway is involved in the biosynthesis of many primary and secondary metabolites; the cloning of *tbhmgr*, *tbfpps* and *tbsqs*, coding for the three most important regulatory enzymes of isoprenoids biosynthesis, have allowed not only to analyse their genetic organisation and the characteristics of their deduced amino acid sequences, but also to obtain new information regarding this important metabolic pathway in *Tuber*, in particular during the

complex process of ascoma maturation. Previous study highlighted the significant presence of isoprenoids and the expression of some genes involved in their synthesis during *T. borchii* fruit body maturation (Zeppa *et al.* 2004; Gabella *et al.* 2005); nevertheless, no information were available regarding the genes coding for the three most important regulatory enzymes of the isoprenoid biosynthesis in *Tuber*.

A quantitative Real-time analysis during the ascoma maturation, evaluating not only unripe and ripe fruit body, but also the intermediate ones revealed a significative increase in the expression of the three genes during the process, providing a real molecular confirmation of the isoprenoids production in the *T. borchii* ascomata.

As reported for the *tbhmgr* gene expression, HMG-CoA reductase enzyme activity is found to be up-regulated in the ripe ascomata, respect to the intermediate and the unripe ones.

Some of secondary metabolites produced by the isoprenoids biosynthetic pathway have been identified by Ion trap GC-MS during ascomata maturation suggesting that a high expression of *tbhmgr*, *tbfpps* and *tbsqs* genes in the ripe fruit body could be explained by an increasing synthesis of the terpenic compounds that could contribute to the flavour of the truffle ascomata and have antimicrobial and antifungal activity.

In conclusion, the isolation of the *T. borchii* isoprenoid biosinthetic genes, their sequence and expression analysis represent important steps towards the understanding of the molecular mechanisms of the isoprenoid pathway in the ectomycorrhizal fungi of the *Tuber* genus.

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A FUNGAL GENE POTENTIALLY SPECIFIC TO MYCORRHIZA ESTABLISHMENT BETWEEN *TERFEZIA BOUDIERI* ISOLATES AND *CISTUS INCANUS* HAIRY ROOTS.

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Abstract

- Four fungus–hairy root clone combinations were cultivated under two sets of conditions, in which the root and the fungus were separated by a cellophane sheet or were allowed physical contact. One of the combinations produced endomycorrhizas, the other three solely ectomycorrhizas. Fragments isolated by cDNA–AFLP analysis from cellophane-separated cultures (pre-infection) were used to identify differentially expressed genes by reverse Northern analysis.
- Genes showing no homology to known sequences constituted the largest group under both growth conditions. Some fungal genes were expressed transiently, while others exhibited altered expression patterns as conditions changed from individually growing through the pre-infection stage to mycorrhizas.
- Genes expressed exclusively under combinations allowing either ectomycorrhiza or endomycorrhiza under a particular condition were detected. Four of these were further analyzed. The four showed, initially, no homology to known sequences. After extension to full length - GenBank matches were found for three.
- The fourth, expressed under pre-infection conditions, turned out to be a short peptide with no match in the databases. In the isolate capable of forming endomycorrhiza under our study conditions this peptide was two amino acids longer than in the isolate incapable of such associations. The significance of this finding is discussed.

Key words: Cell biology; Mycorrhiza specific genes; *Terfezia boudieri*; *Cistus incanus*.

Introduction

Terfezia boudieri Chatin is a desert edible truffle found in Southern Europe, North Africa and the Middle East (Trappe 1990, Lassoe & Hansen 2007).

This species like other *Terfezia* species (e.g. Fortas and Chevalier 1992; Gutierez *et al*. 2003) can establish either endo- or ectomycorrhiza, depending on growth conditions. Fortas and Chevalier (1992) studied *Helianthemum guttatum* mycorrhized by *Terfezia arenaria, T. claveryi* or *Tirmania pinoyi* and found a shift from ectomycorrhizas at high phosphate (P) concentration (about 1.1 mM) to endomycorrhizas at low concentration (about 0.11 mM).

Gea *et al*., (1994), studying a different system, reported the ability of a high auxin-producing mutant of *Hebeloma cylindrosporum* to penetrate the cell wall of *Pinus pinaster* and form a kind of ectendomycorrhiza.

In each of the truffle systems studied, pairing of different fungal species with the same plant species led to similar mycorrhizal types, suggesting that the genetic background plays no part.

We reported earlier, using *Cistus incanus* transformed root cultures (Wenkart *et al*. 2000) and various *Terfezia boudieri* isolates, that the genetic background of both plant (presumably due to differences in sensitivity to auxin) and fungus (presumably due to differences in auxin excretion) contribute to the determination of the mycorrhizal type formed under any given condition, and that the ratio of phosphate to auxin concentration is a major factor in directing the events leading to such determination (Zaretsky *et al.,* 2006a; Kagan-Zur *et al*., 2008).

As a consequence of that study we now had at hand a system with which we could predetermine at will the type of mycorrhiza to be formed, thus enabling the study of gene expression differences under each relevant combination and condition leading to ecto- or endo-mycorrhiza formation.

Two main general approaches for studying changes in gene expression patterns upon passage from free living to the mycorrhizal stage have been tried. Studies designed to target specific genes or gene products (e.g. Kim *et al*., 1999; Nehls *et al*., 1998, 1999; Wright *et al.,* 2000), necessitating prior knowledge concerning the gene product or biochemical pathways under investigation. Alternatively, overall approaches that require no previous knowledge or assumptions may be employed. The latter type of explorations have been undertaken at two expression levels, that of mRNA (in the form of cDNA) (e.g. Voiblet *et al*., 2001; Podila *et al*., 2002; Sundaram *et al*., 2004) and that of protein (e.g. Hilbert *et al.,* 1991; Burgess *et al*. 1995; Tarkka *et al*., 1998). Whatever the method of study, however, very little work has been done on gene expression changes during the establishement of mycorrhizas by truffles (Pierlioni *et al*., 2001; Polidori *et al*., 2002; Menotta *et al*., 2004; Miozzi *et al*., 2005).
We used the system described above to study overall gene expression at the cDNA level, looking to score genes involved in determining the type of mycorrhiza formed. Parts of the results were reported by Zaretzky *et al.,* (2006b), and Kagan-Zur *et al*., (2008).

Materials and Methods
Cultures: *Terfezia boudieri* isolates were routinely grown on Fontana medium (Bonfante and Fontana 1973), pH 6, solidified with 1.5% Difco Bacto Agar. *Cistus incanus incanus* transformed roots were routinely grown on N5 medium [MS medium (Murashige and Skoog 1962) with 20% of the amount of nitrates] solidified with 0.2% Phytagel (Sigma) with 500 mg/l ampicillin.
Growth conditions For this set of experiments, *Cistus* hairy roots and *T. boudieri* isolates were grown as described below on medium M (Bécard and Fortin, 1988) at pH 5.5, solidified with 0.2% Phytagel modified with respect to phosphorus concentration and containing 0.48 mg/l KH_2PO_4 (low P, medium M).
Two root clones, one more sensitive to external auxin (M2) than the other (W51) and two fungal isolates (42a, a low auxin producer, and 27, a high auxin producer) were employed in this study. Only the combination of M2 and 42a produced endomycorrhiza under the above conditions. All other three produced ectomycorrhiza (Zaretsky *et al*., 2006a). For separated cultivation of roots and *T. boudieri* on the same plates 3 transformed root clone tips were transferred to an M medium plate and covered with a piece of cellophane (King, China). A *T. boudieri* isolate was then placed on the cellophane. The plates were kept in the dark for 4 weeks (prior to use, the cellophane sheets were boiled for 10 min in H_2O containing 0.336 mg/l ethylene diamine tetra acetic acid (EDTA), washed in distilled water, and autoclaved). Isolates and roots were then collected separately, frozen in liquid N_2, and stored at -80°C until RNA extraction.
To obtain mycorrhized roots, transformed root clones were grown in tubes for 2.5 weeks before inoculation with a cube of Fontana (Bonfante and Fontana, 1973) agar medium containing the *T. boudieri* isolate. After inoculation, the tubes were maintained in a growth room at 25°C in the dark for an additional 4 weeks. The mycorrhized roots were then collected, frozen in liquid N_2 and stored at -80°C until RNA extraction.
A scheme of the procedure is presented in Fig. 1.

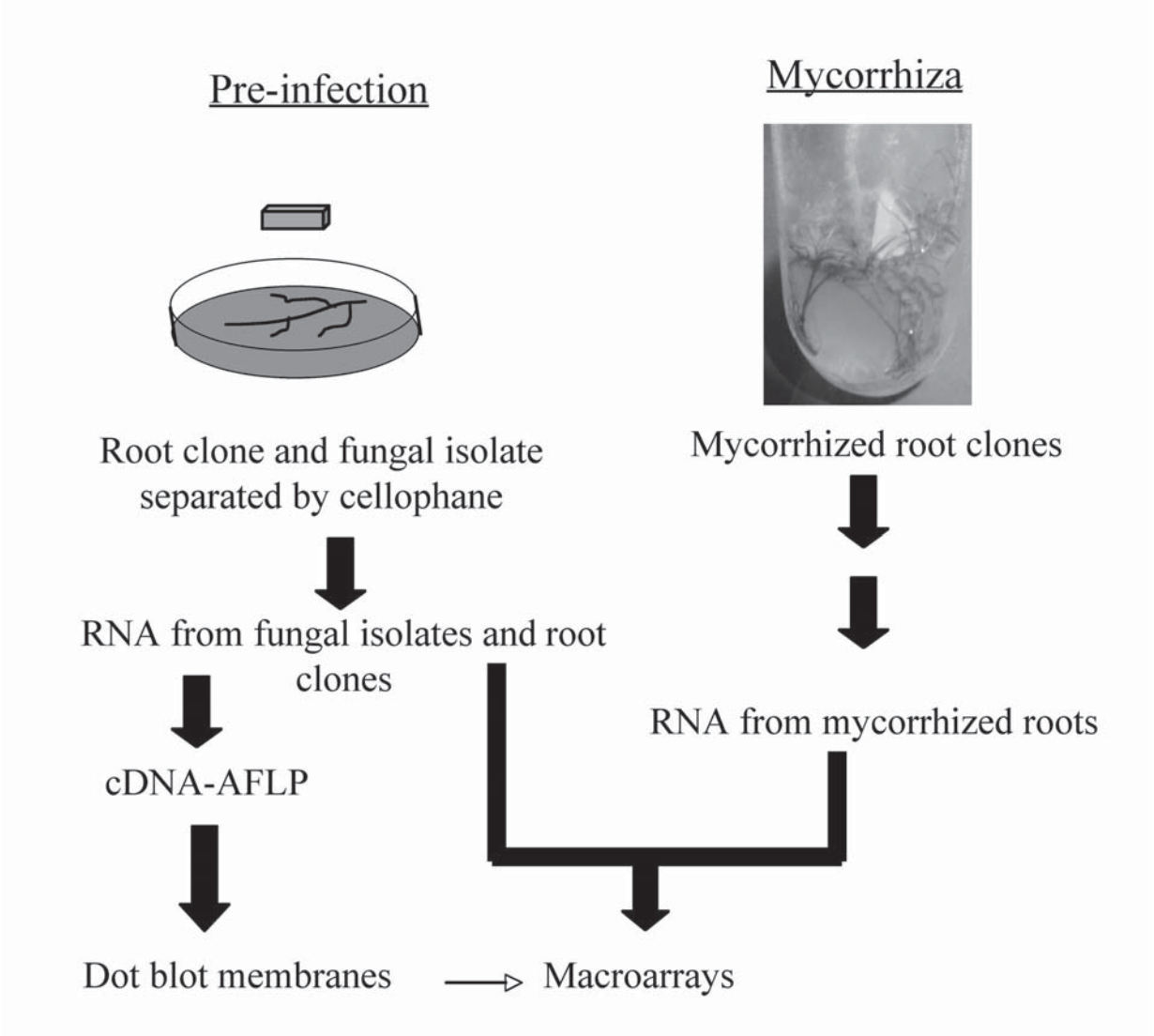

Fig. 1 A schematic representation of the experimental design

RNA extraction, cDNA-AFLP analysis and cloning of excised AFLP fragments were performed as described in detail by Zaretsky *et al.,* (2006b).
cDNA fragments that showed differential display in any of the comparisons were deposited in dbEST at the National Center for Biotechnology Information (NCBI accession numbers DT639222- DT639223, DV205746- DV205817).

Macroarray: Each cDNA fragment (100 ng) was denaturated and dot blotted onto Hybond-N^+ nylon membrane (Amersham Biosciences, London, UK) according to the protocol described by Ausubel *et al*. (2003) using Bio-Dot® microfiltration apparatus (Bio-Rad, CA, USA). Each fragment was spotted twice on the membrane, and three membrane duplicates were prepared.
The RNA for hybridization was extracted from each of the fungal isolates (42a and 27) grown under pre-infection conditions with plant clones (M2 and W51), and also from the mycorrhized roots (each plant clone with each fungal isolate, in all 8 probes). Total RNA (5 µg) was labeled with [□-^{32}P]dCTP (Amersham Biosciences, London, UK) during reverse transcription with the enzyme Superscript II (Invitrogen, Paisley, UK). Unincorporated nucleotides were removed using ProbeQuant™ G-50 micro columns (Amersham Biosciences, Piscataway, NJ). Hybridization procedure is detailed in Zaretsky *et al*., (2006b). An example of a differential expression is presented in Fig 2.

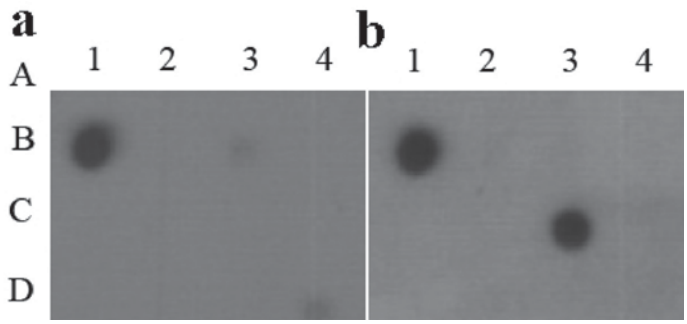

Fig. 2 Samples of a macro-array (partial membranes) stressing differences in hybridization patterns between treatments. **a**: cDNA synthesized from pre-infection stage fungal isolate 27 RNA (cellophane separated) from plant clone M2 and used as a probe for hybridization. **b**: cDNA synthesized from RNA of plant clone M2 mycorrhized by fungal isolate 27 was used as a probe for hybridization.

Gene extension: Four fungal genes showing mycorrhiza-associated expression in macroarrays and no match to the databases were chosen for further gene extension. For this purpose, first cDNA strand was synthesized from fungal isolates grown under preinfection conditions as described in Zaretsky *et al*. (2006b). All bands were extended using cDNA synthesized from isolate 27 grown in the presence of plant clone W51.

Stepwise extensions were performed. Two specific primers were synthesized outward from the known sequence in each direction leaving a short stretch of the known sequence with which to perform alignments between the known and the extended sequences. All primers for gene extension were synthesized by Integrated DNA Technologies (IDT) Inc., Iowa, USA. Two sequential PCR reactions were then performed with one of the external primers and a random primer (synthesized as a mixture of 15 bp long oligonucleotides) for each direction using annealing temperatures between 50°C and 53°C until some amplification was observed. The resulting band or bands were then reamplified using the same PCR conditions with a random primer and a further external specific primer. Control reactions were performed with each of the primers alone. Bands resulting from these amplifications were then ethanol precipitated, cloned and sequenced as described by Zaretsky *et al*. (2006b). The resulting extended sequence was aligned with the previously known sequence, checked for the presence of the overlap between the two, and then translated in all 6 existing open reading frames (ORFs) using the translating tool at ExPASy Proteomics Server (http://www.expasy.org/). Blast search was then performed.

The single fragment (# 229) lacking a GenBank match even after extention to full length was further studied: cDNA samples from all 4 fungus/root- clone combinations, as well as genomic DNA of both fungal isolates were amplified under the same PCR conditions using primers designed from the ends of the extended sequence. All amplification products were then sequenced and compared.

Results

All in all 63 independent fungal cDNA fractions were obtained. These could be divided into three major groups. Genes expressed under preinfection conditions (roots and fungus separated by a cellophane membrane), mycorrhiza enabling conditions, and genes expressed under both growth conditions (Table 1).

Tab. 1. Distribution of expressed genes between the growth conditions.

Growth conditions	No. of genes	Percent of total
Preinfection	37	59
Both stages	17	27
Mycorrhiza	9	14

For most fragments, no match to GenBank genes was found (45 cDNA fragments out of the 63). The expression mode of either preinfection or mycorrhiza-expressed genes could be divided into 6 groups, detailed in Table 2. Genes associated with fungal isolate 27, with root clone W51 and with ectomycorrhiza were encountered, but no genes specifically associated with isolate 42a, clone M2 or endomycorrhiza were detected under either condition.

Tab. 2. Differentially expressed genes under preinfection or mycorrhiza-enabling conditions.

Fragment expression associated with:	Expression mode				Number of clones	
	M2+ 42a	M2+ 27	W51+ 42a	W51+ 27	Preinfection	Mycorrhiza
Isolate 27		+		+	19	2
Clone W51			+	+	1	1
Ecto-mycorrhiza		**+**	**+**	**+**	**5**	**4**
Uniformly expressed	+	+	+	+	9	1
Single combination (a)			+		2	
Single combination (b)		+				1
Complex expression		+	+		1	-

None of the 17 clones expressed under both preinfection and mycorrhiza- enabling conditions, displayed identical patterns under both. Genes associated with Clone M2 (2 genes) or Isolate 42a (a single gene) were encountered only in this group, and only associated with mycorrhiza, not with preinfection (data not shown). Three genes were ectomycorrhiza associated, again only under mycorrhiza-enabling conditions, and two genes were ecto-mycorrhiza associated under preinfection conditions and endo-mycorrhiza associated under mycorrhiza enabling conditions (Table 3).

Tab. 3. Relevant genes expressed differentially under both preinfection and mycorrhiza enabling conditions.

Preinfection					Mycorrhiza				
M2+ 42a	M2+ 27	W51+ 42a	W51+ 27		M2+ 42a	M2+ 27	W51+ 42a	W51+ 27	
+	+	+	+			+	+	+	
	+		+			+	+	+	Ecto under
			+			+	+	+	mycorrhiza
	+	**+**	**+**		**+**				Ecto/Endo
	+	**+**	**+**		**+**				specific

For none of the ectomycorrhiza-specific genes was a matching sequence found in the GenBank. This could have several causes:

1. The sequences represent 5' or 3' untranslated regions (5' or 3' UTR).
2. The cloned fragments are too short to yield an acceptable *e* value.
3. These sequences represent unidentified fungal mycorrhiza-specific genes.

For eliminating the first two possibilities we needed to extend the existing fragments to obtain a full length of each gene. Extension of four of the mycorrhiza-specific genes was undertaken

(1 expressed solely under pre-infection, 1 expressed under both conditions - ecto - under pre-infection, endo - in mycorrhizas - and 2 expressed solely under the mycorrhiza enabling conditions). After extension, GenBank matches were found for three of the four (Table 4).

Tab. 4. cDNA-AFLP fragments selected for gene extension and their modes of expression.

Fragment no.	Mode of expression	Size **before** extension (bp*)	Size **after** extension (bp/**aa****)	Homology
25	**Ecto**mycorrhiza specific under mycorrhized conditions	175	740\ **132**	vps13 Vacuolar sorting-associated protein ($4e^{-22}$)
269	**Ecto**mycorrhiza specific under mycorrhized conditions	334	1990/ **375**	GCD1 Translation initiation factor ($2e^{-50}$)
246-1	**Endo**mycorrhiza specific under mycorrhized conditions, **ecto**mycorrhiza specific under preinfection conditions	81	1675/ **557**	ATPase: Chromosome segregation ($1e^{-12}$)
229	**Ecto**mycorrhiza specific under preinfection conditions	386	997/ **22 or 24**	No Homology

* (bp) base pair; ** (aa) amino acids.

Two of the originally cloned sequences proved to be part of the UTR, while the third lay within the coding sequence but had originally been too short to give an acceptable *e* value. The fourth, expressed under pre-infection conditions, was a short peptide with no match in the databases. In the single isolate found to be capable of forming endomycorrhizas under our study conditions (42a), this peptide was two amino acids longer than in the isolate incapable of such associations (27) (Table 4).

Discussion

The aim of this study was to compare fungal gene expression during preinfection and mycorrhizal growth conditions leading to ecto- or endomycorrhiza formation. We are aware that our differentially displayed gene-isolation system tends to under-represent genes expressed during actual mycorrhizal establishment and maintenance. It should be kept in mind that the excised cDNA–AFLP fragments were originally isolated as differentially expressed under preinfection growth, as compared with genes expressed under free-living conditions, and that although some turned out to be expressed exclusively under mycorrhizal conditions, we may have failed to collect some of the genes present in mycorrhizas. However, the results clearly indicate the transient expression of some genes (absent in free living cultures, present in the preinfection stages, and absent again in mycorrhizas), together with alterations in the expression patterns of others.

Also, at present we cannot discard the possibility that the differentially displayed fragments isolated in this study do not constitute 63 distinct genes. Some may represent different parts of the same gene. Gene extension of each cDNA fragment will have to be carried out to confirm or eliminate this option.

In addition, unconnected to the declared initial aim, our system, using two distinct *T. boudieri* isolates grown in the presence of two distinct *C. incanus* hairy root clones, allowed us to

observe and conclude that the same *Cistus* plant root clone elicits different responses from different *Terfezia* isolates, and likewise that the same fungal isolate responds differently to different plant root clones, without any apparent correlation to the type of mycorrhiza formed.
Fourty five cDNA fragments out of the 63 independent clones showed no homology to any sequence deposited in the GenBank database. There are three possible explanations for the absence of matching.
1. The sequences represent 5' or 3' untranslated regions (5' or 3' UTR).
2. The cloned fragments are too short to yield an acceptable *e* value.
(Again, gene extension of each cDNA fragment will have to be carried out to confirm or eliminate these options).
3. Podila *et al*. (2002) suggested that expression of ectomycorrhizal, match-lacking genes, may be unique to the particular ectomycorrhizal fungus studied, or that they may represent very rare transcripts that have not been identified and/or characterized previously. We suggest that such sequences could represent unidentified fungal mycorrhiza-specific genes. A more detailed study encompassing all of these genes is needed in order to clarify this question.
Extension of four of the mycorrhiza-specific genes was undertaken (1 expressed solely under pre-infection, 1 expressed under both conditions – ecto- under pre-infection, endo- in mycorrhizas – and 2 expressed solely under the mycorrhiza enabling conditions). After extension, GenBank matches were found for three of the four. Two of the originally cloned sequences proved to be part of the UTR, while the third lay within the coding sequence but had originally been too short to give an acceptable *e* value. The fourth, expressed under pre-infection conditions, was a short peptide with no match in the databases. This peptide was two amino acids longer in the isolate exhibiting low auxin synthesis capability, and enabling endomycorrhiza formation with the auxin sensitive root clone, under low phosphate concentration. It is becoming increasingly evident that peptides play an important role in many biological processes (e.g. Negishi *et al*. 1995; Mygind *et al*. 2005; Raaijmakers *et al*. 2006; Selth *et al*. 2006; Sabbatini, 2009). The *Laccaria bicolor* genome-sequencing project provided evidence of small peptides (SSP's) expressed at mycorrhiza forming stages (Martin *et al.* 2008). The SSP's definite role and importance, however, is, at this point, unclear, as is the role and importance of our small peptide. At present we cannot link the peptide to the effects of P and auxin levels on mycorrhizal type determination. However, it may be a part of a signal transduction pathway not yet elucidated.

In conclusion: Several types of fungal genes are expressed in mycorrhizas: Genes specific to a fungal isolate, irrespective of the root clone symbiont; genes expressed in response to one root clone, but not to the other; genes expressed under one of the growth conditions only, either under preinfection (transiently) or mycorrhiza; and genes showing different patterns of expression under different growth conditions. The latter may be involved in choosing the specific cellular pathways that lead to new growth stages. Our results single out, for the first time, some of the genes that may be involved in determining the type of association to be formed: ecto- or endomycorrhizal.

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CHARACTERIZATION OF A *TUBER MAGNATUM* TRUFFLE-GROUND: FROM SOIL MICRORGANISM PROFILES TO *TUBER* DETECTION

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A truffle-ground is a special environment where plants and microbes form a rhizosphere niche within which truffles develop. During a long-term investigation of a natural *T. magnatum* truffle-ground in Piedmont, fruiting bodies were sampled from 1997 to 2002 and genotyped (Mello *et al*., 2005), as well as mycorrhizal tips (Murat *et al.,* 2005). From this survey and historical records, productive and non-productive areas were identified; however, the ecology of *T. magnatum* is still elusive. The molecular mechanisms involved in the truffle body formation are largely unknown, as well as the environmental factors, which may affect such event. For example, we wondered why the mycelium does not lead to the development of a truffle body in some areas, which might be potentially productive due to the presence of well-adapted host plants. In order to reply to some of these questions marks, we developed multiple approaches, among them the identification of soil microorganisms associated to the fruiting body formation, the development of tools to reveal soil mycelial networks and the detection of *Tuber* biodiversity.
It has been hypothesized that soil-borne communities may have an impact on truffle production. In order to address this issue, we investigated bacterial and fungal communities resident in productive *versus* adjacent non-productive areas in the previously investigated truffle-ground by using PCR-DGGE (denaturant gradient gel electrophoresis). Bacterial 16S rRNA gene fragments were amplified with the primers 968F-GC and 1378R (Heuer et al. 1997, 1999), while fungal 18S rRNA genes were amplified using the primers FF390 and FR1-GC (Vainio *et al*. 2000). The bacterial profiles, produced in DGGE, resulted to be generally highly similar across all samples within the grounds, but a *Moraxella osloensis* population appeared to be consistently associated with productive sites.
The relation among this bacterium genus and productive truffle sites is in line with previous results. In 1995, Citterio *et al*., isolated bacteria belonging to *Moraxella*, from *T. magnatum*, *T. maculatum* and *T. borchii* ascomata. On the basis of these findings, *M. osloensis* seems to be a promising marker of a potential truffle presence in a productive soil.
Fungal communities showed no obvious patterns in relation to productivity, although *Mortierella* and *Fusarium oxysporum* appeared to be more abundant in the productive area. In order to obtain a glimpse of the fungal diversity inside the truffle-ground, 21 bands, from the most dominant fungal populations, were excised and sequenced. In only six cases the recovered sequences show a similarity with known taxa (species and genera) - *Fusarium oxysporum* Schltdl., *Clavulina cristata* (Holmsk.) J. Schröt*., Metarhizium anisopliae* (Metschn.) Sorokin*, Cortinarius, Psilocybe, Mortierella* -. Basidiomycota are the most represented phylum; Agaricales with 9 sequences and 3 families (Tricholomataceae, Strophariaceae and Cortinariaceae) were the most represented order.
These results open the question whether microbe-mediated mechanisms may inhibit/facilitate truffle fruiting-body production or, *viceversa*, truffle fruiting-bodies have an impact on the microbes living in the rhizosphere. We are still far away from answering these questions.
The second question was focused on truffle distribution; we wondered whether *T. magnatum* mycelium mirrored the fruiting body presence and whether other truffle species were present in the *T. magnatum* truffle-ground. In order to answer these questions, we designed primers in the ß-tubulin gene*,* testing its potentiality as a genomic region to build *Tuber* species barcodes. DNA Barcoding is, in fact, a technique that uses a short gene sequence from a standardized

region of the genome as a diagnostic “biomarker” for a rapid and accurate species identification (http://www.barcoding.si.edu/).
Tracking the dynamics of a given ectomycorrhizal fungus is considered a difficult task since fruiting bodies do not reflect the distribution of ground networks (Dahlberg, 2001). The ß-tubulin gene has been largely used in *Tuber* phylogenies (Wang *et al.,* 2006; Jeandroz *et al*., 2008) and is considered an excellent candidate to design species-specific primers (McCartney *et al*., 2003). A nested protocol was set up to amplify soil DNAs sampled in January and May 2005 in the same truffle-ground of the previously described studies. Thanks to its sensitivity, the protocol detected *T. magnatum* propagules in productive and non-productive periods and areas, clearly showing that truffle mycelium extends well far from the fruiting-body collection sites.
The design of *Tuber* genus- specific primers allowed us to detect a high *Tuber* diversity in the natural truffle-ground. In fact, at least 5 *Tuber* species were present, belonging to *aestivum, rufum, puberulum* group, according to clades proposed by Jeandroz *et al*. (2008).
In conclusion ß-tubulin gene was found to be a good region to design species- and *Tuber* genus-specific primers. These primers will be useful to understand truffle ecology and biodiversity.

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DIVERSITY OF THE GENUS *TUBER* FROM THE WEST BALKAN AREAS USING MOLECULAR CHARACTERISATION

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Abstract
Compared to well characterized European areas and despite the suitable climate and vegetation conditions of the region, the area of SE Europe, particular the Balkan area, has only recently been more extensively explored for the presence and diversity of hypogeous fungi. Several surveys have been performed in last decade generating substantial collection of hypogeous fungi, particular from the genus *Tuber*, in official herbaria. The latter material, mainly identified by regional specialists, was subjected to molecular diversity assessment and identification. We attempted to characterize selected collections at the molecular level (complete nrDNA ITS and partial tub2 gene for beta-tubulin) and to explore them for a potential new molecular variants as expected due to known high endemic characteristic and diverse ecosystems composition of the Balkan region.
Molecular approach supported morphological identification results and confirmed the presence of all commercially important species including *Tuber magnatum* Pico, *Tuber melanosporum* Vittad., and *Tuber aestivum* Vittad.. We have also observed high diversity (polymorphism) within few other morphological species (*Tuber rufum* Pico*, Tuber brumale* Vittad.), which partially confirmed up to date scientific literature. The presentation aims to point out selected results, new for science and present the high biodiversity aspect of hypogeous fungi in the Balkan region.
Key words: truffles, molecular diversity, molecular identification, Balkan area.

Introduction

The area of SE Europe, particular the Balkan area has not been thoroughly explored for truffle diversity yet. In past several publications or lists of species, showing the presence and diversity of truffles were published mainly focusing on the morphological species delimitation (see Marjanovič *et al*. 2009 for details). The distribution and diversity of truffles may depend on a number of factors including the long-term distribution and migration of host trees, dispersion of spores, climatic conditions, geographic barriers (Jeandroz *et al.* 2008) which can influence diversity also at the molecular level thus making the species diversity of truffles for some species rather problematic. We aimed to apply different regions in the nrDNA for assessment of the molecular diversity of truffles and for conformation of morphological identifications based on the comparison of obtained sequences with data from GenBank for available ascocarps collected from the area of West Balkan.

Methodology

Truffle ascocarps were obtained from official herbaria or hunted with trained dogs at ecologically different sites, and excavated from the soil with much care not to disturb the site. Collections were determined based on morphological characteristics of ascocarps according to Montecchi

and Sarasini (2000), Riousset *et al*. (2001), and Ceruti *et al*. (2003). Ascocarps are kept at public or private herbaria collections: Herbarium at the Real Jardin Botanico – MA-Fungi, Herbarium at the Institute for Multidisciplinary Research – "FHS", Institute for Systematics of Higher Fungi - "AP", and Mycotheca and Herbarium at the Slovenian Forestry Institute - "GIS". DNA was extracted from fully developed identified ascocarps by removing about 50ng of the glebe. Any damaged, rotten or infected areas including any soil particles were avoided to minimise interference in the subsequent amplification. Beta-tubulin gene (tub2) including introns and exons was amplified with primer pairs published in Russo *et al*. (1992), Thon and Royse (1999) constructed for *Basidiomycetes* (B36F, B43F, B41R, B12R) or for *Ascomycetes* (T10, T222; Schroeder *et al*. 2002). Previously published ITS sequences were selected from Marjanović *et al*. (2009), Grebenc *et al*. (in press) and GenBank. PCRs, purification and sequencing were performed as described in Grebenc *et al*. (2009). All sequences were aligned using stand-alone freeware version of MAFFT program (http://align.bmr.kyushu-u.ac.jp/mafft/software/) with L-INS-i aligning strategy for comparison of sequences containing sequences flanking around one alignable domain for beta tubulin and E-INS-I strategy for sequences where unalignable residues are left unaligned at the pairwise alignment stage as applied for ITS region (Katoh *et al.* 2005). PHYML v2.4.5 was applied to produce ML tree with TN93 nucleotides substitution model. Phylogenetic trees were annotated in MEGA 4.

Results

The diversity of the genus Tuber from the West Balkan area based on the nrDNA ITS region

Using the fungal material for analysis of the ITS region in nrDNA compared to selected sequences available in GenBank we managed to support all morphological identifications of truffles collected in west Balkan areas. *Tuber borchii, T. oligospermum, T. maculatum, T. foetidum, T fulgens, T. excavatum, T. macrosporum* and *T. rufum* were confirmed, so as all commercially important species including *Tuber magnatum* Pico, *Tuber melanosporum* Vittad., and *Tuber aestivum* Vittad.. Intraspecific diversity at the ITS level was observed for *T. oligospermum, T. brumale* and *Tuber rufum*, while the resolution of the phylogenetic tree indicates a complex of species in *T. fulgens* and *T. excavatum* group (Figure 1).

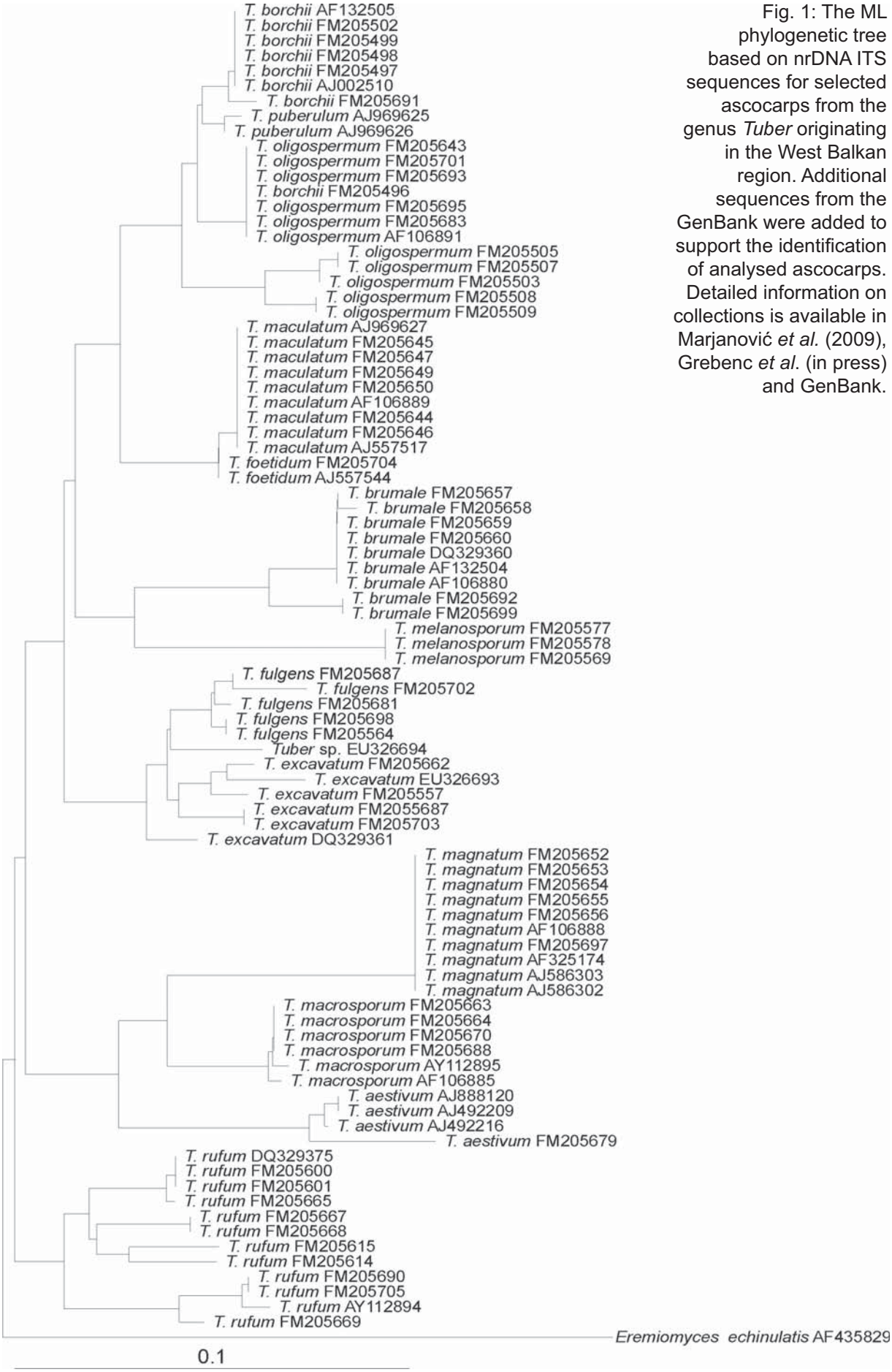

Fig. 1: The ML phylogenetic tree based on nrDNA ITS sequences for selected ascocarps from the genus *Tuber* originating in the West Balkan region. Additional sequences from the GenBank were added to support the identification of analysed ascocarps. Detailed information on collections is available in Marjanović *et al.* (2009), Grebenc *et al*. (in press) and GenBank.

Amplification of the partial beta-tubulin sequence and their diversity

The preliminary amplification of the beta-tubulin region for selected ascocarps (Table 1) with primer combinations T10 & T222, B36F & B41R, B36F & B12R and B43F & B41R yield only moderate success.

Tab. 1 – Characteristics of selected ascocarps.

Species	**GenBank accession number**	**Location**	**Herbarium voucher**
Tuber maculatum	FN555425	Molinicos, Spain	MA-Fungi 46891
Tuber malençonii	FN555426	Molinicos, Spain	MA-Fungi 46892
Tuber mesentericum	FN555427	Molinicos, Spain	MA-Fungi 46894
Tuber oligospermum	FN555428	Boadilla del Monte, Spain	MA-Fungi 39553
Tuber uncinatum	FN555429	Es Barranc, Soller, Spain	MA-Fungi 24605

Specific fragment of expected length were amplified only with primer pair B43F&B41R, while other primer pair combinations yield no or unspecific (multiple) amplifications (data not shown). Obtained sequences of the B43F&B41R primer pair amplified partial beta-tubulin gene (tub2) represented partial sequence of intron 7 & 9, and complete sequence of intron 8 and exons 7&8 (Thon and Royse, 1999). Sequenced region showed separated among *T. maculatum, T. malençonii, T. mesentericum, T. oligospermum* and *T. aestivum/uncinatum* (Figure 2). The comparison to public available sequences was limited to *T. borchii* and *T. magnatum* only, due to low coverage and few available sequences of the particular part of beta-tubulin gene. The variability in beta-tubulin gene was lower then the nrDNA ITS spacers and mainly limited to introns (data not shown).

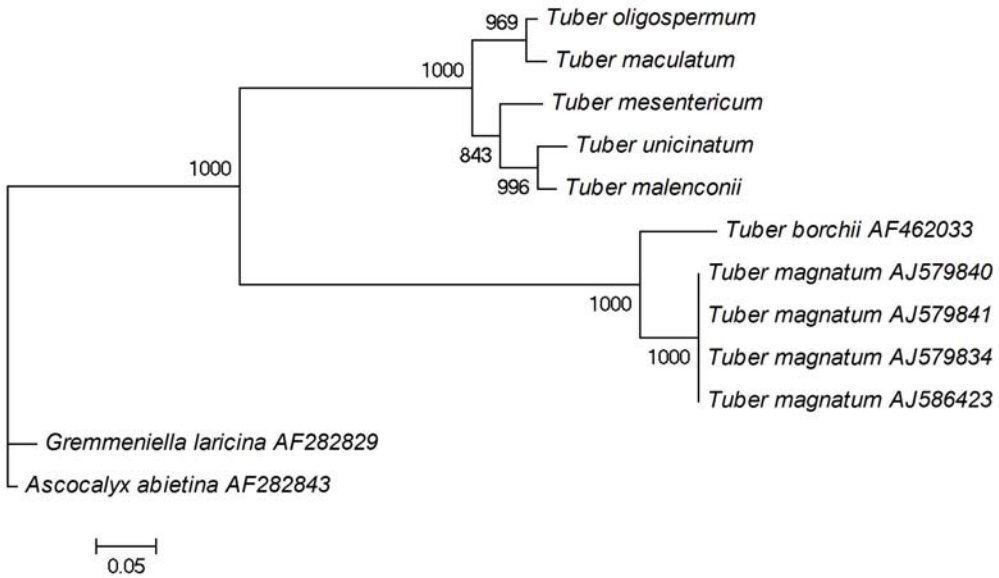

Fig. 2: The ML phylogenetic tree based on partial beta-tubulin gene (partial intron 7 & 9, and complete of intron 8 and exons 7&8 sequence) for selected ascocarps.

Discussion

The western Balkan area has only recently attracted interest of scientific community for more detailed investigation of occurrence and diversity of hypogeous fungi, truffles (the genus *Tuber*). In past, only scattered records existed for Serbia (Lindtner 1935), Croatia (Frančiškovič 1950) and

Slovenia (Scopoli 1772), while since early 90ies of the past century more systematic approaches were published for the area (Milenković *et al.,* 1992; Milenkovic & Marjanovic 2000; Piltaver and Ratoša, 2006; Grebenc *et al.,* 2009; Marjanović *et al.,* 2009). Currently an amount of collections is available in public and private herbaria covering substantial parts of the studied area.
The material obtained from past studies supported with new records was subjected to molecular analyses of ß-tubulin and nrDNA ITS region. Primers applied or amplification of ß-tubulin were either constructed for Ascomycetes – Ophiostomatales (Schroeder *et al*., 2002) or for Basidiomycetes (Thon and Royse, 1999), thus the applicability of primers for the genus *Tuber* was expected and confirmed to be low. The phylogenetic tree based on beta-tubulin sequences supported close relationship among species belonging to the "*T. aestivum* morphology group" (e.g. *T. aestivum/uncinatum*, *T. malençonii* and *T. mesentericum*) and the "*Tuber magnatum* morphology group" Riousset *et al*., (2001).
The phylogenetic distribution of applied *Tuber* sequences based on the nrDNA ITS region indicates similar position of terminal clades (species) as in previously published analyses (Jeandroz *et al*. 2008; Marjanović *et al*., 2009). The intraspecific diversity within *Tuber rufum* has been studies previously (Iotti *et al*., 2007; Wang *et al.,* 2007) while the observed diversity within *T. oligospermum, T. brumale* from the West Balkan area needs more detailed analysis.
The comparison of both approaches confirmed expected high molecular diversity and high biodiversity of the genus *Tuber* including the presence of all commercially important species in the region. We also confirmed that phylogenetic analysis of both sequenced regions (e.g. nrDNA ITS and partial beta-tubulin) generate congruent results and comparable distribution of at interspecific level in the genus *Tuber* as previously mentioned in Wang *et al*., (2006).

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IDENTIFICACIÓN MOLECULAR DE TRUFAS

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Abstract: Molecular identification of truffles

The organoleptic properties of some truffle species have notable importance in gastronomy and therefore significant economic value. Two of these species are *Tuber melanosporum* Vittad., and *T. aestivum* Vittad. the black truffle and the summer truffle, respectively. These hypogeus fungus were first collected in their natural forest habitat, but now are cultivated in high extension fields due to increasing market demand. The species identification for mycorrhization procedure, evaluation of propagation in seedlings, and monitoring of plantations is of critical importance for cultivation, avoiding undesired species as *T. brumale* Vittad. and *T. indicum* Cooke & Massee and assuring that the desired species is present at different stages of plant growth. Morphological characters do not allow in all cases the correct identification of truffles, especially at ectomycorrhizal stages. Therefore, this study has developed an inexpensive and simple DNA-based technique for the characterization of four *Tuber* species. Ribosomal DNA region (ITS) have been analysed using conserved and general primers (ITSI-ITS4), only positive PCR ITS products were digested with a combination of restriction enzymes. The result is distinctive band patterns allowing the identification of each species. This technique can be used for typing fruit bodies avoiding possible frauds in the case of morphological similar glebas as *T. melanosporum* and *T. indicum*, and for typing ectomycorrhizae to detect the presence of the desired species in nurseries and in few months in truffle orchards, rather than wait 4-6 years until the formation of the fruit body. This is an initial step for the characterization of truffles, following steps include a restriction map of the most important species of the genus *Tuber*.

Key words: ITS, Restriction enzymes, *Tuber aestivum*, *T. brumale*, *T. indicum*, *T. melanosporum*, Ectomycorrhizal identification.

Introducción

La identificación morfológica de ciertas especies del género *Tuber* P. Micheli ex F.H. Wigg. es una tarea difícil en multitud de ocasiones. Tanto macroscópica (carpóforos), como microscópicamente (micorrizas y esporas), algunas de estas especies son muy similares entre sí, lo cual se ve acentuado por el estado de maduración y por otro tipo de condiciones edafoclimáticas que alteran dichas estructuras e impiden su clasificación. En un gran número de situaciones se hace necesario llevar a cabo estas identificaciones: control del inóculo a emplear en la elaboración de planta micorrizada, evaluación de la misma, control de micorrización de plantaciones y, en caso de llevarse a cabo, del inóculo esporal que se aplique a los árboles.

En el presente estudio se ha trabajado con carpóforos y micorrizas de *Tuber melanosporum* Vittad., *T. aestivum* Vittad., *T. brumale* Vittad. y *T. indicum* Cooke & Massee. Las dos primeras especies son sobradamente conocidas por sus excelentes propiedades organolépticas, las cuales hacen aumentar día a día su demanda en los mercados europeos y cada vez más en los del resto del mundo, demanda que no puede ser satisfecha por la producción silvestre, motivo principal por el que se llevan a cabo plantaciones de árboles micorrizados por las mismas (Palazón, 2001). Las otras dos especies no están tan bien consideradas, aunque ambas son comercializadas. La presencia de micorrizas de *T. brumale* o *T. indicum* en las plantaciones,

ya se deba a errores en la micorrización o a contaminaciones en las propias parcelas, puede cambiar totalmente la vocación productora de esos árboles (Sáez & De Miguel, 2008).
Como bien es sabido los carpóforos de estos cuatro hongos poseen un peridio de color negro y la gleba va pasando de blanca (*T*. *aestivum*), a gris (*T*. *brumale*) y totalmente negra (*T*. *melanosporum* y *T*. *indicum*) (Montecchi & Sarasini, 2000). Las dos últimas pueden confundirse fácilmente y, si sus carpóforos son inmaduros, pueden parecerse a los de *T*. *brumale* (Fig. 1). Por supuesto, la observación de las esporas, *T*. *aestivum* las tiene alveoladas y las otras tres especies aculeadas (Montecchi & Sarasini, 2000), es determinante e imprescindible en su identificación, pero si no se realiza o si el carpóforo aún no las ha formado las posibilidades de error aumentan.

Fig. 1. Carpóforos de *Tuber melanosporum* (1º y 3º) y *T*. *brumale* (2º), el primero podría confundirse con un carpóforo inmaduro de *T*. *brumale* (Palazón *et al*. 2008).

En cuanto a las micorrizas, *T*. *aestivum* vuelve a ser la que mejor se diferencia del resto, principalmente por su manto con células angulares (Tipo L) y sus cistidios indivisos y largos (Agerer & Rambold, 2008). Las otras tres especies poseen el manto con células redondeadas (Tipo M), *T*. *brumale* posee cistidios cortos, indivisos y rígidos, los de *T*. *melanosporum* y *T*. *indicum* son ramificados en ángulo recto y también rígidos (Agerer & Rambold, 2008), éstos dos últimos, presentan una gran similitud morfológica que complica la identificación correcta de cada especie. A veces las micorrizas carecen de cistidios, por lo que se complicaría la identificación de las últimas tres especies.
Las técnicas de biología molecular pueden ser la clave para evitar todo este tipo de situaciones en las que el investigador, truficultor, certificador o viverista, tiene dudas sobre la identidad del material que está manejando. En la última década el empleo de marcadores moleculares en la determinación inter e intraespecifica ha tomado una gran importancia y relevancia. Las técnicas basadas en el ADN han permitido evaluar y caracterizar la diversidad y estructura genética que en cierta medida se mantenía indeterminada *in situ* (Pérez-Collazos & Catalán, 2006, 2007), ha sido posible comprobar hipótesis de extinción, migración, y de biogeografía (Kebede *et al*. 2007; Pérez-Collazos *et al*. 2009), así como desarrollar metodologías útiles para la caracterización de variedades y especies de uso comercial (Sharief *et al*. 2005). El genero *Tuber* y sus especies han sido objeto de numerosas investigaciones, la finalidad de dichos estudios era optimizar técnicas de extracción de ADN a nivel de ectomicorriza (Di Battista *et al*. 1999), se han utilizado técnicas moleculares como los RAPD AFLP, microsatélites y la secuenciación de regiones ITS en especies como *T. aestivum*, *T. mesentericum* para estudiar la estructura genética de las poblaciones (Wedén *et al*. 2004; Sica *et al*. 2007; Riccioni *et al*. 2008), se han desarrollado técnicas para detectar la presencia de *T. melanosporum* en el suelo (Suz *et al*. 2006) e incluso se han llevado a cabo estudios biogeográficos en el genero *Tuber* (Jeandroz *et al*. 2008). En cuanto al desarrollo de técnicas para la identificación interespecifica en *Tuber*, varias metodologías y técnicas han sido propuestas en diferentes especies. La técnica isoenzimática permitió la diferenciación de 6 especies del genero (Urbanelli *et al*. 1998). El empleo de cebadores específicos ha sido

evaluado en diferentes especies (Paolocci *et al*. 1997; Bertini *et al*. 1999; Sejalon-Delmas *et al*. 2000; Mabru *et al*. 2001; Suz *et al*. 2006) y algunos estudios han empleado digestiones con enzimas de restricción (RFLP) obteniendo patrones característicos de cada especie (Paolocci *et al*. 1997; Mabru *et al*. 2004), sin embargo, hasta el momento ningún estudio había caracterizado las especies mas importantes y problemáticas en la truficultura española; *T. indicum*, *T. aestivum*, *T. brumale* y *T. melanosporum*.

El objetivo de este estudio es desarrollar un método eficiente, repetible y económico que permita determinar la presencia de una de las cuatro especies estudiadas a nivel micorrícico y a nivel de fructificación, permitiendo así una detección precoz de las micorrizas en viveros y en parcelas jóvenes, y evitando posibles fraudes o equivocaciones en glebas de especies similares morfológicamente.

En la búsqueda de metodologías para la caracterización de diferentes especies de trufa, varios investigadores han desarrollado y han propuesto el empleo de cebadores específicos que solo se van a anclar a regiones de ADN especificas de cada especie (Paolocci *et al*. 1997; Bertini *et al*. 1999; Sejalon-Delmas *et al*. 2000; Suz *et al*. 2006). No obstante, nuestra experiencia nos ha demostrado que la amplificación positiva con cebadores específicos determina la presencia de la especie de interés, pero la amplificación negativa puede ser el resultado de una concentración insuficiente de ADN extraída de la ectomicorriza, o el resultado de una inhibición de la PCR como consecuencia de la presencia de compuestos secundarios de la gleba en la muestra (falso negativo en ambos casos), o debido a la ausencia de la especie de interés (negativo verdadero). Por otro lado, el uso de cebadores específicos no nos permite determinar el número de especies que se encuentran en la muestra de estudio. Por consiguiente y teniendo en cuenta lo mencionado anteriormente, decidimos emplear cebadores conservados capaces de amplificar todas las especies de *Tuber* estudiadas, para lo cual se seleccionó el estudio de la región ribosomal (ITS1-ITS4), una región conservada en un gran numero de especies vegetales y de hongos, seguida de una región intermedia variable e informativa (Torrecilla & Catalán, 2002). De esta manera se tomó como punto de partida solamente aquellas muestras con un amplificado positivo de la región ITS, evitando así los falsos negativos comentados anteriormente. Estos fragmentos fueron digeridos con enzimas de restricción y los resultados mostraron patrones diferentes permitiendo la correcta identificación de cada especie.

Métodos y Material

Nuestro material de trabajo inicial consistió en glebas y micorrizas colectadas en 4 localidades diferentes de la Península Ibérica de las especies *T. melanosporum*, *T. brumale* y *T. aestivum*. Las glebas de la especie *T. indicum* se obtuvieron de un mercado de Zaragoza y las micorrizas proceden de ensayos en planta de vivero llevados a cabo en el invernadero de la Escuela Politécnica Superior de la Universidad de Zaragoza (Huesca).

Las secuencias empleadas para realizar los alineamientos se descargaron de la base de datos del National Center for Biotechnology Information (NCBI).

Extracción de ADN y amplificación de los ITS

Se evaluaron diferentes métodos para la extracción de ADN, entre ellos el CTAB (Doyle & Doyle, 1990) con una trituración previa en nitrógeno líquido y los productos comerciales E.Z.N.A Fungal DNA miniprep kit® (Omega) y el DNeasy Plant Mini Kit® (Quiagen).

Se evaluaron diferentes cebadores específicos para las especies estudiadas (Paolocci *et al*. 1997; Bertini *et al*. 1999; Sejalon-Delmas *et al*. 2000; Suz *et al*. 2006). Sin embargo, tal como se ha comentado en la introducción los resultados no fueron los deseados. La baja concentración de ADN en la extracción a partir de material ectomicorrícico se convirtió en un factor limitante en la amplificación de la región ITS con cebadores específicos, ya que la totalidad del volumen obtenido en la extracción se empleaba para la posterior amplificación. Por lo tanto, se decidió emplear cebadores generales y tomar la amplificación positiva como punto de inicio para evitar así los falsos negativos. Se emplearon los cebadores ITS1 e ITS4, estos cebadores

han mostrado su estabilidad y ser capaces de encontrar grados de polimorfismo en diferentes especies, géneros y familias (Torrecilla & Catalán, 2002).

Secuenciación y Alineamiento
Las regiones ITS amplificadas fueron secuenciadas por MACROGEN. Con el fin de asegurarnos de la identificación que se había hecho por métodos morfológicos y de etiquetado, las secuencias se compararon en la base de datos BLAST del NCBI.
Se descargaron un total de 195 secuencias de las cuatro especies estudiadas (30, 20, 10 y 6 secuencias para *T. melanosporun*, *T. aestivum*, *T. indicum* y *T. brumale*, respectivamente) y de 10 especies relacionadas. Se realizó un alineamiento en conjunto utilizando el programa MEGA4 (Tamura *et al*. 2007) y posteriormente uno por separado para cada una de las cuatro especies estudiadas. Dichos alineamientos permitieron determinar las zonas en donde las secuencias entre diferentes especies son idénticas y las posiciones polimórficas de cada especie.

Restricción de los fragmentos ITS
Se determinaron los sitios de restricción con más de 50 enzimas diferentes en las secuencias de cada una de las especies estudiadas utilizando el programa Sequence Manipulation Suite (Stothard, 2000). Se elaboraron mapas de restricción que permitieron caracterizar a las cuatro especies de *Tuber*.

Resultados y discusión
Extracción de ADN y amplificación de los ITS
Todos los métodos de extracción estudiados generaron resultados positivos para las glebas y ectomicorrizas de *T. melanosporum, T. indicum* y *T. aestivum*, sin embargo, el ADN obtenido a partir de las glebas de *T. brumale* no generó una correcta amplificación de la región ITS, muy probablemente debido a la presencia de compuestos secundarios que podrían inhibir la PCR. Solamente mediante el empleo del DNeasy Plant Mini Kit® (Quiagen) fue posible eliminar dichos compuestos secundarios y por consiguiente obtener una amplificación óptima.
Los cebadores no específicos ITS1- ITS4 generaron amplificaciones positivas en el 85% de los casos, en el 15% restante no se obtuvo una banda correspondiente a la región estudiada, probablemente debido a una escasa concentración de ADN inicial, a la presencia de compuestos secundarios inhibidores o a la presencia de contaminantes en la muestra inicial. Ese 15% de casos de amplificación negativa se excluyeron de los análisis subsiguientes.

Restricción de los fragmentos ITS
Mediante el empleo del programa Sequence Manipulation Suite fue posible determinar los sitios de restricción en las secuencias de las cuatro especies estudiadas. En *T. melanosporum* se encontraron 20, 4 y 5 enzimas de restricción diferentes con uno, dos y más de dos sitios de restricción, respectivamente. En *T. aestivum* 6, 12 y 11 enzimas diferentes con 1, 2 y más de 2 sitios de restricción. En *T. brumale* 20, 5 y 7 enzimas diferentes con 1, 2, y más de 2 sitios de restricción. En *T. indicum* se detectaron 8 enzimas con un solo sitio de restricción, y 10 y 2 con 2 y más de 2 regiones de corte, respectivamente.
La selección de las enzimas de restricción se fundamentó en dos principios.
1.- Los sitios de restricción deben estar conservados en cada una de las especies, ya que si se emplea una enzima de restricción vinculada a una región polimórfica, todos los individuos que presenten cambios en su secuencia de bases no van a ser digeridos por la enzima y por consiguiente la especie no será identificada como tal,
2.- Los patrones de bandas procedentes de cada corte enzimático deben caracterizar a cada una de las especies estudiadas.
Por lo tanto, a nivel intraespecifico se alinearon las muestras estudiadas junto con las secuencias descargadas del NCBI y se seleccionaron las enzimas que presentaron patrones diferenciales en cada una de las especies y que actuaban sobre regiones conservadas. De

esta manera se asegura la viabilidad y fiabilidad de esta técnica. Se seleccionaron tres enzimas de restricción ECORI, BSP1407I y Sma I.
La enzima ECORI permitió la caracterización de *T. brumale,* con un solo sitio de restricción y dos fragmentos resultantes de aproximadamente 350 y 500 pares de bases (Fig. 2), esta enzima también permitió la separación de *Tuber melanosporum* y *T. indicum* (en los que presentó un solo corte produciendo dos fragmentos de aproximadamente 300 y 600 pares de bases) de *T. aestivum* (sin sitio de restricción) y de *T. brumale* (Fig. 2).

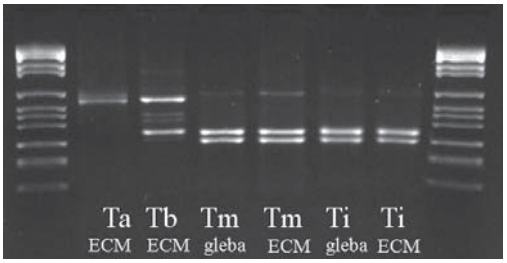

Fig. 2. Patrón de bandas tras el corte enzimático de ECORI.
Ta: *T. aestivum*; Tb: *T. brumale*; Tm: *T. melanosporum*; Ti: *T. indicum*; ECM: ectomicorriza. Marcador de peso VI Roche.

La enzima BSP1407I permitió la separación entre las dos especies más conflictivas de este estudio, debido a sus similitudes morfológicas. *Tuber melanosporum* presenta un solo sitio de restricción para BSP1407I y el producto del corte son dos fragmentos de 460 y 200 pares de bases aproximadamente (Fig. 3). En contraposición *T. indicum* no presenta ningún sitio de restricción para esta enzima. *Tuber aestivum* y *T. brumale* tampoco presentan sitio de restricción para BSP1407I (Fig. 3).

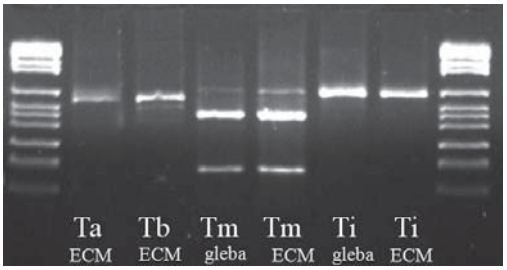

Fig. 3. Patrón de bandas tras el corte enzimático de BSP1407I.
Ta: *T. aestivum*; Tb: *T. brumale*; Tm: *T. melanosporum*; Ti: *T. indicum*; ECM: ectomicorriza. Marcador de peso VI Roche.

La enzima SmaI permitió la identificación de *T. aestivum*, ya que esta especie presenta un sitio de restricción para esta enzima y como producto de dicho corte se obtuvieron dos fragmentos de 385 y 235pares de bases aproximadamente (Fig. 4). Las demás especies estudiadas no presentan sitios de restricción para SmaI (Fig. 4).

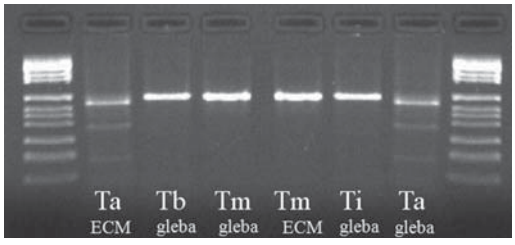

Fig. 4. Patrón de bandas tras el corte enzimático de SmaI.
Ta: *T. aestivum*; Tb: *T. brumale*; Tm: *T. melanosporum*; Ti: *T. indicum*; ECM: ectomicorriza. Marcador de peso VI Roche.

Tras el empleo de las tres enzimas de restricción se obtuvo un mapa RFLP mediante el cual es posible identificar de manera rápida y sencilla cada una de las especies estudiadas. Dicha identificación es posible gracias a que cada especie presenta un patrón de bandas diferente que le caracteriza (Fig. 5).

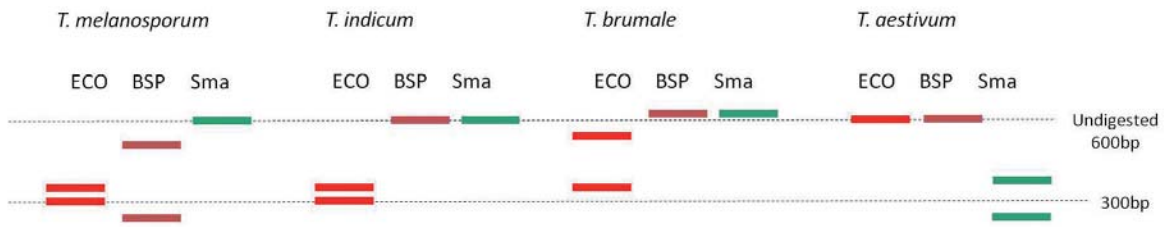

Fig. 5. Patrón RFLP de las cuatro especies de *Tuber* estudiadas
Enzimas de restricción empleadas ECO: ECORI (rojo); BSP: BSP1407I (azul); Sma: SmaI (verde).

A pesar que la técnica RFLP y sus variaciones (utilización de enzimas de restricción) han demostrado ser eficientes, reproducibles (siempre y cuando los sitios de restricción estén conservados) y fiables en diversos estudios (Mabru *et al*. 2004; Paolocci *et al*. 2004; Cordes *et al*. 2008; Ubeda *et al*. 2009), quisimos corroborar la validez de la técnica descrita a la hora de identificar cada una de las diferentes especies de trufas. Para lo cual, las regiones ITS amplificadas se secuenciaron y mediante un BLAST se corroboró que correspondieran a la especie en cuestión. En el 100% de los casos, los patrones de RFLP y los BLAST de las secuencias coincidieron, identificando a cada una de las cuatro especies de *Tuber* estudiadas, confirmando así la fiabilidad de la técnica desarrollada en este estudio.

Conclusiones

Los resultados de nuestro estudio han demostrado que es posible identificar cuerpos fructíferos morfológicamente similares, tal como sucede con las glebas de *T. melanosporum* y *T. indicum*, pudiendo evitar de esta manera posibles fraudes o confusiones. De igual manera, la técnica descrita, además de ser rápida, efectiva y económica permite detectar la presencia de cada una de las cuatro especies estudiadas a nivel de micorriza, permitiendo así, una evaluación de la planta procedente de vivero y un seguimiento constante de las plantaciones truferas a los pocos meses de haber sembrado las plántulas, en contraposición a los 4 a 6 años que habría que esperar para la formación del cuerpo fructífero.

Este trabajo es un paso inicial en la evaluación precoz de la presencia de las especies deseadas y de las indeseadas en las plantaciones truferas. Entre las perspectivas se encuentra la elaboración de una mapa de restricción para las especies más importantes del genero *Tuber*.

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DEVELOPMENT OF BIOTECHNOLOGICAL TOOLS IN *TUBER BORCHII*

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Abstract

We are interested in developing biotechnological tools necessary for molecular and cellular biology investigation of the filamentous ascomycetes *Tuber spp*. The first genomic sequence of *Tuber* is coming up due to a French-Italian collaboration, however the possibility of developing functional genomic is limited by the absence of a protocol for *Tuber spp* transformation. Here we report a protocol based on *Agrobacterium tumefaciens* hypervirulent strains and on binary plasmids constructed for *Tuber* colonies transformation. We constructed two T-plasmids containing hygromycin resistance (HygR) and a variant green fluorescent protein (EGFP) as markers, in order to select transformants by partial HygR and screen for epifluorescence by confocal microscopy. We obtained a mosaic of mycelial cells transformed and untransformed and this at the moment is the limit of the system. However, the transformants are stable and continue to grow on selective medium and to produce EGFP at least for 70 days. Next step will be to try to strongly increase homologous integration events.

Key words: genomics and cell biology, transformation vectors, EGFP, *Agrobacterium tumefaciens*.

Advances in the molecular analysis of *Tuber* during the last ten years have seen the cloning and characterization of several genes (Soragni *et al.,* 2001; Gabella *et al.,* 2005), the development of a molecular phylogenetic framework for these fungi and, lately, the launching of an international genome sequencing project (Bohannon, 2009). Gene discovery/expression studies have led to the identification of unexpected (or previously unknown) genes as well as regulatory traits that may be unique of the symbiotic life style. Each of these genes has provided novel information and working hypotheses on truffle biology/metabolism. For example, a photoreceptor (TbWC-1) homologous to the well characterized *Neurospora crassa* NcWC-1 and the discovery of blue-light controlled morphogenesis in an underground, conceivably "dark-growing" fungus, have raised new scenarios as to the role that light (particularly, light avoidance) might play in plant-root tropism and in *Tuber* growth in general.

These data will ultimately require confirmation *via* gene disruption/overexpression studies. Initial steps in this direction have been undertaken recently. Among them, the setting up of a prototype *Agrobacterium*-mediated transformation system in *Tuber borchii*, relying on *in vitro* cultured mycelia, a hypervirulent *A. tumefaciens* strain (AGL-1) and a binary vector (pBGgHg) (Chen, 2000) bearing the hygromycin resistance (HygR) and the enhanced green fluorescent protein (EGFP) gene under the control of the *Agaricus bisporus* GAPDH promoter and the CaMV 35S terminator (Grimaldi *et al.,* 2005). About 20% of the treated mycelia became partially hygromycin-resistant and displayed an unequal hyphal edge profile, with only ~1% of EGFP fluorescence-positive hyphae which failed to propagate under selective (+ Hyg) conditions. Low transformation efficiency and a highly heterogeneous population of untransformed and transformed hyphae (the latter carrying a mixture of transformed and untransformed nuclei) thus appeared to be the main limitations of this procedure.

Here we report on more recent experiments, in which we tried new combinations of *Agrobacterium* strains (AGL-1, G3101 and LBA4404) as well as different vector/promoter constructs (e.g., the

strong *Pyrenophora* ToxA promoter - Ciuffetti *et al.,* 1997) fused to a portion of a *Tuber* gene (e.g., *TbWC-1*) as a way to promote homologous integration.

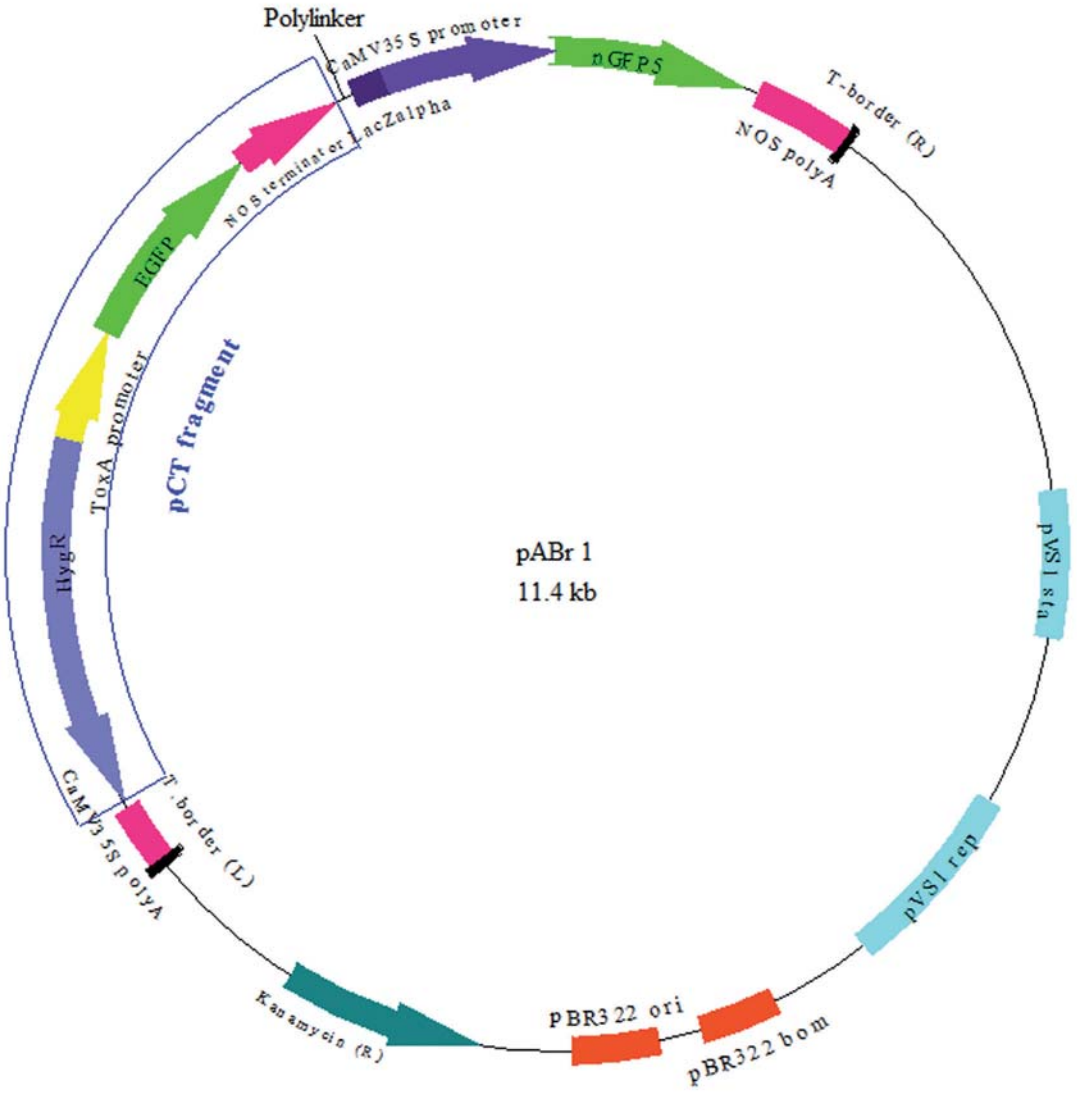

Fig. 1 pABr1 is derived from a pCAMBIA 1300 vector, where the T-DNA has been replaced by a fragment from pCT74 (pCT fragment) containing the EGFP gene (EGFP) under the control of the ToxA promoter (ToxA) and the hygromycin B resistance cassette (HygR). The polylinker contains the following restriction sites: Hind III, Sph I, Pst I, Sal I, Xba I, Bam HI, Sma I, Kpn I, Sac I, Eco RI.

We constructed two plasmids, pABr1 (Fig. 1) and pABr2 (map not shown). The first is a construct of 11425 bp, the second plasmid is 13101 bp long and contains a fragment of the truffle TbWc-1 gene, as a means to enhance homologous integration. Their purpose during the set up of this protocol is only to demonstrate that *Tuber* colonies treated with *Agrobacterium* containing the recombinant plasmids have indeed been transformed. For this reason, the plasmids contain two transformation "markers": hygromycin resistance (HygR) and EGFP expression. The second marker is necessary due to the incomplete hygromycin resistance of the transformed colonies, which are actually mosaics of transformed and untransformed hyphae. With the previously used plasmid (pBGghg) the percentage of fluorescent transformed hyphae showing fluorescence was very low (see above). To further improve transformants detection, we therefore decided to enhance EGFP expression with the use of a strong, constitutive promoter. We chose the ToxA promoter of *Pyrenophora tritici-repentis* (Ciuffetti *et al.,* 1997), whose activity has been tested in a large number of fungi, and in all cases led to very high transgene expression levels.

***T. borchii* transformation protocol**

1. Put *T. borchii* mycelia plugs on PDA plates. Place the mycelia on dialysis tubes in order to allow an easy plate to plate transfer.
2. Incubate 10 days at 25°C in the dark.
3. The day before the transformation, inoculate *A. tumefaciens* in LB from a single colony and incubate O.N. at 28°C with shacking.
4. Check $O.D._{600}$ of the culture, pellet and resuspend the cells in fresh LB in order to have an $O.D._{600}$=0.075, add 200 µM acetosyringone to induce the *vir* gene expression.
5. Incubate at 28°C for 4h (2 replicative cycles).
6. Transfer *T. borchii* mycelia to fresh PDA plates containing 200 µM acetosyringone.
7. Check $O.D._{600}$ of the *A. tumefaciens* culture, which should be around 0.3.
8. Transform *T. borchii* by pipetting 50 µl of *A. tumefaciens* culture onto every single *Tuber* colonies.
9. Co-cultivate for 3 days at 22°C in the dark.
10. Wash the *Tuber* colonies with sterile H_2O and transfer them to PDA plates supplemented with 15 µg/ml hygromycin, 100 µg/ml ampicillin, 200 µM cefotaxime, in order to allow transformants selection (HygR) while killing *A. tumefaciens* cells (Amp and Cefotaxime).
11. Screen *T. borchii* colonies for partial hygromycin resistance.
12. Visualize EGFP expression, preferably with a confocal microscope (excitation at 488 nm; emission at 530nm).

We then performed a transformation experiment, using pABr1, to test if the new construct was more stable. We routinely screened the *Tuber* mycelia for the presence of fluorescent hyphae 3 and 6 days after the transformation, obtaining a percentage of fluorescent hyphae, comparable to that of pBGghg (~ 0.7%, see Fig. 2 in Grimaldi *et al.,* 2005).
To test if the EGFP expression was stable over time (an important feature to confirm that the T-DNA was integrated in the *Tuber* genome), we monitored hyphal fluorescence after a long period of incubation at 25°C in the dark.
We used a maximum 70 days-incubation, at which, as shown in Fig. 2, the number of fluorescent hyphae per microscope field was high and the EGFP expression intact, thus indicating that the transformants are stable.
Finally we carried out a Southern blot analysis on genomic DNA extracted from transformed colonies. In the case of pABr1 we confirmed the integration of the T-DNA in *Tuber* genome (data not shown). The mode of integration of pABr2 has not been analyzed yet.

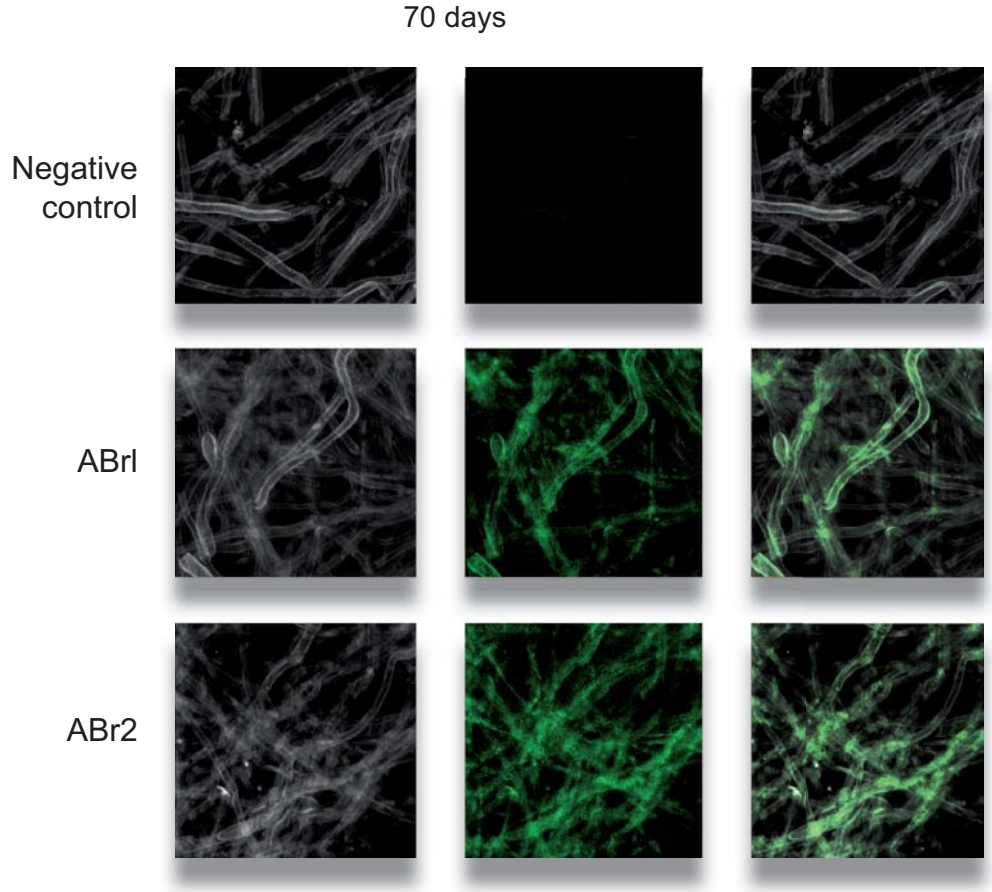

Fig. 2 Screening after 70 days from transformation. The samples are the same as those visualized after 3 and 6 days, recovered from the microscope slide, washed and cultured for an additional 63 days under sterile conditions. A large number of fluorescent hyphae is visible.

In this work we provide preliminary documentation of an improved *Tuber* transformation procedure, which yielded a higher stability of the transformants compared to that reported in Grimaldi *et al*. (2005). Moreover, the inserted transgene now appears to remain integrated in the genome for at least for 70 days, as demonstrated by epifluorescence monitoring. While transformant stability was clearly improved, the problem of the mosaicism of transformed and untransformed colonies is still unsolved.

The recent sequencing of the *Tuber* genome has revealed the presence of the homologs of two *Neurospora* genes that enhance heterologous recombination, which could be knocked out in order to eliminate, or strongly reduce, this event. This will ensure that a very large number of recombination events will be homologous.

The set up of an efficient transformation system will also provide a way to overexpress a heterologous gene, another important tool (alternative but also complementary to gene disruption) for the study of truffle biology.

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A FIRST STEP TO THE UNDERSTANDING OF MYCELIAL DATA IN *TUBER MELANOSPORUM-QUERCUS ILEX* ORCHARDS

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Black truffle cultivation has become an important alternative in marginal agricultural lands, but understanding the biology and ecology of this fungus is essential to achieve successful production. We carried out observational studies (Suz *et al.* 2006, 2008) in three *Tuber melanosporum* truffle orchards in Spain, representing critical phases in truffle cultivation (*burn* onset, productivity onset and truffle production phase). In each orchard, trees were randomly selected to study the relationships between soil mycelial abundance, tree growth and surface rock cover, with the presence and extension of the *burn* and with truffle production. Molecular tools, beginning with extraction of total soil DNA and followed by conventional and Real-time PCR with *T. melanosporum*-specific primers, were used to estimate mycelial distribution and abundance in soil.

Mycelium of *Tuber melanosporum* was found mostly in the first 35 cm of the soil profile, although it was also present as deep as 60 cm but in lower quantities. DNA from this mycelium was detected as far as almost 90 cm outside the visual edge of the *burn*. Moreover, it could be detected before the appearance of the *burn*.

Trees displaying a *burn* were taller (p=0.013), tended to have greater diameters (p=0.07) and presented higher biomass of black truffle mycelium in soil (p=0.016) than trees with no *burn*. It seems that the allelopathic effect of *T. melanosporum* mycelium initially became evident on the surface around the trees, after a certain biomass of *T. melanosporum* mycelium had formed belowground. Tree growth was positively related to the appearance of the *burn*, possibly because bigger trees potentially have more carbon available for the fungus.

We found that the amount of black truffle mycelium was greater in soils from newly productive trees (p=0.014) than in soils from nonproductive trees, suggesting that a certain amount of mycelial biomass is needed for the fungus to start producing the first truffles. However, when trees were productive for many years, we observed a decrease in the soil mycelial biomass (soil from productive trees presented lower amounts of DNA from *T. melanosporum* mycelium than soils from non-productive trees, p=0.04), perhaps because in the productive phase, the investment of fungal resources is shifted from vegetative growth to ascomata (truffle) formation.

Lower surface rock cover was associated with recently productive trees (p=0.08; average of the orchard: 78.8%) while truffle-producing trees presented higher rock coverage values than non-productive trees (p=0.007; average of the orchard: 43.3%). Thus, surface rock coverage was dissimilarly related to the productivity of truffles, depending on the average rock cover of the orchard.

Further studies are needed to explain and interpret our results and to generalize our observations to the life cycle of this still mysterious fungus.

Key words: *Tuber melanosporum*, soil mycelium, truffle cultivation, DNA, *burn*, rock cover.

Acknowledgements

We are indebted to Doñate and Tudel families for the open access to their truffle orchards. This

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ECOLOGY AND POPULATION BIOLOGY SESSION

SOILS SUITABLE FOR THE WHITE TRUFFLE

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Abstract
Soil suitability evaluation for truffle species is aimed at recognizing places where they reproduce on the basis of two essential conditions: the presence of a host plant and a suitable soil environment. Truffle species propagate whenever they meet a host, but they will produce truffles if and only if the right soil environment is present. In the case of *T. magnatum* Pico;right conditions are met in well-drained soils that have a moderately alkaline pH, a high amount of calcium carbonate and enough water all over the year. Further compulsory characteristics are the lack of water stagnation in the system, a very soft consistence, and more than 10% of the soil volume occupied by interconnected macropores. From the pedogeographical point of view, this combination of characteristics is fulfilled only in valley bottoms and hill slopes where geomorphological processes rejuvenate soils, originating a disordered distribution of particles due to frequent depositions of alluvial materials or to accumulation of soil aggregates transported along slopes by mass movement. The knowledge gained in soils for white truffle is essential to map areas for *T. magnatum* cultivation whose management, waiting for more effective nursery mycorrhization, is currently possible only in the wild.
Key words: truffle landscapes, soil environment, soil suitability, *T. magnatum*.

The landscapes of *T. magnatum*

The environmental investigations carried out from 1990 in Italy and, later on, in Croatia found a high selectivity of *T. magnatum* Pico for the soil environment to grow and produce ascocarps, and different environmental processes originating an environment.
From the geological point of view, the areas of diffusion of *T. magnatum* approximately correspond to regions characterized by the outcrop of terrigenous sedimentary rock formations belonging to Eocene-Miocene turbidites or Pliocene shallow sea sediments. At the contact with the atmosphere, these formations easily release fine silica sediments rich in $CaCO_3$. The relatively high erodibility of these sedimentary rocks is one of the main factors producing the two most widespread, often connected, landscapes where *T. magnatum* is found in Italy (Lulli *et al.,* 1991, Bragato *et al.,* 1992a) and Croatia (Bragato *et al.,* 2004), whereas a third slightly different landscape on sedimentary rock outcrops is limited to some areas of the Marche (Lulli *et al.*, 1995) and Abruzzo regions (Chiuchiarelli *et al*., 2010) in Italy.
The most common landscape related to *T. magnatum* production is formed by networks of small valley bottoms joining larger fluvial plains still affected by floods/flood depositions (Lulli *et al.,* 1991; Bragato *et al*., 2004). In this kind of landscape, the presence of *T. magnatum* looks related to fluvial dynamics and some of the fluvial landforms shown in Figure 1.

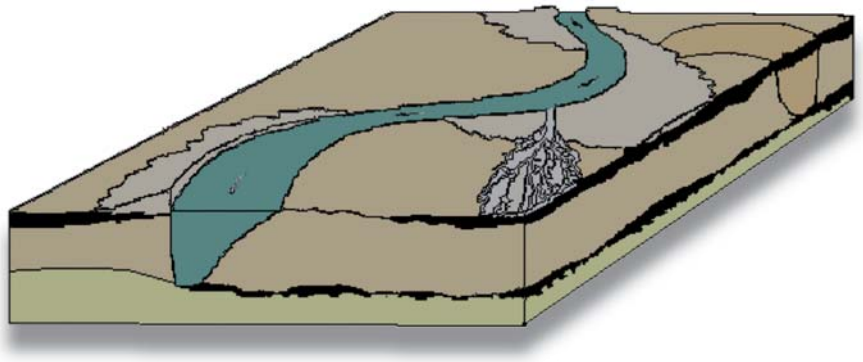

Fig. 1 The fluvial landscape model

In general terms, the areas of production - in light gray - coincide with the natural levees of the river, where flood energy is highest and a chaotic sedimentation of relatively coarse mineral particles takes place. Since flood energy and flow velocity partly depends on the geomorfological characteristics of the environment surrounding a riverbed, the size of productive areas can range from meters to tens of meters, in the former case making difficult to contour truffle-producing areas in soil maps.
The second main landscape of truffle-producing areas is located on hillslopes (Bragato *et al.,* 1992a). It is partially affected by fluvial dynamics – which specifically acts along seasonal streams on slopes – but the most important process is the gravitational movement of soil aggregates along slopes and their accumulation whenever slope gradient suddenly decreases. Mass movement is set up by small landslides (Raglione *et al.,* 2010) or seasonally surfacing water tables that allow individual soil aggregates to move downwards like in Figure 2.

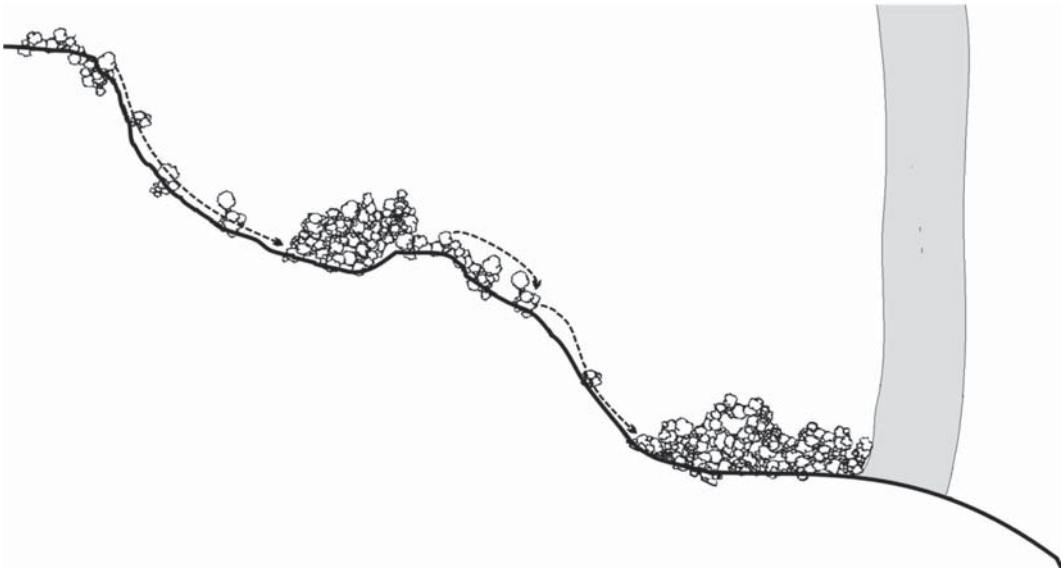

Fig. 2 Downward movement of soil aggregates on slopes.

Both landscapes are locally characterized by a chaotic accumulation of particles/aggregates that substantially increases the presence of large voids. As an example, the presence and distribution of pores larger than 50 μm in a productive and an unproductive area are shown in Figure 3.

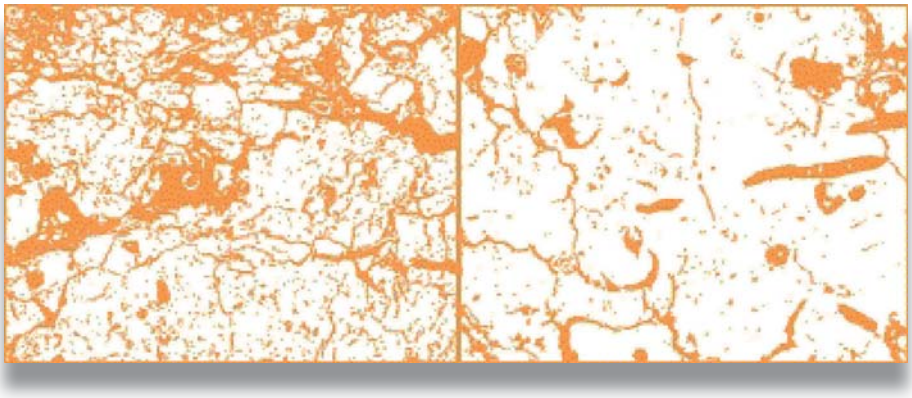

Fig. 3 Pores larger than 50 μm (in orange) recorded in soil thin sections from a productive (left side) and an unproductive area (right side). Corresponding soil sections were 35mm wide.

The productive area not only displays a higher macroporosity, but also shows a much branched network of macrovoids that give specific features of high aeration and softness to

soil (Bragato *et al.,* 1992b). In the absence of further perturbations, the natural rearrangement of soil structure would decrease macroporosity to a steady state value specific of the type of soil considered. The high-macroporosity condition always found in truffle-producing sites of hillslopes and fluvial plains is instead guaranteed by the accumulation of new particles/aggregates that continuously rejuvenates the soil.
Comparable macroporosities are obtained in a further "truffle landscape" observed in Acqualagna, Marche region, where soil aeration is provided by a rock formation that produces an increasing amount of macrovoids as the load of upper strata decreases near the earth surface (Lulli *et al.,* 1995).
In this specific case, the rearrangement of soil structure in time is limited and high macroporosity values are maintained also in more developed soils like those observed in the Abruzzo region (Chiuchiarelli *et al.,* 2010).
Even if soil structure plays a fundamental role in making a soil suitable for white truffle production, soil micromorphometry measurements are too espensive to be used in practice and bulk density, which is inversely related to total porosity in soil, can partially replace them. For example, the data of Table 1 refers to a comparison between productive and unproductive soils of a flood plain and an unproductive one of a nearby slope (Bragato *et al.,* 2004). Bulk density specifically distinguishes soils of the valley bottom from that on slope, with values respectively corresponding to 48% and 58% of soil volume occupied by voids.

Tab. 1 Comparison of productive and unproductive soils in relation to some physical and chemical attributes.

	Valley bottom		Slope
	Productive	unproductive	unproductive
Bulk density, kg L^{-1}	1,10	1,10	1,37
pH	7,69	7,29	7,23
Total $CaCO_3$, %	29,2	27,8	1,4

In general, the combined use of the attributes reported in Table 1 allows to discriminate *T. magnatum* soil environment. The high content in $CaCO_3$ that for instance characterizes many truffle-producing soils increases pH to slightly alkaline values not tolerated by other competing fungi, neutralizes oxalic acid resulting from fungal metabolism, and stabilizes soil structure preventing the shrinking/swelling effect of silicate clays. However, in soils of temperate regions rich in $CaCO_3$, one of the earlier soil forming processes is the solution and removal of carbonates from soil surface strata. From this point of view, the unproductive soil on slope is paradigmatic. The decrease of both $CaCO_3$ and pH is coupled with the rearrangement of soil structure indicated by bulk density, emphasizing the enhanced evolution of this soil with respect to those of the valley bottom. In the absence of new depositions, decarbonation and structure rearrangement are among the causes responsible for the loss of *T. magnatum* production in truffle-producing regions.
Unlike the soil on slope, the decrease of pH values recorded the unproductive soil of the valley bottom is not related to $CaCO_3$ content but to the accumulation of organic C in soil surface strata caused by a slowdown of wood litter mineralization. In this case, the high content of organic C is indicative of seasonal water stagnation that favours the release of low-molecular-weight organic acids capable to lower soil pH despite the high $CaCO_3$ content.

Can we map and manage soils suitable for white truffle production?
T. magnatum grows in a soil environment whose specific characteristics explain its limited production also in regions renowned for the white truffle. Its environmental selectivity affects

the size and distribution of truffle-producing areas, which range from meters to few hundred meters and can be recognized only in very detailed, 1:5.000 - 1:10.000 scale soil surveys.
This notwithstanding, researches done in the last twenty years has made the mapping of soils suitable for *T. magnatum* feasible. Productive and unproductive soils can be distinguished in the field thanks to morphological characteristics like those of Figure 4: accumulation of organic matter on the surface and symptoms of water stagnation in form of grey-coloured spots characterize the unproductive soil of the valley bottom, whereas well-developed aggregates are present all over the profile in the unproductive soil on slope.

Fig. 4 Upper 25cm cores of the soils reported in Table 1.

In many environmental conditions, detailed surveys made by skilled surveyors can be exhaustive. It is for instance the case of soils in landscapes not yet modified by man activity. Conventional surveys are effective for truffle-producing areas on slopes, where white truffle production displays a spotted distribution in space and the surveyor identify suitable areas from surface morphology.
A more complex approach is instead required when landforms have been obliterated by man, like in reclaimed fluvial plains. In such cases, field observations may be coupled with geostatistical techniques to draw up maps like that reported in Figure 5, that shows the probability to find a fine and friable structure predicted with indicator kriging interpolation.

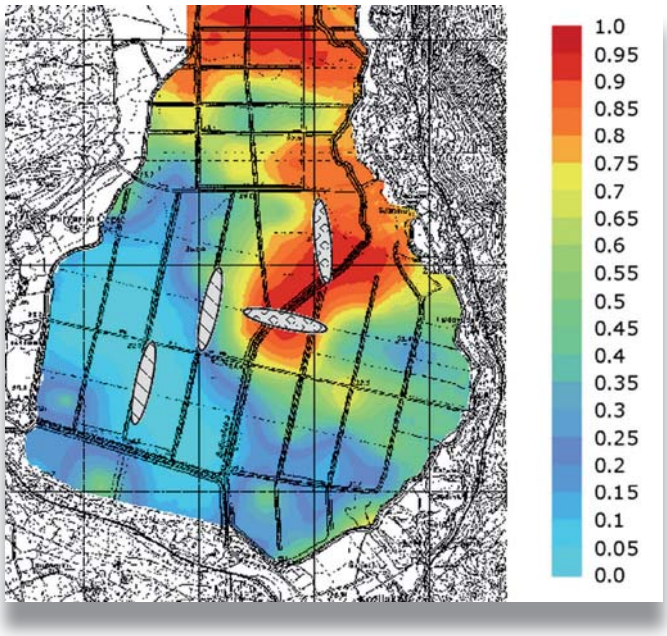

Fig. 5 Map of the occurrence of a fine and friable structure in an area suitable for *T. magnatum* production.

This probability is larger than 0.7 in the truffle producing areas – i.e. the two ellipses to the east of the map – whereas unproductive areas on the west side of the map display probabilities lower than 0.5 to find a soil structure suitable for white truffle production.
Once mapped, soils suitable for truffle production should be managed according to the environmental requirements of *T. magnatum*. Some of them are visualized in the following Figures in terms of fitness landscapes (Kauffman, 1993) under the assumption that the requirementes for *T. magnatum* colonization are less restrictive than those for white truffle production: peaks and valleys correspond to low and high suitabilities, respectively. Figure 6 summarizes the requirements of *T. magnatum* for soil structure characteristics.

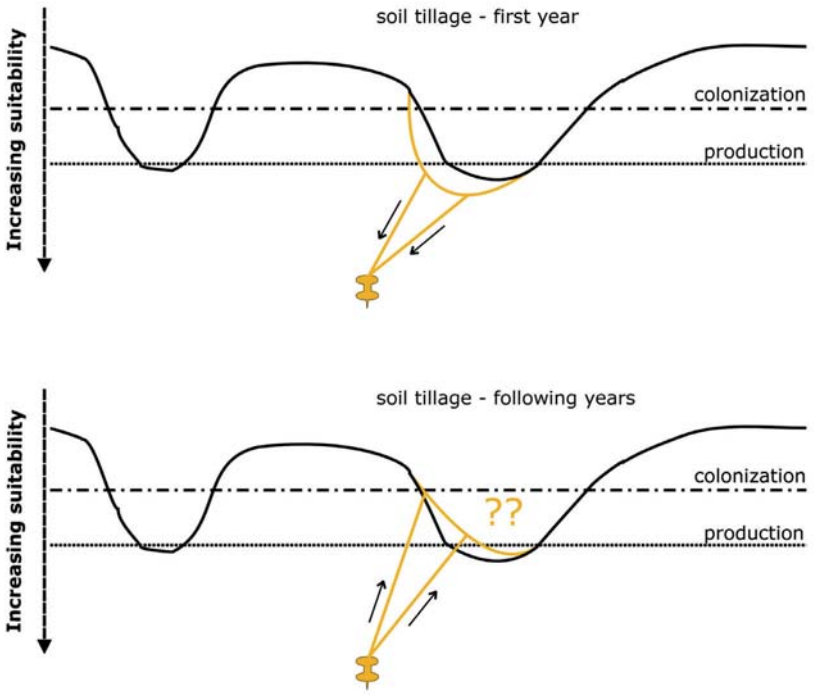

Fig. 6 The fitness landscape of *T. magnatum* in relation to soil structure and tillage practices.

In natural conditions, soil structure development needs tens of years, but tillage affects it almost imediately. Soil tillage is aimed at increasing soil porosity and, in theory, it would originate the right conditions for *T. magnatum* production. Unfortunately, its effect lasts for limited periods of time and, if wrongly done, it can decrease rather than increase soil suitability for white truffle production, for instance when soil encrustment takes place. Structure degradation is also the reason why liming should be avoided in soils for *T. magnatum*: apart from its expensiveness in neutral to slightly alkaline soils, the need to bury large amounts of lime induces soil compaction and a long-lasting loss of soil macroporosity.
Since *T. magnatum* production is strongly influenced by climate fluctuations, the fitness landscape shown in Figure 7 depicts the effect of soil moisture on white truffle production in dry and wet years.

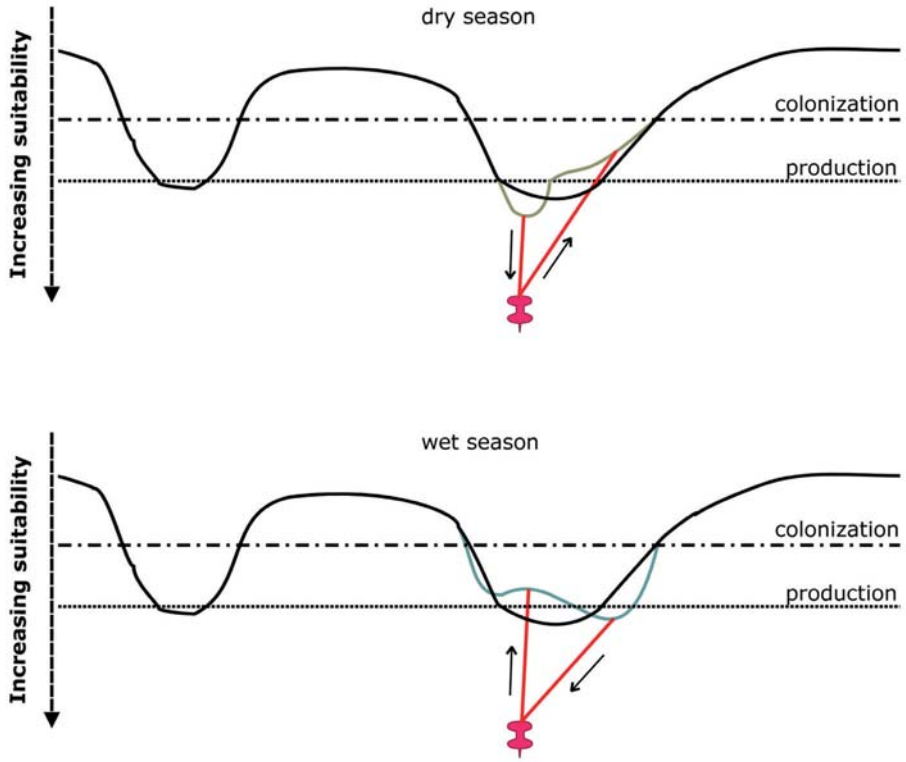

Fig. 7 The fitness landscape of *T. magnatum* in relation to soil moisture.

Soil moisture variability not only affects the size of truffle production, but also controls the spatial variability of collection sites in truffle-producing areas. Several agroforestry practices aimed at controlling the density of the vegetation cover have been already tested, but white truffle production may be managed also with irrigation practices capable prevent both soil encrustment and an excess of water in the surroundings of the mycorrhized roots.

Outstanding issues
Despite the knowledge acquired on *T. magnatum*, a lot of research is still needed to understand the biological characteristics and the environmental requirements of this hypogeous fungus. From the soil biology point of view, I think the following issues should be dealt with in the near future :

a) how the life cycle of *T. magnatum* matches the continuous rejuvenation of soil surface?
b) which are the ecological relationships of the fungus with the other living organisms of the rizosphere?
c) what kind of soil environment is required for *T. magnatum* colonization, and does it differ from that found in production areas?

Two further issues will in the end concern field experimentation when nursery mycorrhization will become feasible:

d) the test of suitable tillage techniques and devices capable to increase soil macroporosity while limiting encrustment and compactation;
e) researches on irrigation practices well-suited for the environmental requirements of *T. magnatum*.

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A CONTRIBUTION TO THE CHARACTERIZATION OF THE SOILS SUITABLE FOR *TUBER MELANOSPORUM* AND *TUBER AESTIVUM* GROWTH THROUGH MINERALOGICAL ANALYSIS OF THE CLAY FRACTION

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Abstract
A mineralogical analysis of the clay fraction of 30 and 26 soil samples coming from epipedons of natural truffle beds of *Tuber melanosporum* Vittad. and *Tuber aestivum* Vittad. respectively was carried out. The aim was to investigate on possible relationship between the type of mineralogical clay and the granulometric clay content. The results of X-ray diffraction patterns indicate that the main types of mineralogical clays found in soils suitable for *Tuber melanosporum* and *Tuber aestivum* growth are represented by kaolinite, illite-mica minerals, smectites and hydroxy-Al interlayered vermiculite (HIV). Since the various families of mineralogical clays present different physical and chemical properties we have compared the mineralogical analysis of the clay fraction with the percentage of granulometric clay of the fine earth (< 2 mm fraction) taking also into account the bulk presence of rock fragments. This comparison indicates that, in the case of *Tuber melanosporum*, expansible clays (smectites) are dominant when the granulometric clay content does not exceed 27% in the fine earth and rock fragments are abundant. If the fine earth represents the whole bulk of soil, the prevalence of smectites is associated with low or very low content of granulometric clay. On the contrary, the dominance of non dynamic clays, kaolinite plus HIV and illite, corresponds to higher content of granulometric clay (> 27%) with a moderate quantity of rock fragments. With regard to the soils of *Tuber aestivum* the prevalence of smectites can be associated with a decidedly clayey texture even if very high contents of granulometric clay (> 50%) are associated with the dominance of kaolinite. The results of the mineralogical analysis of the clay fraction confirm so the field observations, where the presence of *Tuber aestivum* is usually associated with soil characteristics indicating physical dynamicity. This is evidenced by tendency of the soil to hardening and compaction when dry, by great plasticity in moist state and by the prevalence of medium blocky structures with respect to granular and crumby ones.
Key words: soil, truffle ecology, clay mineralogy.

Introduction

The studies carried out till now on soils of natural production of "black truffles" can be considered sufficient to understand the differences between the *Tuber melanosporum* Vittad. and *Tuber aestivum* Vittad.; the main soil physical and chemical parameters predisposing or limiting the production of these two species have been well identified (De Simone *et al*., 1993; Lorenzoni *et al.,* 1995; Owczarek *et al.,* 2007; Raglione *et al.,* 1992, 2001, 2005). Nevertheless some questions have to be clarified: for instance the particle size composition (texture of the fine earth plus rock fragments) of the soils has a very high variability for both species; many soils can have, for example, the same clay content, but can be otherwise suitable for the two truffles. This fact makes impossible to use granulometric parameters to establish the suitability of a soil for one of this two truffles. Field investigations show, however, that the productive soils of the two species are characterized by peds of different size and hardness and it is well known that these properties are associated not only with the quantity of granulometric clay but also the type of clayey minerals which are present in it. For this reason it is right to presume that the quality of the clay must be different in the two cases. The aim of this work is to show that the contribution of the mineralogical analysis of the clay fraction can help to a better

characterization of the soils suitable for *Tuber melanosporum* and *Tuber aestivum* growth.

Materials and methods

The mineralogical analysis of truffle soils involved 30 and 26 samples of soils suitable for the production of *Tuber melanosporum* and *Tuber aestivum* respectively. Soil samples were collected from epipedons (A2 horizons) or subsurface horizons (B horizons) when the top horizon was very thin. In any case the depth ranges of sampling are representative of the soil portion where the fungus mycelium and the carpophore grow. Soil samples were air dried and sieved with a 2 mm mesh. The mineralogical analysis was carried out by X-ray diffraction on the clay fraction (< 2μm) obtained from the fine earth (fraction < 2 mm) with dispersion with sodium hexametaphosphate and sedimentation in deionized water. Specimens were then Mg-saturated, washed free of chlorides and freeze-dried. Clay-aggregate samples, oriented on glass slides from a water suspension, were analysed with a 3-kW Rigaku D/MAX III C diffractometer, equipped with a horizontal goniometer, a curved-beam graphite monochromator and Cu radiation. Slides were step-scanned from 2° to 15° 2θ with steps of 0.02° 2θ at 2 second intervals. The following five separate treatments according to the "Official Methods of Mineralogical Analysis of the Soil" (MiPAF, 2005) were performed for each sample in order to identify the different clay minerals (phyllosilicates): Mg-saturation, ethylene glycol solvation, K-saturation, K-saturation followed by heating for 2 hours at 335°C and K-saturation followed by heating for 2 hours at 550°C.

Results and discussion

The results of the mineralogical analysis evidence that the main types of mineralogical clays found in the granulometric clay fraction of soils suitable for *Tuber melanosporum* and *Tuber aestivum* growth are represented by kaolinite, illite-mica minerals, smectites and hydroxy-Al interlayered vermiculite (HIV). Phyllosilicates kaolinite, illite, and HIV are considered very little expansible clay minerals while smectites are tipically expansible clays. Generally kaolinite and smectites are mutually exclusive. Because the intrinsic characteristics of the clay minerals influence the macroscopic physical properties of the soil, we have compared the mineralogical analysis of the clay fraction with the percentage of granulometric clay of the fine earth (< 2 mm fraction) taking also into account the volumetric content of rock fragments in the soil.

A few examples of X-ray diffraction patterns which compare, from a mineralogical point of view, two soils suitable for *Tuber melanosporum* and *Tuber aestivum* respectively, are shown in Figures from 1 to 4. In the comparisons we have considered different or similar contents in granulometric clay and rock fragments. Usually the kaolinite is dominant in *Tuber melanosporum* soils and associated generally with HIV, while the smectites are dominant in *Tuber aestivum* soils (Fig.1).

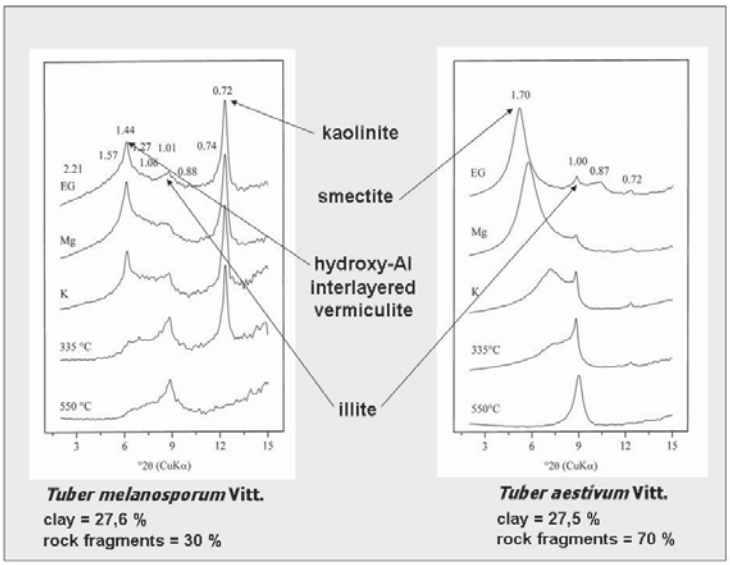

Fig. 1 Under the same granulometric clay content the soil of *Tuber melanosporum* shows a clay

mineralogy completely different from that relative to the soil of *Tuber aestivum*.

Tuber melanosporum soils can have smectites if the percentage of granulometric clay is low (Fig. 3) or if the percentage of rock fragments is high (Fig. 2).

Tuber aestivum soils can have also a very high content of granulometric clay without rock fragments if the kaolinite is the dominant clay mineral (Fig. 2).

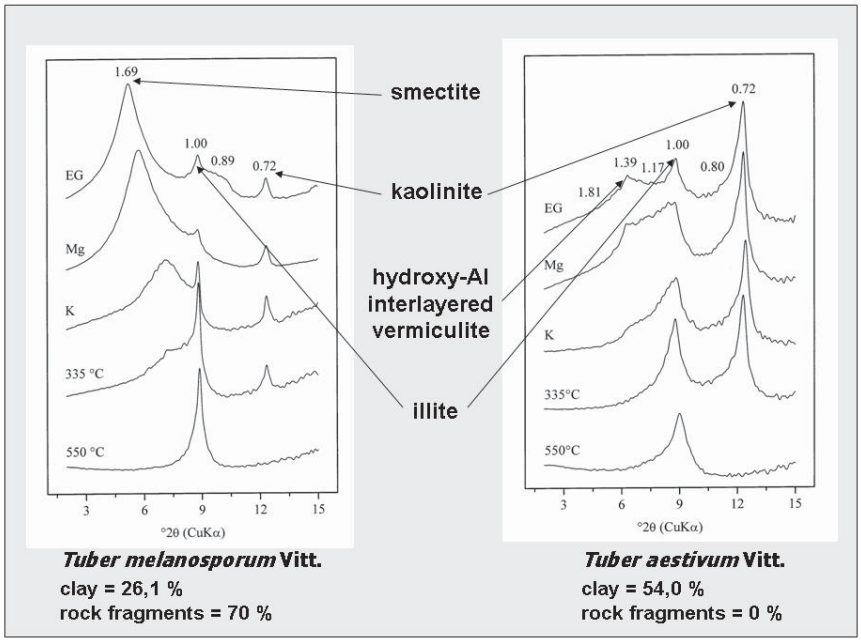

Fig. 2 A reasonable content of granulometric clay with prevalence of smectite is associated with abundant rock fragments in the soil of *Tuber melanosporum*. In the soil of *Tuber aestivum* the effects of a very high content in granulometric clay and of the absence of rock fragments are mitigate by the prevalence of non-expansible clays.

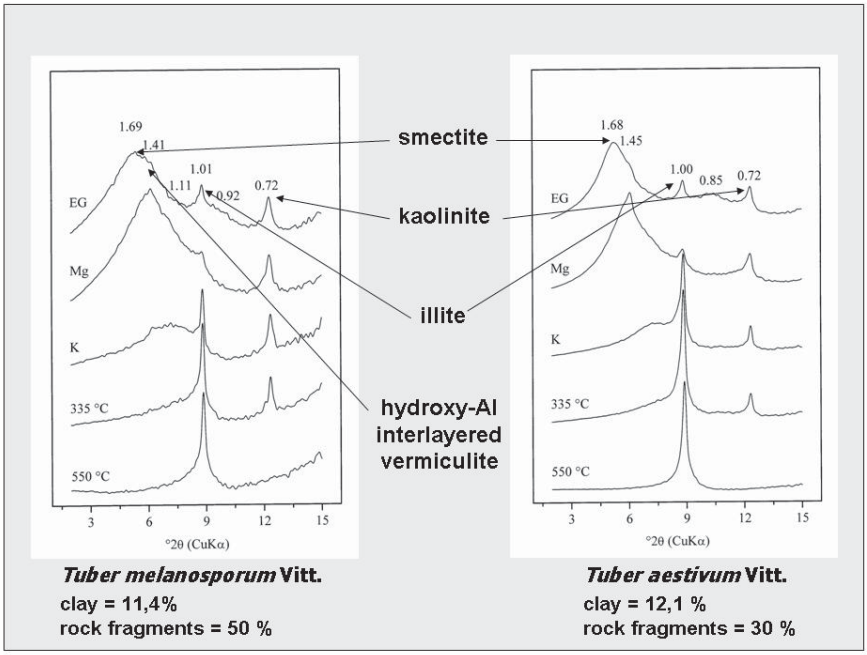

Fig. 3 Under the same granulometric clay content and type of mineralogical clays the soils of *Tuber melanosporum* tend to have a greater quantity of rock fragments compared to those of *Tuber*

aestivum.

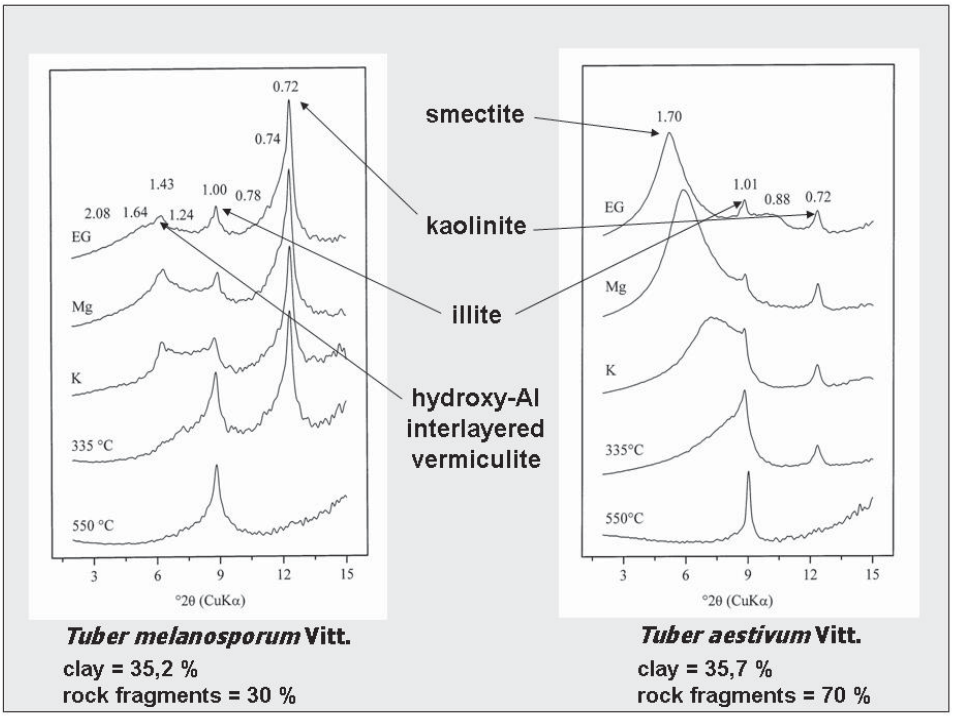

Fig. 4 When the granulometric clay content is quite high and the rock fragments little abundant the soils of *Tuber melanosporum* present non-expansible clays. The same granulometric clay content with presence of expansible clays is mitigate by abundant rock fragments in soils of *Tuber aestivum*.

Our analyses indicate that, in the case of *Tuber melanosporum*, expansible clays (smectites) are present when the granulometric clay content does not exceed 27% in the fine earth and rock fragments are abundant. If the fine earth represents the whole bulk of soil, the prevalence of smectites is associated with low or very low content of granulometric clay. On the contrary, the dominance of non dynamic clays (kaolinite, HIV and illite) corresponds to higher content of granulometric clay (> 27%) associated with a higher content of rock fragments. With regard to the soils of *Tuber aestivum* the dominance of smectites can be associated with a decidedly clayey texture (granulometric clay content > 40%) even if very high contents of granulometric clay (50-85%) are combined with the prevalence of kaolinite.

Conclusions

In summary, for both *Tuber melanosporum* and *Tuber aestivum*, there is a common trend which combines the increase of granulometric clay content with the prevalence of kaolinite and HIV compared to the smectites. The percentage of granulometric clay, in correspondence of which this change of the type of mineralogical clay occurs, is lower in the soils of *Tuber melanosporum* than the ones of *Tuber aestivum*.

The mineralogical analysis of the clay fraction confirms the field observations: *Tuber melanosporum* is usually present in soils without physical dynamicity, while *Tuber aestivum* is usually associated with soil characteristics indicating physical dynamicity. This latter is evidenced by tendency of the soil to hardening and compaction when dry, to have great plasticity in the moist state and by the prevalence of medium blocky structures.

In conclusion we can affirm that the mineralogical analysis of the granulometric clay fraction can help to best establish the soil suitability for *Tuber melanosporum* or *Tuber aestivum* growth. The presence of rock fragments is fundamental in soils of *Tuber melanosporum*; their percentage has to increase with the granulometric clay content, particularly if the expansible clays (smectites) are dominant.

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ESTUDIO DE LA FLORA VASCULAR Y LA MICOBIOTA MICORRÍCICA EN QUEMADOS TRUFEROS DE NAVARRA (ESPANA)

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Abstract: Study of the vascular and mycorrhizal flora in truffle brûles in Navarra (Spain).
In spite of the continuous advances in trufficulture there are still many questions about truffle growing, hence the big importance of studying the characteristics of the environment in which it best develops.
In this study we have analysed the main ecological characteristics of the vascular flora growing in 24 holm oak *brûles*. This flora is special, very influenced by the alelopatic substances produced by the mycelium of the fungus, modifying the composition and the characteristics of the flora. As a result, plants grow smaller and with a lower covering. Sometimes they even present changes in their life-cycle. Therophytes typical of disturbed areas are dominant in plantations. Xerophile plants are also common. Besides, the species found in truffiers are proper of shaken, removed and generally sunny, dry and stony places, and many of them weeds. This corroborates the fact that the brûle is a very disturbed environment, both by fungus mycelium as by human intervention.
On the other hand, monitoring the mycorrhizae is very important in truffle growing since it allows us to know if the plantations are properly developing or if truffle mycorrhizae have been replaced by other competing fungi. That is why we have carried out a below-ground study of the mycorrhizae appearing in the roots of the same holm-oaks, to check the presence of truffle mycorrhizae and to know if other mycorrhizal fungi are colonizing the roots.
Key words: *Tuber melanosporum*, flora, ectomycorrhizae, *brûlé*, trufflegrowing.

Introducción

La trufa negra (*Tuber melanosporum* Vittad.) es un Ascomicete ectomicorrícico de desarrollo subterráneo, muy apreciado por su alto valor gastronómico y comercial, sin embargo, en las últimas décadas, su producción ha disminuido drásticamente en Europa, de ahí su alto valor de mercado (Bonet *et al*., 2006). El micelio de la trufa forma micorrizas con las raíces de ciertas plantas leñosas, especialmente del género *Quercus*, por lo que, para intentar abastecer la alta demanda del producto, en la actualidad se están realizando numerosas plantaciones con encinas micorrizadas con trufa negra. Sin embargo, los resultados obtenidos en estas plantaciones muchas veces no alcanzan las producciones esperadas y, a pesar de la experiencia acumulada, sigue habiendo muchas preguntas sin resolver acerca del desarrollo y fructificación de este preciado hongo. Para intentar resolver estos problemas, es imprescindible conocer el ambiente tan especial en el que se desarrolla la trufa. Esto nos ha impulsado a realizar un estudio en profundidad de la flora vascular y micorrícica de los quemados de varias plantaciones truferas, con el objetivo de conocer mejor las condiciones en que se desarrolla la trufa.
La trufa se asocia de forma natural a encinas, robles y avellanos, por lo que éstos son los 3 simbiontes que más se han utilizado en las plantaciones realizadas hasta el momento. Sin embargo, el simbionte que mejores resultados está dando en Navarra es la encina, por lo que actualmente, en algunas plantaciones los avellanos están siendo cortados. A la hora de realizar una plantación, las raíces de las plantas procedentes de viveros, deberían estar micorrizadas exclusivamente con *Tuber melanosporum*. Sin embargo, con el paso del tiempo,

las especies competidoras aumentan, incrementando la diversidad micorrícica en las raíces y compitiendo con las micorrizas de la trufa. Por ello, es esencial llevar a cabo un seguimiento exhaustivo de los árboles, su estado de micorrización y su evolución a lo largo del tiempo, para saber el estado de las plantaciones y para entender las condiciones que promueven la fructificación de la trufa (Baciarelli-Falini *et al*., 2006). Este proceso natural e inevitable es un serio problema, ya que los competidores pueden disminuir la capacidad micorrícica de la especie deseada, pudiendo llegar a desplazarla. Sin embargo, identificar las especies fúngicas que forman parte de la simbiosis o hacer una aproximación taxonómica es difícil debido a las escasez de rasgos diagnóstico, que además dependen del hospedador y de las condiciones ambientales (Baciarelli-Falini *et al*., 2006).

Por otro lado, algunas micorrizas, presentes en las raíces de las encinas, producen sustancias alelopáticas que inhiben el crecimiento de la mayor parte de la vegetación herbácea que crece alrededor de los árboles truferos, de forma que sólo permanecen aquellas especies capaces de resistir dicho efecto, las cuales podrían actuar como plantas indicadoras de zonas potenciales para la producción de trufa (Olivier *et al*., 2002). A esta zona prácticamente desprovista de vegetación se le conoce como “quemado” y pueden llegar a ser muy aparentes dependiendo de la estación.

Por ello, uno de los objetivos principales planteados en este estudio es **analizar el espectro ecológico de la flora** que consigue desarrollarse en los quemados. Así mismo, los objetivos propuestos en relación con la flora micorrícica son **comprobar si las micorrizas de la trufa negra continúan presentes** en las raíces **o** han sido **desplazadas** por otras especies y, de ser así, **identificar cuáles son estas micorrizas competidoras**.

Material y métodos

Durante cuatro años se ha muestreado la flora vascular y la micobiota ectomicorrícica en quemados de encinas (*Quercus ilex* subs*. ballota*).

Para el estudio de la flora vascular, ésta se ha muestreado mensualmente en cuatro plantaciones y en 2 truferas silvestres. Las zonas de estudio se han establecido en las dos principales áreas de producción trufera de Navarra: Tierra Estella y Valdorba. En Tierra Estella, se realizaron estudios en dos plantaciones (una en la localidad de Ollogoyen (30TWN7027) y otra en Metauten (30TWN7226)), así como en una zona de producción natural adyacente. En La Valdorba, se realizaron estudios en dos plantaciones situadas en la localidad de Olóriz (30TXN1321) y en una zona de producción silvestre cercana. En todas ellas, se han prospectado los quemados de 6 encinas, lo que supone un total de 24 encinas para el muestreo de flora vascular.

En todas estas zonas se realizaron inventarios fitosociológicos (Braun- Blanquet, 1979) en el interior de los quemados. Una vez en el laboratorio, se procedió al prensado y conservación de la flora vascular, tras lo cual se procedió a la identificación de todas las especies recolectadas y al posterior análisis del total de la flora. Estos ejemplares se encuentran depositados en la Sección Botánica del Departamento de Biología Vegetal de la Universidad de Navarra y serán incluidos en un futuro en el herbario PAMP de la Universidad de Navarra. Los especímenes se han determinado siguiendo a Castroviejo (1986-2005), Aizpuru (2000) y Tutin *et al*. (1964-1980). Después se ha realizado un catálogo florístico de las especies recolectadas, a partir del cual se han realizado los espectros taxonómico, biológico (siguiendo el sistema de Raunkiaer, en Site *et al*., 2004) y corológico. A la hora de representar los resultados, se presentan en forma de gráfica los porcentajes totales obtenidos a lo largo de todo el estudio, y en forma de tabla se recogen comparativamente los resultados obtenidos en las parcelas y en la zona natural.

Para analizar el hábitat de las especies (Aizpuru, 2000), se ha distinguido entre: a) plantas ruderales y arvenses, propias de lugares alterados y nitrogenados (hábitat 1), b) plantas propias de pastos y matorrales secos, luminosos y pedregosos (hábitat 2) y c) resto de hábitats (hábitat 3), para calcular y analizar su porcentaje, ya que esos son previsiblemente los hábitats más comunes para las plantas que crecen en los quemados. Algunas especies aparecen indistintamente en los dos primeros tipos de hábitat, por lo que se han señalado aparte (hábitat 1 y 2).

Para el estudio de las ectomicorrizas (ECM), se recolectaron muestras estacionalmente en primavera y otoño, cuando las ECM son más abundantes y activas (Bueé et al., 2005), en un total de 10 plantaciones. En todas ellas, se han prospectado los quemados de 6 encinas, lo que supone un total de 60 encinas. La extracción se realizó cavando con una pequeña azada hasta encontrar las raíces de la encina. Se tomaron varios fragmentos de raíces micorrizadas de 4-6cm de longitud. Tras el tamizado y limpieza de las muestras se almacenaron a 4º C hasta el momento de su identificación. Para la identificación de las ECM se ha utilizado la caracterización morfológica de las mismas, comparando las muestras con las descripciones de otros autores (Ingleby *et al*., 1990; Bencivenga *et al*., 1995; Granetti, 1995; Saez y De Miguel, 2008; Agerer *et al*., 1987-1998; Agerer y Rambold 1998; Goodman *et al*., 1996-2000), ya que no existen claves que permitan identificar cualquier ECM. Aunque se sabe que más de 5000 especies de hongos forman ECM (Horton y Bruns, 2001), sólo poco más de 300 han sido descritas (De Román *et al*., 2005). Por ello, en nuestro estudio hemos encontrado algunos morfotipos no identificados, cuya morfología no coincide con ninguna descripción. Las ECM se han descrito de acuerdo con la terminología de Agerer (1987-1998), teniendo en cuenta la ramificación, el tipo de manto y las características de su superficie, y la presencia de hifas que emanan o cistidios, muy útiles para el diagnóstico de las micorrizas de trufas y otre especies ounque puden cambiar según la estación.

Resultados

Flora Vascular

El total de especies vegetales asciende a 280 táxones: 1% gimnospermas (con sólo tres taxones, pertenecientes al género *Juniperus*), 82% dicotiledóneas (el grupo mejor representado) y 17% monocotiledóneas (correspondientes a las familias *Cyperaceae, Liliaceae, Orchidaceae* y *Poaceae*) (Fig. 1).

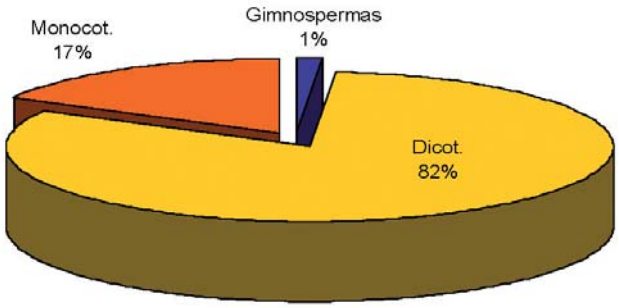


Tabla 1: Porcentaje de grupos en cada zona.

	Parcelas	Natural
Gimnosp.	1	1
Dicot.	82	87
Monocot.	17	12

Fig. 1: Grupos de flora vascular de los quemados.

Comparando el espectro taxonómico de las parcelas y de las zonas de producción natural (Tabla 1), vemos que en las truferas silvestres el porcentaje de monocotiledóneas disminuye, debido principalmente a que, a pesar de que algunas especies de Orquídeas aumentan, el número de Poáceas es notablemente menor.

Los 280 taxones pertenecen a 45 familias (Fig. 2), siendo las Asteráceas la familia mejor representada en todas las zonas, seguidas de Poáceas y Fabáceas. Este hecho no es extraño ya que son las familias mejor representadas en los encinares de la región (Alberdi, 2003). En las truferas silvestres (Tabla 2) el porcentaje de Poáceas disminuye bastante en favor de otras familias como Labiadas o Cistáceas, familias características de las series de vegetación propias del ambiente natural en el que se localizan.

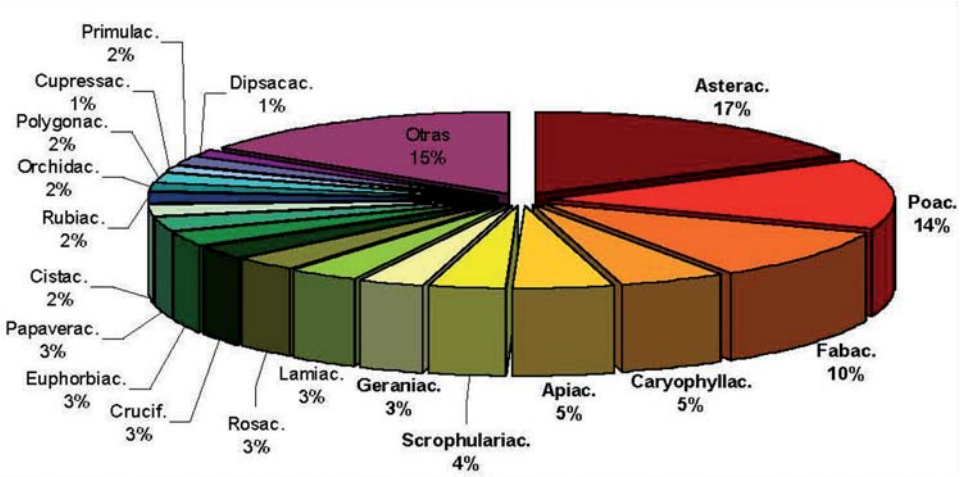


Fig. 2: Espectro taxonomico de la flora de los quemados.

Tabla 2: Espectro taxonómico de cada zona (%).

	Parcelas	Zona natural
Asteraceae	17,80	12,00
Poaceae	15,18	5,33
Fabaceae	9,95	10,67
Caryophyllaceae	5,76	4,00
Apiaceae	4,71	5,33
Geraniaceae	3,66	1,33
Scrophulariaceae	3,66	2,67
Euphorbiaceae	3,14	-
Rosaceae	3,14	2,67
Cruciferae	2,62	6,67

	Parcelas	Zona natural
Papaveraceae	2,62	-
Rubiaceae	2,62	4,00
Lamiaceae	2,09	9,33
Polygonaceae	2,09	-
Primulaceae	2,09	5,33
Cupressaceae	1,57	1,33
Cistaceae	0,52	4,00
Orchidaceae	0,52	4,00
Otras	16,23	21,33

Las especies más abundantes, como *Andryala integrifolia, Convolvulus arvensis, Medicago lupulina, Senecio vulgaris, Calendula arvensis, Sinapis arvensis* subsp. *arvensis, Anagallis arvensis, Desmazeria rigida* subsp. *rigida, Picris echioides, Mercurialis huetii, Quercus ilex* subsp. *ballota* y *Petrorhagia prolifera*, son especies comunes en la vegetación natural de la zona, sin embargo su presencia podría ser indicadora de zonas adecuadas para el establecimiento de la trufa en Navarra. *Desmazeria rigida* subsp. *rigida* ha sido citada anteriormente por otros autores como una de las especies más frecuentes, lo que podría indicar que es característica de los quemados (Reyna, 2007).

Llama la atención el hecho de que las dos especies más frecuentes, *Andryala integrifolia* y *Convolvulus arvensis*, aparecen en todas las plantaciones inventariadas; sin embargo, no se han encontrado nunca en las zonas de producción silvestre. Por otro lado, *Convolvulus arvensis* es una especie que, a pesar de ser muy abundante, ve alterado su ciclo vital cuando se desarrolla en el interior del quemado, como se verá más adelante.

En cuanto al espectro biológico (Fig. 3), el 54% de las especies son terófitos; es decir, especies anuales adaptadas a ambientes abiertos, productoras de bancos de semillas abundantes, que

completan su ciclo vital rápidamente y pasan la estación desfavorable en forma de semilla. Este dato corrobora el hecho de que el quemado es un ambiente alterado, tanto por el micelio del hongo como por las técnicas culturales que los truficultores llevan a cabo para mejorar la producción. Los terófitos adaptan su ciclo vital al de la trufa, evitando el periodo en el que el micelio es más agresivo (Martegoute, 2002).

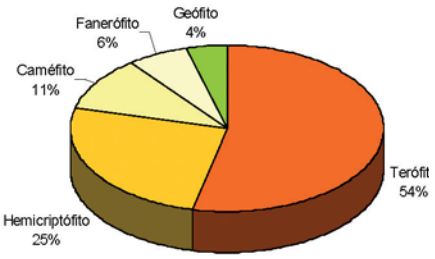


Tabla 3: Espectro biológico de cada zona (%).

	Parcelas	Zona natural
Teróf.	58,73	32,35
Hemicriptóf.	25,40	22,06
Caméf.	6,35	27,94
Faneróf.	6,88	10,29
Geóf.	2,65	7,35

Fig. 3: Espectro biológico de los quemados.

Por otro lado, un 46% son especies vivaces, sin embargo se trata en su mayoría de especies que no se desarrollan adecuadamente. En las zonas de producción natural, menos antropizadas (Tabla 3), el número de especies vivaces aumenta considerablemente (68%), especialmente los caméfitos que no conseguían implantarse adecuadamente en las plantaciones.
En cuanto al espectro corológico (Fig. 4), la mayor parte de la flora inventariada se corresponde con taxones mediterráneos y eurosiberianos. En las plantaciones (Tabla 4) un alto porcentaje de especies mediterráneas son sustituidas por especies subcosmopolitas o plurirregionales, especies de amplia distribución, que se instalan con facilidad en los ambientes degradados, en este caso en las plantaciones, sustituyendo a las especies autóctonas.

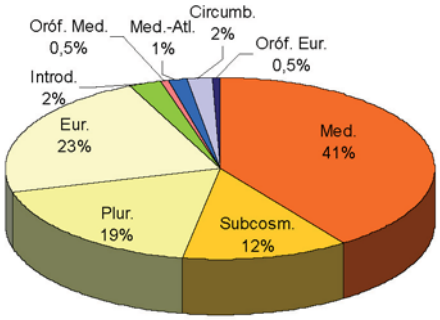


Fig. 4: Espectro corológico de la flora en los quemados.

Tabla 4: Espectro corológico de cada zona (%)

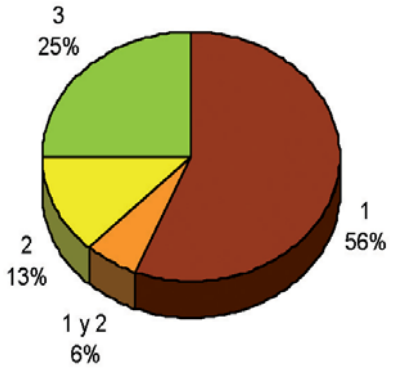
	Parcelas	Zona natural
Med.	35,75	55,93
Subcosm.	13,97	10,17
Plur.	20,11	13,56
Eur.	23,46	11,86
Introd.	2,79	0,00
Oróf. Med.	0,00	1,69
Med.-Atl.	1,68	0,00
Circumb.	1,68	5,08
Oróf. Eur.	0,56	1,69

En cuanto al substrato preferente (Tabla 5), dominan las especies indiferentes o que se desarrollan preferiblemente sobre suelos calizos.

Tabla 5: Substratos preferidos por la flora vascular de los quemados.

Substrato	**%**
calizo	7,7
calizo (silíceo)	0
silíceo (calizo)	0,5
silíceo	0
Indiferente	91,8

En relación al hábitat (Fig. 5), abundan las especies xerófilas, propias de cunetas, lugares alterados y removidos, así como las plantas ruderales y arvenses (un 56% del total), lo que refleja el ecosistema tan especial de las truferas cultivadas. Si a esto le sumamos las especies típicas de pastos y matorrales secos, soleados y pedregosos, estos dos tipos de hábitats suman un 75% del total, lo que corrobora el hecho de que las zonas propicias para la producción de trufa son lugares generalmente soleados, secos y pedregosos. Estos resultados concuerdan con los obtenidos por Reyna (1992).



1. Plantas ruderales y arvenses. propias de barbechos y baldíos. Lugares alterados, removidos y nitrificados.
2. Pastos y matorrales secos, luminosos y pedregosos.
3. Otros hábitats.

Fig. 5: Hábitats preferidos por la flora de los quemados.

En cuanto al efecto alelopático que ejerce la trufa sobre la flora vascular, éste se puede apreciar en 2 aspectos diferentes:

- En primer lugar, muchas de las especies leñosas que consiguen germinar en el interior del quemado no consiguen florecer ni fructificar, por lo que no completan su ciclo vital. Este es el caso de especies como *Quercus ilex ballota, Rhamnus alaternus, Juniperus communis, J. oxycedrus, Corylus avellana, Buxus sempervirens, Hedera helix, Cornus sanguinea* subsp. *sanguinea*, *Rosa* sp. y *Rubus* sp. Este hecho es especialmente destacable en los quemados de las parcelas, ya que en las zonas de producción silvestre, sí consiguen desarrollarse algunos arbustos (caméfitos en Tabla 3).
- Por otro lado, muchas de las especies anuales que sí consiguen completar el ciclo, presentan un desarrollo mucho menor, es decir, no alcanzan el tamaño que sería normal para la especie. Éste es el caso de especies como *Geranium molle, Petrorhagia prolifera, Calendula arvensis, Helianthemum sp., Veronica sp., Anthemis arvensis, Anagallis foemina, Lamium purpureum* y *Sinapis arvensis*. Este fenómeno de enanismo fue observado también por Martegoute, 2002 y Rosell, 1997.

El estudio e interpretación de la flora vascular y su morfología demuestran y corroboran el efecto alelopático causado por la trufa sobre las plantas del quemado, observado también por otros autores (Plattner y Hall, 1995; Olivier *et al*., 2002).

Micobiota ectomicorrícica

En cuanto a los resultados del estudio de los hongos ectomicorrícicos, se han identificado un total de 19 tipos micorrícicos pertenecientes a los géneros *Tuber, Scleroderma, Pisolithus, Hebeloma-Cortinarius, Hymenogaster, Genea, Sphaerosporella, Tomentella* y a los grupos Teleforoide y Boletal (Tabla 6). El resto de tipos reconocidos no han podido ser determinados y ascienden a 20.

Tabla 6: Morfotipos presentes en las parcelas.

	Num. de plantación										
Tipo	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Total**
T. melanosporum	3	2	3	0	1	7	1	3	1	6	27
T. brumale	2	5	0	2	3	0	3	0	4	6	25
T. aestivum.	2	2	6	5	1	0	0	7	2	1	26
T. mesentericum	3	4	1	0	0	0	0	0	0	1	9
T. rufum	0	0	0	0	0	0	2	0	0	0	2
Tuber	4	3	1	0	1	3	1	3	1	1	18
Tomentella.	4	10	10	7	1	3	5	0	4	4	48
Q. squamosa	0	0	3	1	0	0	2	0	0	4	10
Q. cumulosa	0	0	2	1	0	1	0	0	0	0	4
SB	0	0	0	1	0	0	0	0	0	0	1
T39	0	0	0	0	1	0	0	0	0	1	2
AD	1	3	13	5	5	11	11	9	3	4	65
Scleroderma.	0	0	3	0	4	13	3	0	1	15	39
Hebeloma/ *Cortinarius*	0	0	3	0	1	1	3	1	10	0	19
Pisolithus	0	0	4	1	0	1	1	0	0	1	8
Sphaerosporella.	0	0	0	1	0	0	0	0	0	0	1
Hymemenosgaster	3	2	2	0	1	0	0	0	0	0	8
Boletal	0	0	1	0	0	0	0	0	0	0	1
Genea	0	2	0	0	0	0	0	0	0	0	2
No identificados	5	3	3	5	0	5	5	1	4	2	33

De entre los morfotipos identificados destacan las especies del género *Tuber*, 5 en total (tabla 6). Además de *Tuber melanosporum*, en las parcelas se han encontrado también otras especies de trufas, entre las que destacan *T. brumale, T. aestivum* y *T. mesentericum* (Fig. 6).

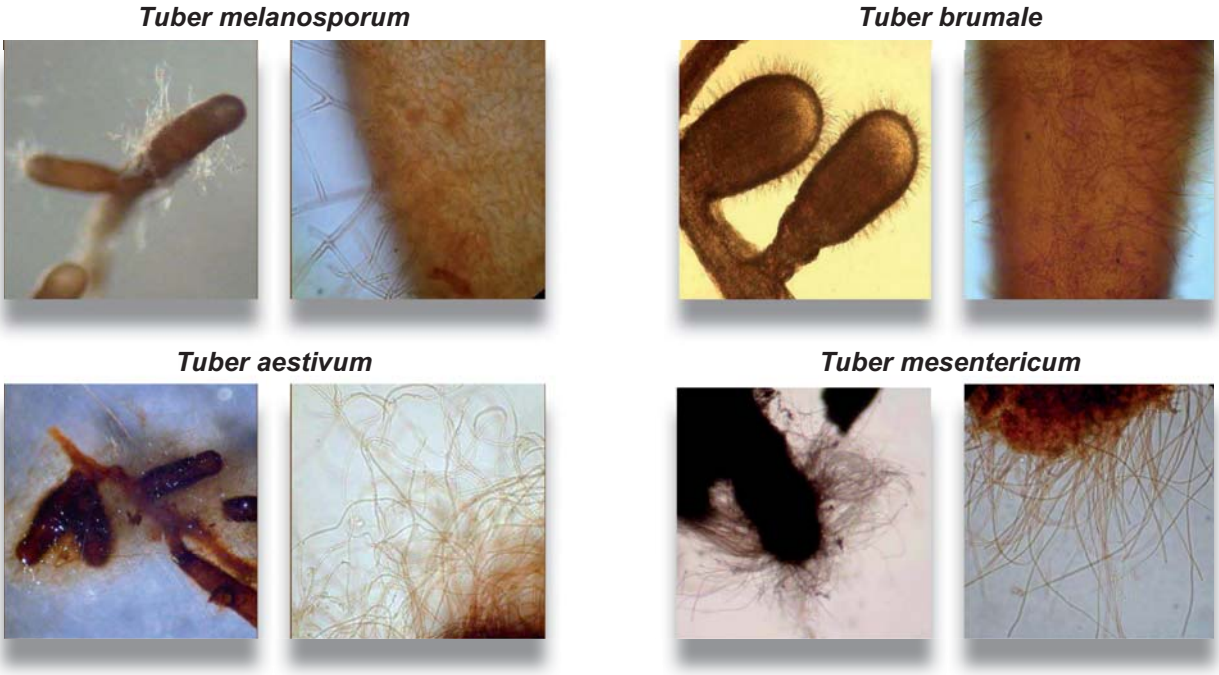


Fig. 6: Imágenes de las distintas especies de trufas presentes en las parcelas.

AD (“Angle droit” Giraud, 1988) y *Scleroderma* son también morfotipos muy frecuentes (Fig. 7). El morfotipo AD es competidor de *T. melanosporum*. Produce quemados creando falsas expectativas (De Miguel y Sáez, 2005). Recientemente, esta micorriza ha sido identificada como un Ascomicete (Baciarelli Falini *et al*., 2006) y confirmada y descrita como *Quercirhiza quadratum* (Águeda *et al*., 2008 a y b). Por otro lado, el género *Scleroderma*, en algunas parcelas, llega a aparecer en 5 de los 6 árboles, 4 de los cuales presentan también *T. melanosporum*. Este resultado concuerda con la experiencia de campo de que pese al desarrollo de *Scleroderma* en muchos de los árboles, se recogen trufas.
La diversidad por plantación no suele ser muy alta. En una de las plantaciones se han llegado a encontrar 15 morfotipos diferentes, pero se trata de una plantación no productiva que ha sido invadida por morfotipos procedentes del entorno natural.

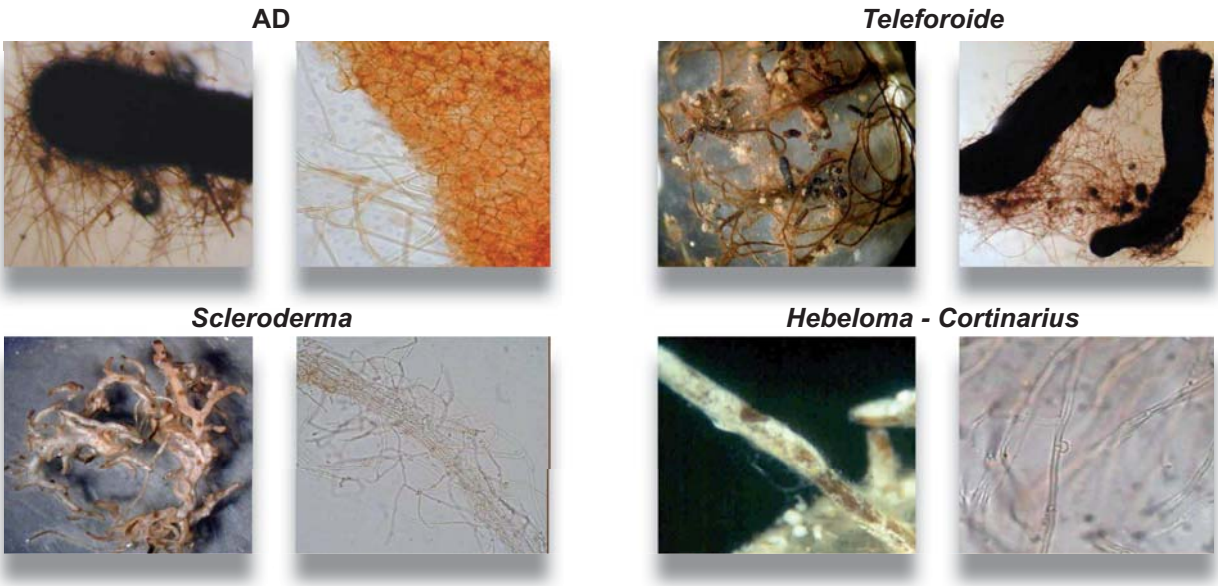


Fig. 7: Otras micorrizas abundantes en las parcelas.

En cuanto a la presencia de la micorriza de la trufa negra (Tabla 7), ésta se ha encontrado en 9 de las 10 plantaciones y en 22 de los 60 árboles estudiados, lo que concuerda con el hecho de que a la hora de la entrada en producción de una plantación, son relativamente pocos los árboles productores, pero a pesar de la baja frecuencia de las micorrizas de trufa negra en muchos de los árboles abarcados en el estudio, en las plantaciones se recoge trufa.

Tabla 7: Presencia de las distintas especies de trufas en cada parcela.

Parcela	*T. melanosporum*	Frecuencia absoluta	*T. brumale*	Frecuencia absoluta	*T. aestivum*	Frecuencia absoluta
1	2	0,33	2	0,33	2	0,33
2	2	0,33	4	0,67	1	0,17
3	3	0,50	0	0,00	3	0,50
4	0	0,00	1	0,17	3	0,50
5	1	0,17	3	0,50	1	0,17
6	4	0,67	0	0,00	0	0,00
7	1	0,17	3	0,50	0	0,00
8	4	0,67	0	0,00	4	0,67
9	1	0,17	3	0,50	1	0,17
10	4	0,67	5	0,83	1	0,17
Árboles totales	22	0,37	21	0,35	16	0,27

Si agrupamos los morfotipos en: género *Tuber*, Teleforoides, AD, *Scleroderma*, otros tipos identificados y tipos no identificados (Fig. 8), se observa la gran representación del morfotipo AD y morfotipos teleforoides en todas las plantaciones, que en conjunto superan a los morfotipos del género *Tuber*. Esta gran abundancia de teleforoides es normal en los bosques de encina de esta región (De Miguel y Sáez, 2005). El género *Tomentella* incluye numerosas especies muy frecuentes en Navarra, especialmente en bosques naturales (De Román, 2003). Además, los hongos ectomicorrícicos con esporocarpos inconspicuos, como *Tomentella*, suelen ser dominantes en las comunidades ECM de bosques boreales (Baier *et al*., 2006).

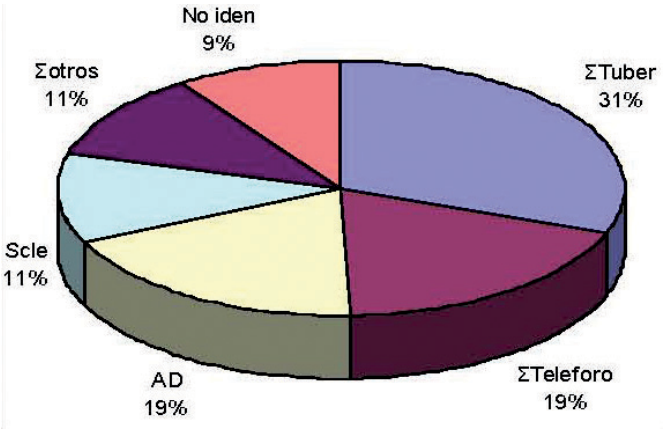


Fig. 8: Porcentaje de los distintos grupos micorrícicos.

Por último, destaca el hecho de que las especies que van colonizando los árboles de las plantaciones son en general, especies del entorno, con redes de hifas amplias, como el morfotipo AD, o micorrizas con rizomorfos potentes, como *Quercirhiza squamosa*, otras especies Teleforoides o *Scleroderma*, que hacen que sean grandes competidoras. Las características de los elementos que emanan son clave en la exploración del suelo, ya que conllevan una importante extensión del sistema radical, permitiendo una mejor dispersión y colonización (Agerer, 2001). Estas micorrizas son inevitables y compiten con la trufa negra, pero no llegan a desplazarla.
Generalmente tienen un amplio rango de adaptabilidad a las diferentes condiciones ecológicas o requerimientos ecológicos similares a los de las trufas cultivadas (Iotti *et al*., 2005).

Conclusiones

Flora vascular:

- Los resultados obtenidos ponen en evidencia que las plantas que crecen en el interior del quemado se ven muy influenciadas por las exudaciones de los hongos micorrícicos que crecen en el mismo, de forma que se ve alterada tanto la diversidad como las características fenológicas de la flora vascular, llegando en algunos casos a interrumpir su ciclo vital.

Micobiota micorrícica:

- El morfotipo AD y los tipos teleforoides son muy abundantes en las plantaciones. Si sumamos la frecuencia de aparición del morfotipo AD y de los tipos teleforoides, resultan los más frecuentes superando incluso a las especies de *Tuber*
- Muchas de las nuevas especies competidoras que se instalan en las parcelas presentan redes de hifas amplias y rizomorfos potentes, que facilitan su expansión en el sistema radicular del hospedador.
- Las micorrizas competidoras son tan inevitables como esenciales para alcanzar un equilibrio dinámico que permite el correcto funcionamiento de los ecosistemas truferos.

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TUBER ECTOMYCORRHIZAE ON PECAN TREES (*CARYA ILLINOINENSIS*; *JUGLANDACEAE*)

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Abstract
Juglandaceae is an ectomycorrhizal plant lineage that includes important nut trees such as *Carya illinoinensis*, the pecan tree. Abundant fruiting of the ectomycorrhizal truffle species *Tuber lyonii* was first noted in pecan orchards in the 1980's, but over the past decade truffle production appears to have declined. To assess whether ectomycorrhizae of *T. lyonii* are still present, a survey of the ectomycorrhizal communities across five pecan orchards was conducted. In addition to finding ectomycorrhizae of *T. lyonii*, ectomycorrhizae from three other *Tuber* species was detected. Phylogenetic analyses place these three novel *Tuber* species within the Maculatum clade.

Introduction
Pecan trees (*Carya illinoinensis*) are an economically important nut tree in the southeastern US. The state of Georgia is one of the top pecan producing states in the USA and has over 57,000 hectares under pecan cultivation (www.cpes.peachnet.edu/ugapecan/). In the mid-1980's, edible truffles in the genus *Tuber* were found fruiting abundantly in commercial pecan orchards (Fig. 1) in southern Georgia, USA (Hanlin *et al.* 1989).

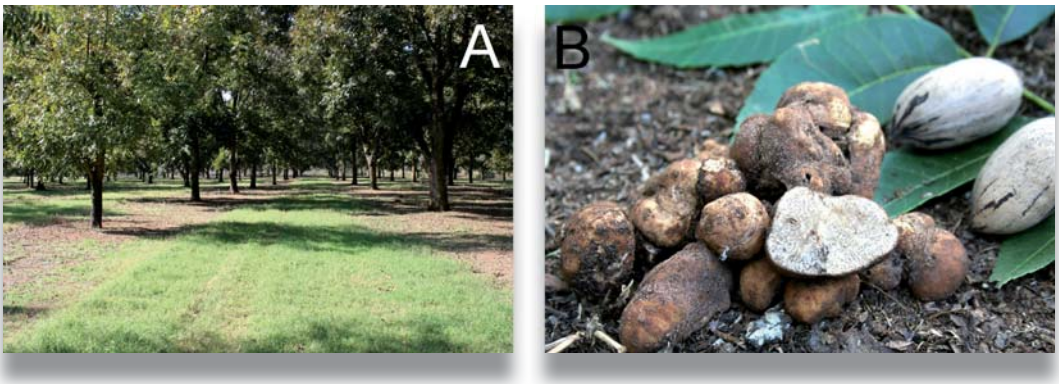

Fig. 1 Characteristic pecan orchard where truffles are harvested. A) Many of the pecan orchards where truffles are found have maintained herbicide strips in tree rows with grass strips in-between. B) *Tuber lyonii* fruitbodies shown with leaf and fruits of its host, *Carya illinoinensis*. The fungus and host appear to have a similar phenology.

The truffle species was identified as *Tuber texense*, a spiney-spored species native to Eastern North America that was later synonymized with *T. lyonii* (Trappe *et al*. 1996). Although not as pungent in aroma as prized European *Tuber* species, a local commercial market developed around this species which sold for between $200-$400 per kg.
In the past decade, it appears that truffle fruiting at formerly productive sites has declined, even at sites where truffles have been found but not actively harvested. The cause of this decline has not been explained, but is believed to have coincided with changes in the management of pecan orchards in the region that included rejuvenation of orchards by significant pruning and thinning.
To determine whether *T. lyonii* mycorrhizae have persisted in orchards where this truffle species was regularly found, root samples collected from pecan orchards were analyzed visually and with molecular tools. Among the 48 phylotypes detected were 4 species of *Tuber*, including *T. lyonii*. The placement of these species in the *Tuber* phylogeny is presented here.

Methods

Site and soil characteristics
Five sites were chosen for this study. One site has a 20-year history of consistent production of the truffle *T. lyonii* in the past (Magnolia+). An adjoining site where truffles had not previously been collected was also sampled (Magnolia-). Two other sites having truffle production in years past, but far less production in recent years were also sampled (Nilo+ and Pine Knoll+). The fifth site sampled was the UGA Tifton Experimental Station (Ponder-) and there is no record of truffle production at this orchard.
Management records were compiled from each orchard including their date of establishment, area planted, varieties planted, spacing between trees, irrigation method, and pesticide applications, and soil applications. Soil classification, pH, and mineral elemental analyses were taken from a composite sample (20-30 cm deep) for each site.

Root sampling
A total of 50 pecan trees (10 trees per site) were randomly sampled across five sites. From each of these trees three soil/root samples were taken at approximately 1 meter from the tree bole at north, northeast, and northwest. Samples were taken using a 2.5 cm core to a 10 cm depth (NW & NE) and by an auger shovel (N). Samples were stored in polypropylene bags, transported on ice, and stored at 4 C° and were processed over the following 10 days. Root samples were soaked in tap water for 1 hour before placing them in a 1 mm sieve and washing them under a gentle stream of tap water. Sections of cleaned mycorrhizal roots were then placed in a Petri dish with tapwater and viewed with a stereoscope. Single roottips were placed in eppendorf tubes containing 250 µl of CTAB 2x extraction buffer for DNA extraction.

Molecular analyses
DNA from one of six mycorrhizae samples taken from each core was randomly selected and extracted with 24:1 chloroform: isoamyl alcohol and PCR amplified using the primer set ITS1 and LR5 (Vilgalys and Hester 1990; White *et al.* 1990). The PCR protocol began with an intial denaturation at 94 C (3 min), followed by 35 cycles at 94 C (2 min), 50 C annealing (30 sec), and a 72 C extension (1.5 min), with a final extension at 72 C (7 min). Each 25 µl PCR reaction consisted of 4.5 µl ddH20, 4 µl dNTPs (1.25 uM), 2.5 µl PCR buffer, 1 µl BSA, 1.25 µl forward primer (10 uM), 1.25 reverse primer (10 uM), and 0.15 µl TAQ polymerase (5 U/µl), and 10 µl of DNA extract (~10 ng / µl). Two µl of each PCR product was loaded into a 1% agarose gel buffered with TAE buffer and stained with 2 µl SYBR safe (Invitrogen, Carlsbad, CA) per 80 ml gel. Gel electrophoresis products were viewed on a GelDoc XR imager (BioRad Laboratories, Inc., Hercules CA). Qiagen quick-clean columns were used to clean PCR products. One µl of cleaned PCR product was used in the sequencing reaction. Sanger sequencing was performed in both directions using BigDye Chemistry version 3.1 (Applied Biosystems, Foster City, CA) with the forward primer (ITS1) and reverse primer (LR5). DNA sequences were determined on an ABI3700 DNA sequence analyzer (Applied Biosystems, Foster City, CA).
DNA sequences were manually edited using Seqencher 4.0 (Gene Codes, Ann Arbor, MI) and ambiguous regions at the ends were trimmed. Both the ITS and LSU sequences were queried against the NCBI public database Genbank using the blastn algorithm. In order to better identify sequences that blasted to *Tuber*, these sequences were aligned with other *Tuber* species with the software MacClade 4.0 (Maddison and Maddison 2002). Ambiguously aligned regions were excluded from the alignment. Parsimony and maximum likelihood analyses using a 6 parameter model were used to determine phylogenetic affiliations of the recovered *Tuber* sequences with PAUP* 4.0b10 (Swofford 2002). Species designations or phylotypes are defined as sequences sharing 99% similarity at the LSU and 96% similarity at the ITS.

Results
A total of 137 ectomycorrhizae were sequenced from 50 trees across 5 orchards. Forty-seven

phylotypes were detected, and included four species of *Tuber*: *T. lyonii* and three other species in Maculatum group (Fig 2).

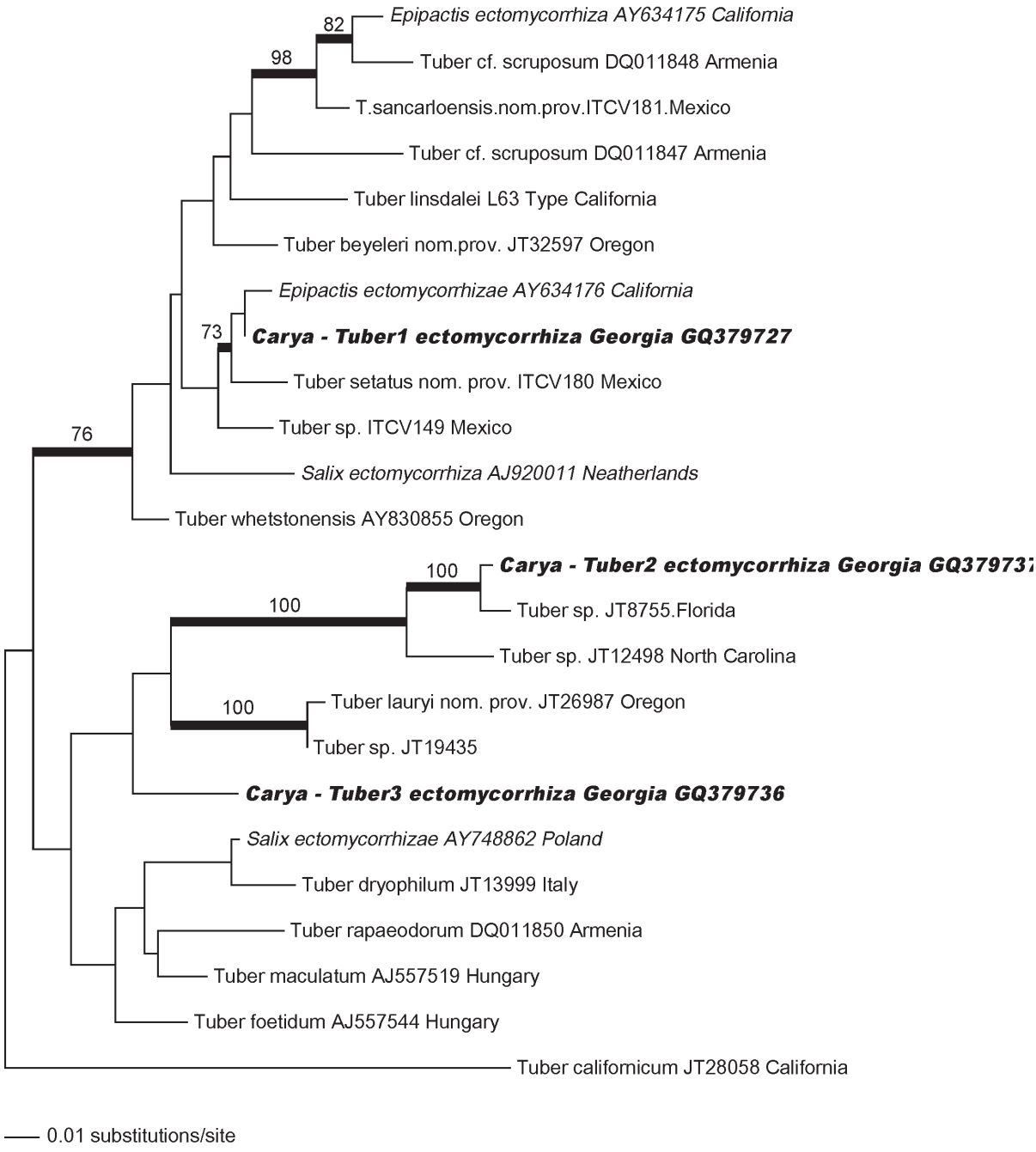

Fig. 2 Most likely tree of the Maculatum clade based on a general time reversible model of nucleotide substitution. Names of taxa are followed by collection or Genbank number and geographic origin (when known). Sequences from ectomycorrhiza are italicized and labeled with the host plant. Sequences produced in this study are in bold. There were 411 characters included in the analyses; 256 were constant; 58 were variable but parsimony-uninformative; and 97 were parsimony-informative. Parsimony and likelihood analyses were congruent, so only the likilihood tree is shown.

No polymorphisms were apparent in the chromatographs of *Tuber* ectomycorrhizae, and for two of the species ITS haplotypes could be distinguished. The most abundant phylotype belonged to an undescribed *Tuber* species (*Carya-Tuber*1). This species was detected 26 times (19%) on 15 trees and at 3 orchards. Nine haplotypes of this species were detected. Nilo+ had the highest haplotype diversity (5), four of which were unique to this site. Only one

of the 9 haplotypes was found in more than a single site. Two haplotypes were detected on a single host tree in 5 of the 7 times that *Carya-Tuber*1 was found in multiple cores from around the same tree. Some of the ectomycorrhizae from *Carya-Tuber*1 were ornamented by short and straight cystidia (Fig 3).

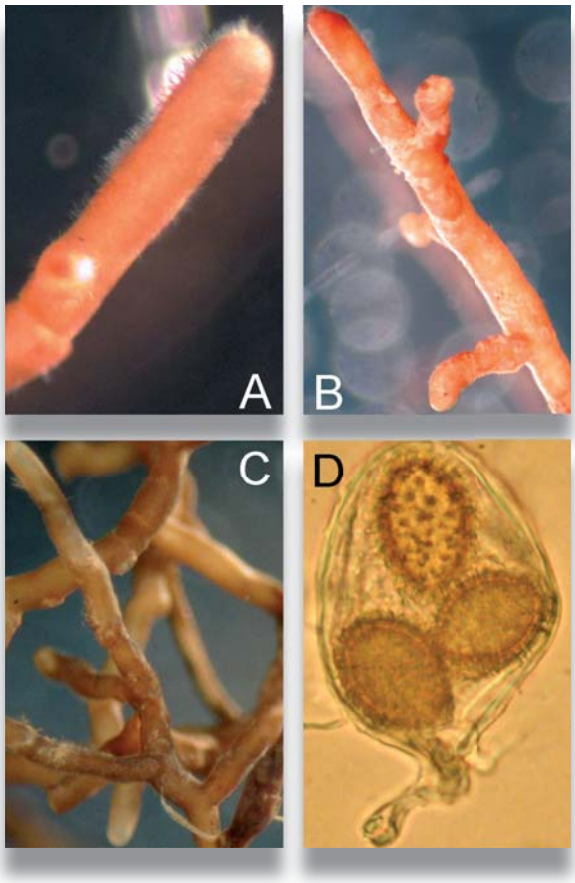

Fig. 3 Characteristics of *Tuber* species associated with *Carya*. Ectomycorrhizae of *Tuber*1 (A), *Carya-Tuber*2 (B), and *Tuber lyonii* (C) on *Carya illinoinensis*. A stemmed ascus with spiney-spores characteristic of *Tuber lyonii* (D).

Tuber lyonii was the second most common phylotype and was detected 23 (17%) times, on 15 trees and at 3 orchards. Six haplotypes were detected for *T. lyonii*, two of which were present at multiple sites. Magnolia+ had the highest haplotype diversity (4). Two haplotypes were detected on a single host tree in 3 of the 4 times that *T. lyonii* was found in multiple cores from around the same tree. Ectomycorrhizae of *Tuber lyonii* on *Carya illinoinensis* appear to have a mantel that is light to dark tan in color (Fig 3).
Sequences from ectomycorrhizae belonging to the two other *Tuber* species (*Carya-Tuber*2 & *Carya-Tuber*3) place these species within the Maculatum clade (Fig 2). *Carya-Tuber*2 ectomycorrhizae were found at the Magnolia+ and Magnolia- pecan orchards, while *Carya-Tuber*3 was only recovered once at Nilo+. These both appear to be novel species. Some of the ectomycorrhizae from *Carya-Tuber*2 were ornamented by short and straight cystidia (Fig 3).

Discussion
Tuber lyonii is a known associate of pecan trees, and was well represented in the analyzed root samples from Magnolia+, Magnolia-, and Pine Knoll+ orchards. Although fruiting of *T. lyonii* has declined (or not been observed in the case of Magnolia-) this species maintains a strong presence in the belowground community. The Magnolia+ orchard had the highest haplotype diversity. The fact that multiple haplotypes were recovered on single trees and across orchards suggests this species is recombining and that the genet size may be small. This species has

likely spread into these orchards from surrounding forests.
Three other species of *Tuber* were found as ectomycorrhizal associates of the pecan tree *Carya illinoinensis*. Sequence analysis places them within the Maculatum clade. Although these species are only known from ectomycorrhizae, their fruitbodies are likely to be small in size, pale in color, with oval-shaped, aveolate-reticulated spores, similar to other species in Maculatum clade (Badalyan *et al.* 2005). These three *Tuber* species appear to be novel phylotypes.
*Carya-Tuber*1 was the most abundant phylotype recovered in this study. Although nine haplotypes were identified only 1 of these was found in more than 1 orchard, suggesting that its population is structured at small geographic scales. The most similar sequences to *Carya-Tuber*1 are from an ectomycorrhizae of *Epipactis* from California, and from a small white truffle from Mexico, *Tuber setatus* nom. prov., known from *Quercus* forests (Gonzalo Guevara *personal communication*).
*Carya-Tuber*2 is most similar to a collection of unidentified truffles found in a *Nyssa* and *Quercus* dominated plant community in Florida. *Carya-Tuber*3 does not cluster tightly with any other fruitbodies we have sampled or sequences on Genbank. Unidentified environmental sequences of *Tuber* in Genbank that fall within the Maculatum group are from *Salix* or the orchid *Epipactis* (Fig 2). In general, species in the Maculatum group appear to be associated with broad-leaf trees and orchids, rather than with conifers as is more common of species in the Borchii group.
In summary, four species of *Tuber* were found associated with *Carya illinoinensis*. Three of these species are novel phylotypes in the Maculatum group and are known only from ectomycorrhizae, however *Carya-Tuber*1 and *Carya-Tuber*2 are closely related to existing fruitbody collections. It seems that *Tuber lyonii* is a major component of the belowground ectomycorrhizal community at the Magnolia+, Magnolia-, and Pine Knoll orchards. The populations of both *T. lyonii* and *Carya-Tuber*1 appear to be structured at a fine-scale.

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HYPOGEOUS FUNGI IN THE ANTHROPOGENIC SITES IN POLAND

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Abstract
Ecology of hypogeous fungi is a matter of increasing interest to professional mycologists, amatours and producers of edible fungi. Some examples of forest study on hypogeous fungi are given in the paper. It is significant that two species of edible truffles (*Tuber aestivum* and *Tuber mesentericum*) we found in anthropogenic sites. It is indicated that *small-scale disturbance*, like wild boar rooting or limited digging to some extend can stimulate occurrence and development of hypogeous fruitbodies. *Large-scale disturbance*, like extensive excavations, can result in loosing the conditions suitable for one group of hypogeous species but can create the habitats suitable for other species. Urban areas sometimes provide good conditions for hypogeous fungi and can be used as experimental sites.
Key words: truffles, false truffles, ecology, tradition.

Introduction

Hypogeous fungi and especially truffles, have been of increasing interest in Poland in the recent years from the mycological, cultural and economical point of view.
Poland is situated rather far from the centre of edible truffle occurrence regions in the Mediterranean zone. In the Baltic region the most common among hypogeous fungi are species of the *Elaphomyces* (Ławrynowicz, 1989). More and more frequent and easy contacts of people from the North and South and the possibility for exchanging experiences result in new ideas and programs to widespread the truffle cultivation to the North of Europe (Chevalier, 2008).

Truffles tradition in Poland

In Poland truffles have been a subject of general interest since centuries owing to church, especially monasteries and scientific contacts with Mediterranean countries. In the vicinity of Cracow and in Polish Jura the agriculture and knowledge of forests were stimulated by the religious orders of Benedictins, Cistersians and Canonas of Holy Spirit (of Montpellier) coming from France. From Italy there came Regular Canons of St. Augustin and Franciscans, from Spain - Blackfriars (Dominicans), Carmeliteo and Jesuits. The Jagiellonian University in Cracow, founded in 1364, had tight scientific contacts with Universities in those countries - Sorbonne, Bologna, Alcalá de Henares and Salamanca. For more than two hundred years the French kitchen became famous and fashionable in Poland. The dishes with truffles prepared according to original recipe symbolized not only the culinary quality but also indicated high social prestige of the family. Till now the duck with truffles can be sometimes ordered in most excellent restaurants.
Where were the truffles coming from? In a small part they were imported from the South of Europe. They were also sold at the market e.g. in Warsaw, but in wide sense “truffles” includes also *Hydnotrya, Rhizopogon, Melanogaster* and *Scleroderma citrinum* called in Polish a truffle till now. In some regions *Choiromyces meandriformis*, called white truffle, is used in the kitchen, too.
Recently, a lot of questions arise on the possibility to cultivate edible truffles in Poland. In this short communication some examples of field observations during our hypogeous studies are presented to support the knowledge on the ecological requirements of different hypogeous fungi growing in natural and man made habitats.

Some examples of forest studies on hypogeous fungi

Long time scientific investigations of fungi were concentrated in the protected areas: national

parks and nature reserves. During joined mycological investigations in the nineties of the last century in the Białowieża National Park more than two thousand species of fungi were recorded, but only one hypogeous species *Elaphomyces asperulus* was found (Bujakiewicz *et al*. 1995). The structure of forest floor makes hypogeous observation very difficult because of great cumulation of woody debris. Also, digging in National Parks is not allowed. Only sometimes wild bears rooting for food can take out the carpophores of the soil. Also *Cordyceps* an epigeous parasite of *Elaphomyces,* indicates the presence of underground host.
The investigations on sampling plots were carried out not far from Białowieża National Park in the area penetrated by wild bears in the Knyszyńska Forest. The qualitative and quantitative investigations using the method of sampling plots were carried out on rooted and unrooted places (Fig. 1 a, b). In this area up to 16 carpophores of *Elaphomyces asperulus* were found in the investigated 1 m² rooted plots. On the other hand, they were absent in 80% of unrooted plots this means that wild boars activity can create better conditions for development of *Elaphomyces*. Moving the humus layer has a positive effect on the production of hypogeous fruit bodies, it means small-scale natural disturbance (Ławrynowicz *et al*. 2006).

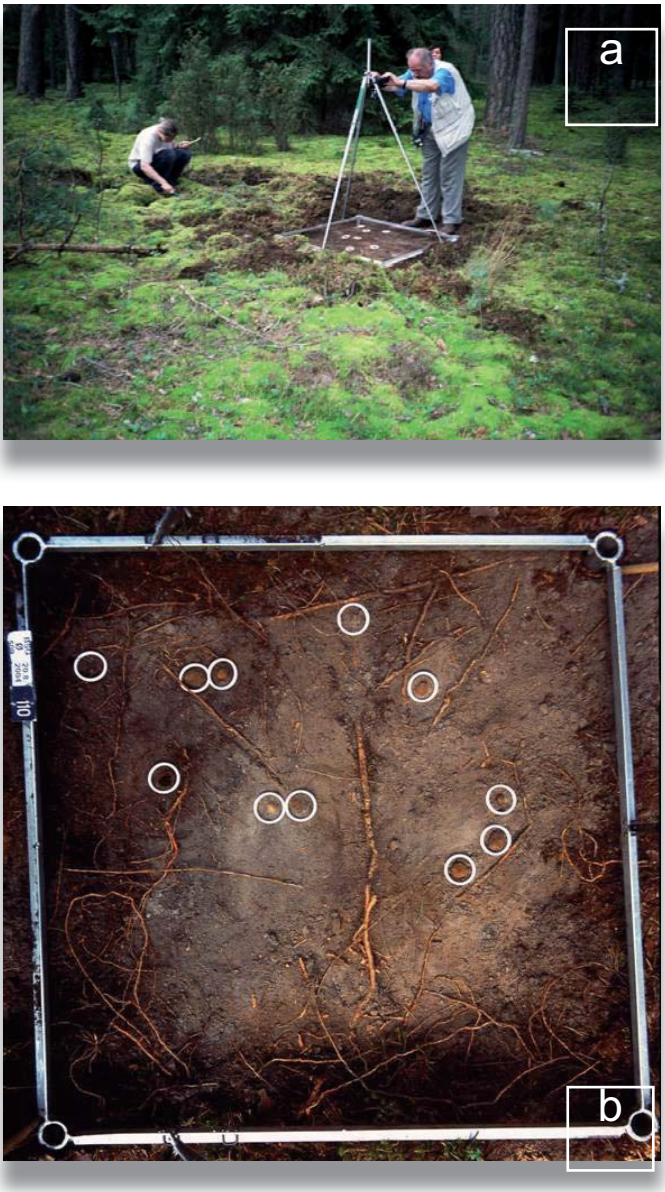

Fig. 1 Permanent plots for quantitative investigation of *Elaphomyces asperulus*
a - documentation of the plot
b - fruit bodies among the roots in soil

An example of a large-scale disturbance is an archeological natural reserve-Odry in Tuchola Forest. Originally, before disturbance, it was a pine forest, *Peucedano-Pinetum*. Under herb, moss and humus layers, a great number of *Elaphomyces asperulus* specimens were found. After archeological excavations mainly to the depth of 1 m on sandy soil another hypogeous fungus dominated namely, *Rhizopogon obtextus*.

Searching for truffles

The interest in truffles by Polish people it is possible to notice as early as the second part of nineteen century (Fig. 2).

Fig. 2 Truffle's hunter with dog.
acc. to Kossak, *Tygodnik Ilustrowany* 1865.

There are the papers by: Caspary (1886), Błoński (1888), Alexandrowicz & Błoński (1894) summaried the data concerning 8 species of truffles, among them one edible species, *T. aestivum* on 6 localities in different, even in Northern, parts of Poland (Lubelska 1953), but no exsiccates to verify determinitions of mentioned species.

Hypogeous fungi produce fruit bodies only in selected sites called hypogeous oasas or nests with suitable ecological conditions. Usually it is possible to find fruit bodies of several other species of hypogeous fungi.

In the seventies of the last century the contents of stomachs of rodents caught by zoologists in the area of the Ojców National Park was investigated. As result were discovered there spores of *Tuber aestivum*. This was the first signal leading to assumption that edible truffles could occurre in the country. It is remarkable, that the oases of black truffles were found in the area in forests intensively used by the people, sometimes along the roots, tourists trails and pathes tramped every day.

These signal, given by mice stimulated mycologists to concentrate their investigations on the jurassic area-of the Cracow-Częstochowa Upland. On August the 26-th, in 1981, it means 27 years ago *Tuber mesentericum* was found as confirmation of occurrence of black truffles in our country. The fruit bodies were found at the tourists trail with scanty vegetation cover.

16 years later, on July the 15-th the next locality was discovered at the distance of 6 km in an anthropogenic site. On the hill used in the past as a pasture the forest was planted 40 years ago: a part with oak *Quercus robur* and a part with birch *Betula pendula*. In thirty-two years old plantation the carpophores of *Tuber mesentericum* were collected. The locality still exists.

Sites of *Tuber mesentericum* are monitor every year and there are some years when no fruit bodies occur. It is remarkable in temperate climatic zone, that only in some years the meteorological conditions are suitable to formation of hypogeous fruit bodies.
The year 2007 was exceptional. In course of systematic digging of the vicinity of *T. mesentericum* sites, two localities of *T. aestivum* were discovered. Paralelly searching by amatours, in some cases with dog's help, yielded of great numbers of carpophores of black truffles in South of Poland. As result of taxonomical analyses it was identified: *Tuber aestivum* (6 localities), *T. aestivum* forma *uncinatum* (3 localities), *Tuber mesentericum* (4 localities) and *Tuber bellonae* (3 localities) (Ławrynowicz *et al.* 2008).
There are also some data from further collections by mycologists and amatours with dogs, as well as investigators from Forestry Institute (Hilszczańska *et al*. 2008; Ławrynowicz 2009).
Finely, the list of Polish species of *Tuber* including recent data is as following: *Tuber aestivum* Vittad., *T. aestivum* Vittad. forma *uncinatum* (Chatin) Montecchi et Borelli, *T. bellonae* Quél., *T. borchii* Vittad., *T. dryophilum* Vittad., *T. excavatum* Vittad., *T. ferrugineum* Vittad., *T. fulgens* Quél., *T. maculatum* Vittad., *T. mesentericum* Vittad., *T. puberulum* Berk. et Br., *T. rapaeodorum* Tul. and *T. rufum* Pico.
The locality of *T. rapaeodorum* is situated within the private garden, under *Corylus avellana*. It has been monitored for 30 years and protected by law as a nature monumental site.

False truffles in urban areas
Some species of hypogeous fungi are known from the occurence on urban areas. The investigation in Łódź - town (Central Poland) permited to observe production of *Melanogaster broomeianus* in great quantities it is easy to see the carpophores without digging in the University campus. On the plots of 10 m² even as many as 50-100 fruit bodies of *M. broomeianus* were collected. This species seems to prefere anthropogenic sites. Because they produce a very pleasant small, some people collect them as truffles.
In some years it is common in green belts along the streets, in city parks. Altrough the carphophores in the town areas are collected or simply damage, they continue to produce the fruit bodies by suitable weather conditions in the next seasons.
In the literature we can find other examples that human activity can stimulate the growth of hypogeous fungi. Hawker (1954) wrote: *Slight disturbance of soil may stimulate some species, presumably through improved aeration. Thus, large number of young fruit bodies of T. puberulum were found on returning to a patch which when searched, and therefore disturbed, some months earlier had yielded only few. Tuber aestivum was found in quantity in the grounds of the University of Bristol one year after the ground had been dug, after having been undisturbed for many years.*
Long term searching for hypogeous fungi in Poland support studies on biology and ecology and natural distribution of that fungi (Ławrynowicz 1988, 1989, 1990; Ławrynowicz *et al*. 2008).

Conclusions
It is remarkable that such famous sanctuarium of nature as Białowieżą National Park is not friendly for hypogeous fungi because of deep deposite of decaying wood.
Several species of hypogeous fungi develop quite well on anthropogenic sites. Consequent, detaliled observations and analysis of ecological conditions of hypogeous fungi fructification could support our knowledge on their life requirements. This knowledge can be used in truffle cultivation as well.
- Small-scale disturbance, like wild boar rooting or limited digging to some extent can stimulate occurrence and development of hypogeous fruitbodies.
- Large-scale disturbance, like extensive excavations, can result in loosing the conditions suitable for one group of hypogeous species but can create the habitats suitable for other species.
- Urban areas are diversified into many microhabitats. Sometimes they provide good conditions for hypogeous fungi and can be used as experimental sites.

- Most of hypogeous fungi are included in the European Red Lists and they deserve proper conservation activities.

Through occurate observations of hypogeous fungi in the natural and anthropogenic sites can help to chose proper methods of conservation and also cultivation of these fungi. Efforts towards the truffle cultivation have been undertaken recently in Poland in programme realized in Forestry Institute in cooperation with Italian colleagues (Hilszczańska *et al*. 2008).

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CARATTERIZZAZIONE ECOLOGICA DELLE TARTUFAIE NATURALI DI *TUBER MELANOSPORUM* VITTAD. E *TUBER MAGNATUM* PICO IN ABRUZZO (ITALIA CENTRALE)

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Abstract: Ecological characterization of natural sites with *Tuber melanosporum* Vittad. and *Tuber magnatum* Pico in the Abruzzo region (central Italy)

The results of a study carried out between 2006 and 2008 dealing with the ecological characterization of natural sites with *Tuber melanosporum* e *Tuber magnatum* are presented[1]. The study consisted of three different analysis: bioclimatic, pedological and vegetational.

The bioclimatic analysys highlights a relatively wide ecological value of the two species, which were found in several bioclimatic horizons. However, *T. melanosporum* appears to be mostly correlated with the Lower Supratemperate horizon, and *T. magnatum* with the Lower Mesoteperate horizon. The *Tuber magnatum* sites are characterized by more abundant summer precipitations and a shorter dry period. The pedological analysis shows a good separation between the two truffles sites. Texiture, organic matter and total carbonates resulted to be the best parameters to describe the different ecological requirements by the two species: *T. melanosporum* requires more sand, organic matter and total carbonates (and also scheleton); *T. magnatum* more silt and clay. The phytosociological analysis of vegetation highlights the clear cenological autonomy of the vegetations related to the two truffle species. Dry open *Quercus pubescens* associations referred to *Roso sempervirentis-Quercetum pubescentis* and *Cytiso sessilifolii-Quercetum pubescentis* associations are present in the sites of *T. melanosporum*. The vegetation of *T. magnatum* sites is highly heterogeneous, i.e. sub-mountain semi-mesophilous *Quercus pubescens* subsp. *pubescens* woods*, Ostrya carpinifolia* or *Quercus cerris* dominated mixed woods, *Corylus avellana, Pyrus pyraster, Populus tremula* or *Ulmus minor* subsp. *minor* pre-woods, igrophilous *Populus alba* or *Populus canadensis* woods. Its phytosociological placement is consequently various. In all cases it reguards fresh formations on deep soils, as underlined by the constant presence of mesophilous and meso-igrophilous species characterizing the *Erythronio dentis-canis-Carpinion betuli* alliance and the *Fagetalia sylvaticae* order; in several cases, also the igrophilous species of the *Salici purpureae-Populetea nigrae* class were found to be abundant.

Key words: *Tuber melanosporum, Tuber magnatum,* Truffle, Bioclimate, Soil, Vegetation.

Introduzione

Nell'ambito della "Carta delle potenzialità tartuficole della Regione Abruzzo", al fine di meglio definire le aree di pertinenza delle due specie di tartufo pregiato (*Tuber magnatum, T. melanosporum*), è stata condotta la caratterizzazione ecologica delle tartufaie naturali censite dall'ARSSA. La costruzione della Carta può avvalersi così, oltre che dell'utilizzo di tematismi già in possesso dell'Ente o comunque reperibili nella letteratura di settore, quali la Carta Fitoclimatica d'Italia (Blasi & Michetti, 2005), la Carta dell Uso del Suolo (Regione Abruzzo, 2000), la Carta dei suoli della Regione Abruzzo (Chiuchiarelli *et al*., 2008), la Carta geologica dell'Abruzzo (Vezzani & Ghisetti, 1998), ecc., di analisi ambientali di maggior dettaglio condotte presso o nelle immediate vicinanze di tartufaie naturali accertate e georeferenziate.

1 *Research carried out within the "Carta della vocazionalità tartuficola della Regione Abruzzo" project, co-financied by Regione Abruzzo with the three-yearly 2001-2003 and 2004-2006 "Interventi di forestazione e valorizzazione ambientale" Programs and by ARSSA with the 2001-2002-2004-2005 Technic Assistance Programs.*

La ricerca, oltre ad approfondire le conoscenze sulle caratteristiche autoecologiche e sinecologiche delle due specie di tartufo nell'ambito della regione, rappresenta inoltre una base conoscitiva, certamente da approfondire ulteriormente, da utilizzare per la costruzione di modelli ecologici, funzionanti a scala regionale, cui fare riferimento nell'impianto di tartufaie coltivate sia relativamente alla specie da utilizzare, sia per ciò che concerne eventuali cure colturali da adottare.

Materiali e metodi

Sono stati elaborati i dati mensili di temperatura e precipitazioni, relativi al periodo 1967-1996, di 48 stazioni termo-pluviometriche, 26 per *T. magnatum* e 22 per *T. melanosporum*, individuate nelle vicinanze delle tartufaie naturali. Su tali dati sono stati calcolati diversi indici bioclimatici e sono stati confrontati i relativi risultati.

E' stata condotta, nel periodo primavera-estate 2007, l'analisi vegetazionale di un campione (27 unità) di tartufaie naturali di *Tuber melanosporum* e *T. magnatum*, attraverso il metodo fitosociologico classico della scuola sigmatista di Zurigo-Montpellier (Braun-Blanquet, 1964). La nomenclatura delle specie segue la Check-List della flora vascolare d'Italia (Conti *et al*., 2005), ad eccezione di *Pyrus pyraster*, per la quale si fa riferimento alla Flora d'Italia di Pignatti (1982).

Per la caratterizzazione pedologica sono stati analizzati i dati relativi a minipits di 50 cm. Essi sono stati "ripuliti", al fine di renderli confrontabili numericamente: sono state eliminate le variabili nominali (descrizione dei singoli strati, classi di tessitura, colore, ecc.) e le informazioni relative ai diversi strati di campionamento sono state "condensate" in una sola riga della matrice sostituendo i valori delle variabili dei diversi strati con valori medi, pesati sull'altezza degli strati stessi. Ai fini di ottenere una matrice di dati numerici utile per l'analisi multivariata, è stata calcolata la correlazione tra le diverse coppie di variabili ed è stato eliminato il campo "carbonati attivi" in quanto piuttosto ben correlato con "carbonati totali". Parimenti, riguardo alle quantità relative di sabbia, limo ed argilla, essendo espresse in percentuale, sono state ritenute sufficienti due sole variabili (la terza rappresenta il complemento a 100%). La scelta è avvenuta, anche in questo caso, attraverso l'analisi della correlazione. Sabbia e limo hanno mostrato la miglior correlazione e tra le due è stato scelto il limo in quanto meno correlato all'argilla rispetto alla sabbia.

L'intera matrice, di dimensioni 7 x 156, standardizzata attraverso la deviazione standard, è stata analizzata attraverso cluster analysis (distanza sulla corda come coefficiente di dissimilitudine tra gli oggetti e legame medio come strategia di clustering) e ordinamento di tipo PCA.

Risultati

<u>Analisi bioclimatica</u>

L'analisi ha evidenziato un'ampia valenza ecologica delle due specie ripetto al clima: entrambe le specie sono presenti, infatti, in diversi piani ed orizzonti bioclimatici individuati attraverso gli indici e la classificazione bioclimatica di Rivas-Martinez (1987, 1996, 2002). Tuttavia, *Tuber melanosporum* mostra una chiara preferenza per l'orizzonte Supratemperato inferiore mentre *T. magnatum* è diffuso prevalentemente nel Mesotemperato inferiore. Il valore medio dell'indice di termicità (It) di Rivas-Martinez è, infatti, decisamente più basso per le aree individuate per la prima specie (It = 166,3) rispetto a quelle di pertinenza della seconda (It = 231,6).

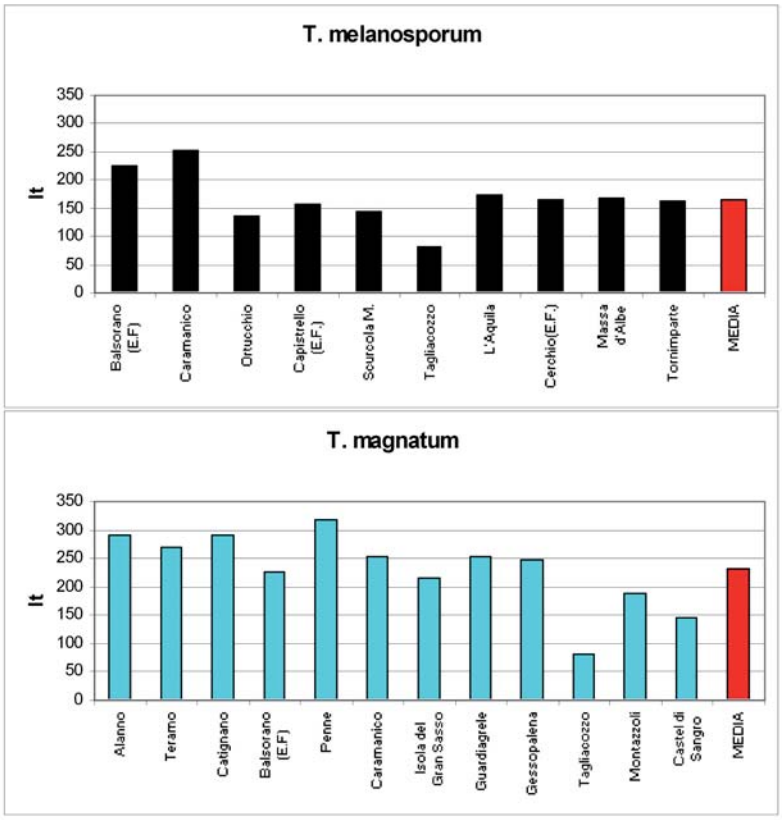

Fig. 1 Andamento, nelle stazioni considerate, dell'indice di termicità (It)

Anche l'applicazione dell'indice di stress da freddo mensile (MCS) di Mitrakos (1982) conferma la preferenza di *T. melanosporum* per stazioni caratterizzate da una maggior incidenza del freddo invernale (fig. 2).

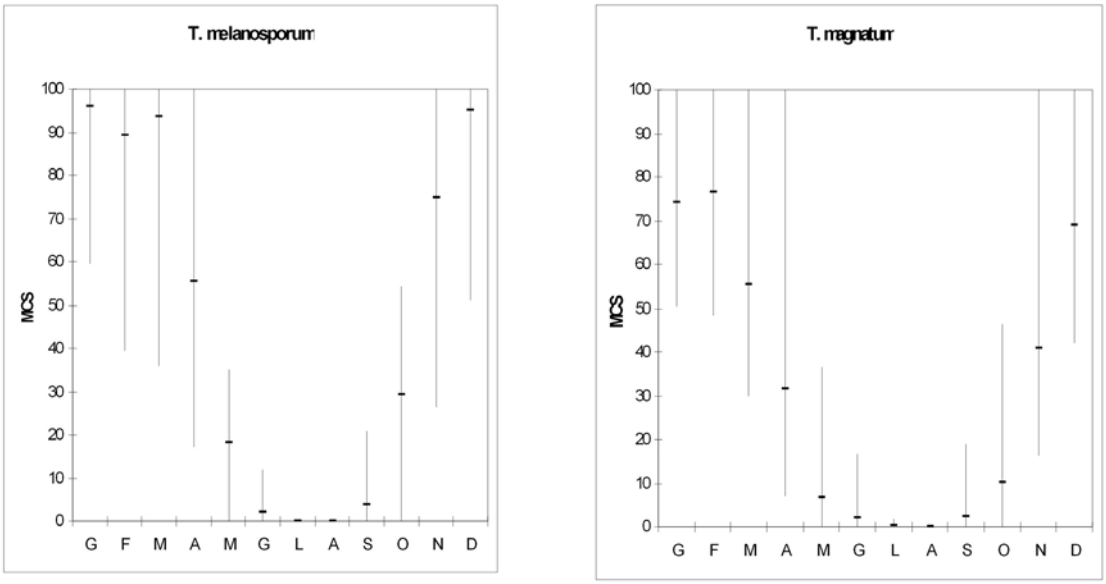

Fig. 2 Andamento, nelle stazioni considerate, dell'indice mensile di stress da freddo (MCS)

Per quanto riguarda l'andamento delle precipitazioni, le aree con presenza di *Tuber magnatum* risultano caratterizzate da precipitazioni estive più elevate rispetto a quelle di *T. melanosporum*. Ciò è messo bene in evidenza anche dai valori assunti dall'indice di stress da aridità mensile (MDS) di Mitrakos (1980) che raggiunge per la seconda specie valori medi ed estremi

decisamente più elevati (fig. 3). Nelle aree di pertinenza di *T. melanosporum* lo stress da aridità mensile è presente, inoltre, seppur con modesta intensità, per un numero di mesi più elevato, non ristretto al solo periodo estivo.

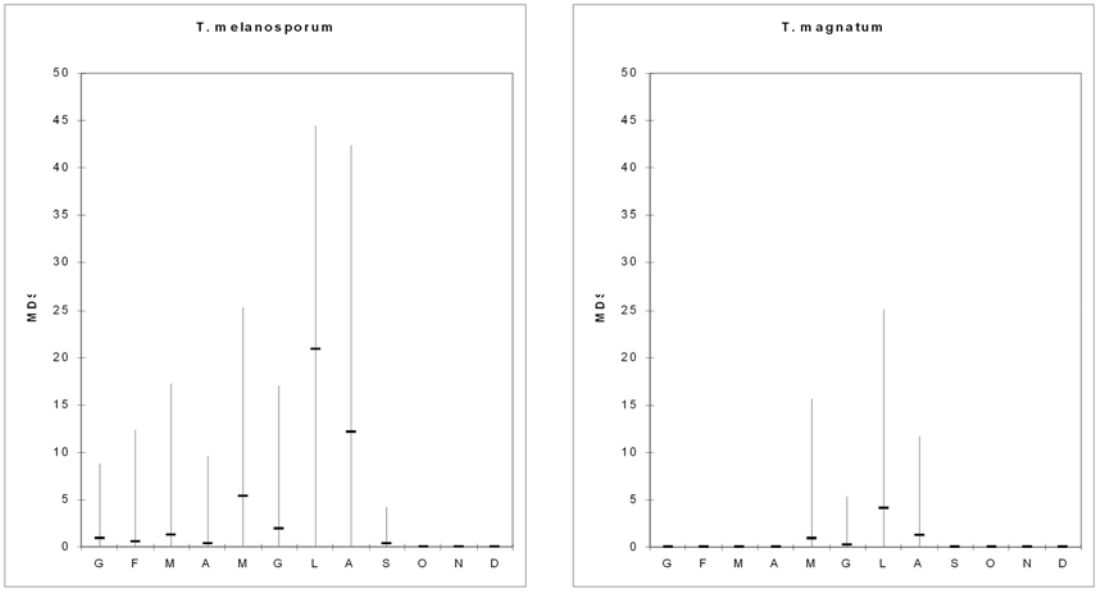

Fig. 3 Valori, nelle stazioni considerate, dell'indice mensile di stress da aridità (MDS).

Analisi vegetazionale

L'analisi dei rilievi fitosociologici ha evidenziato la netta autonomia delle formazioni vegetali sede di tartufaie di *Tuber melanosporum* rispetto a quelle di *T. magnatum*.

Relativamente alle tartufaie di *T. melanosporum,* si tratta di boscaglie più o meno aperte di roverella (*Quercus pubescens* subsp. *pubescens*), riferibili alle associazioni *Roso sempervirentis-Quercetum pubescentis* e *Cytiso sessilifolii-Quercetum pubescentis*, descritte rispettivamente per il M. Conero e per l'Appennino centrale (Biondi, 1986; Blasi *et al.*, 1982), in cui si osserva la presenza, più o meno abbondante, di elementi xerofili delle classi *Cisto-Micromerietea, Festuco-Brometea* e *Quercetea ilicis*.

Le tartufaie di *T. magnatum* si rivelano essere molto più eterogenee, sia per fisionomia, sia per composizione floristica. Il gruppo comprende, con riferimento alle specie dominanti e all'ecologia di massima, boschi di roverella (*Quercus pubescens* subsp. *pubescens*) submontani semi-mesofili, boschi misti a dominanza di carpino nero (*Ostrya carpinifolia*) o di cerro (*Quercus cerris*), boscaglie di pre-bosco a dominanza di nocciolo (*Corylus avellana*), perastro (*Pyrus pyraster*), pioppo tremolo (*Populus tremula*) o olmo campestre (*Ulmus minor* subsp. *minor*), formazioni igrofile a dominanza di pioppo bianco (*Populus alba*) o pioppo ibrido (*Populus canadensis*).

Si tratta comunque, in ogni caso, di formazioni fresche che si affermano su suoli più o meno profondi, come messo in evidenza dalla presenza di specie caratteristiche dell'alleanza *Erythronio dentis-canis-Carpinion betuli* e dell'ordine *Fagetalia sylvaticae*. Sempre molto ben rappresentate, in questi rilievi, le specie nemorali dell'ordine *Quercetalia pubescenti-petraeae* e della classe *Querco-Fagetea*. In numerosi rilievi si osserva, inoltre, una buona rappresentanza di specie caratteristiche delle formazioni ripariali della classe *Salici-Populetea nigrae*, che dimostrano una buona disponibilità idrica nel terreno.

I boschi semi-mesofili di roverella non trovano, al momento, un riferimento fitosociologico a livello di associazione ed anche una loro collocazione nella suballeanza *Laburno anagyroidis-Ostryenion carpinifoliae*, relativa ai boschi mesofili submontani (Ubaldi *et al.*, 1987; Blasi *et al.*, 2004) sembra incerta per via della carenza delle specie differenziali.

Le formazioni miste a dominanza di carpino nero e/o cerro, mostrano una certa affinità con le

associazioni *Melittio melissophylli-Ostryetum carpinifoliae,* descritta per l'Appennino Laziale-Abruzzese (Avena *et al.*, 1980) e *Scutellario columnae-Ostryetum carpinifoliae* descritta da Pedrotti *et al.* (1979) per l'Appennino umbro-marchigiano, dalle quali però si differenziano sia per un minor contributo delle specie caratteristiche dei *Fagetalia sylvaticae,* sia per la presenza di un contingente di specie meso-igrofile che testimoniano la posizione ecologica di transizione con le cenosi tipiche dei terrazzi fluviali. Il rilievo eseguito a Campli (TE), ad una quota più bassa rispetto agli altri (370 m s.l.m.), mostra invece maggiori affinità con l'*Asparago acutifolii-Ostryetum carpinifoliae*, associazione termofila descritta da Biondi (1982) per il M. Conero differenziata da specie sempreverdi dei *Quercetea ilicis*.
A Morino (AQ) è stato rilevato un pre-bosco a dominanza di nocciolo (*Corylus avellana*), la composizione floristica mostra affinità con l'associazione *Carpino betuli-Coryletum avellanae*, descritta da Ballelli *et al*. (1979) per l'Appennino Umbro-Marchigiano, della quale rappresenta un aspetto di transizione verso i boschi igrofili della classe *Salici-Populetea*.
Sul terrazzo di un torrente, a Brittoli (PE), è stata rilevata una boscaglia rada a dominanza di olmo campestre (*Ulmus minor* subsp. *minor*) riferibile all'associazione *Aro italici-Ulmetum minoris*.
Un pre-bosco a dominanza di perastro (*Pyrus pyraster*), di difficile inquadramento sintassonomico, è presente nel sito ubicato a Castellafiume (AQ), mentre un'altra formazione di pre-bosco, a dominanza di pioppo tremolo (*Populus tremula*), è stata rilevata a Quadri (CH). Dal punto di vista fitosociologico, anche quest'ultima comunità risulta di difficile collocazione: rispetto alle associazioni inquadrate nella suballeanza appenninica *Aceri obtusati-Populenion tremulae,* si nota qui una quasi totale mancanza delle specie caratteristiche dell'ordine *Fagetalia sylvaticae,* mentre risultano ben rappresentate quelle dei *Quercetalia pubescenti-petraeae*. Una ulteriore complicazione è data dalla presenza di diverse specie caratteristiche dei boschi igrofili ripariali e golenali dei *Salici-Populetea* che testimoniano, anche in questo caso, una posizione ecologica di transizione tra le formazioni di versante e quelle dei terrazzi fluviali.
I rilievi eseguiti a Torrebruna e Schiavi d'Abruzzo (CH) sono relativi a pioppeti a dominanza di pioppo bianco (*Populus alba*) cui si associa, con diversi gradi di abbondanza, la roverella (*Quercus pubescens* subsp. *pubescens*).
La compresenza delle due specie dimostra, anche in questo caso, una posizione ecologica di transizione tra i boschi dei *Quercetalia pubescenti-petraeae* e quelli tipici dei terrazzi fluviali della classe fitosociologica *Salici-Populetea nigrae*. Di quest'ultima unità, tuttavia, manca completamente il corteggio floristico.
Una maggior maturità strutturale e floristica è presente nel pioppeto di Canistro, a dominanza di pioppo ibrido (*Populus canadensis*), ma l'inquadramento fitosociologico a livello di associazione risulta, anche in questo caso complicato. A livello di alleanza si può fare riferimento, per quest'ultima comunità, all'alleanza *Alnion incanae*.

Analisi pedologica

L'analisi multivariata separa con buona approssimazione le tartufaie di *T. melanosporum* e quelle di *T. magnatum*. Il biplot ottenuto dalla PCA, in particolare, mette inoltre in evidenza il ruolo delle diverse variabili nel determinare questa separazione: le principali risultano essere il contenuto in limo, il contenuto in sostanza organica, il contenuto in carbonati totali e, in misura minore, la quantità di scheletro totale. Ciò è confermato anche dalle analisi univariate delle singole variabili. Non si notano, infatti, differenze sostanziali nei suoli delle due specie di tartufo relativamente a diversi parametri quali pH, contenuto in fosforo, ecc.
In dettaglio, i valori relativi al contenuto in sostanza organica (fig. 4) sono più elevati per *T. melanosporum* che per *T. magnatum*. Ciò è verosimilmente da mettere in relazione con le diverse caratteristiche climatiche dei siti. La più elevata e prolungata incidenza del freddo invernale nei siti di *T. melanosporum* rallenta l'attività della catena del detrito e, di conseguenza, la mineralizzazione della sostanza organica. Trattandosi di suoli sottili su substrato calcareo, ciò non determina, come sarebbe invece lecito aspettarsi, una diminuzione del pH.

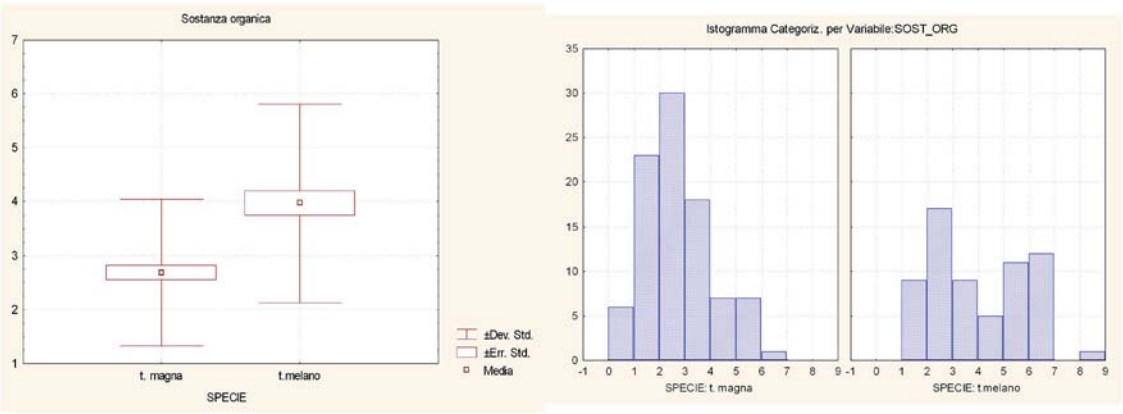

Fig. 4 Contenuto in sostanza organica nei suoli delle due specie di tartufo.

Relativamente alla tessitura, i suoli di *Tuber magnatum* mostrano contenuti più elevati di limo (fig. 5) e di argilla (fig. 6), mentre quelli di *Tuber melanosporum* sono caratterizzati da contenuti elevati di sabbia (fig. 7) che garantiscono una buona aereazione, concordemente con le esigenze della specie (Callot & Jaillard, 1996; Lulli *et al*., 1999; Castrignanò *et al*., 2000). Come già rilevato altrove (Bragato *et al*., 2004), le tartufaie di *T. magnatum* si collocano, infatti, in prossimità dei corsi d'acqua o in corrispondenza di linee di impluvio dove è presente un suolo umido con un contenuto elevato in sedimenti fini di natura alluvionale. Il tartufo nero pregiato, invece, si rinviene per lo più sui versanti o sulle conoidi di detrito alla base degli stessi, dove è presente, inoltre, un maggior contenuto in scheletro, (fig. 8).

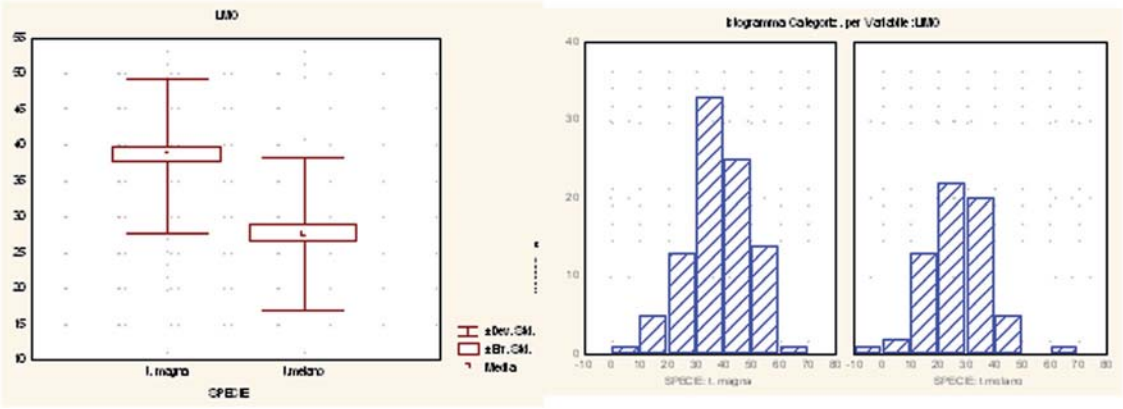

Fig. 5 Contenuto in limo nei suoli delle due specie di tartufo.

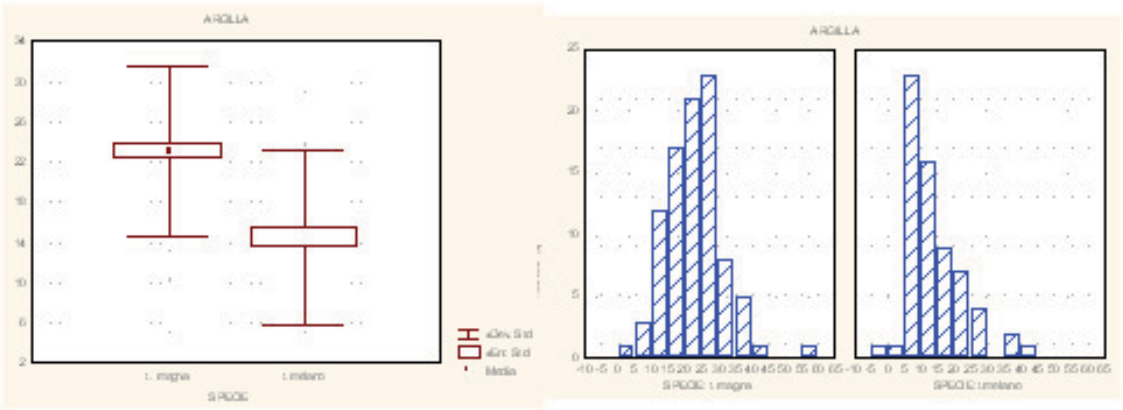

Fig. 6 Contenuto in argilla nei suoli delle due specie di tartufo.

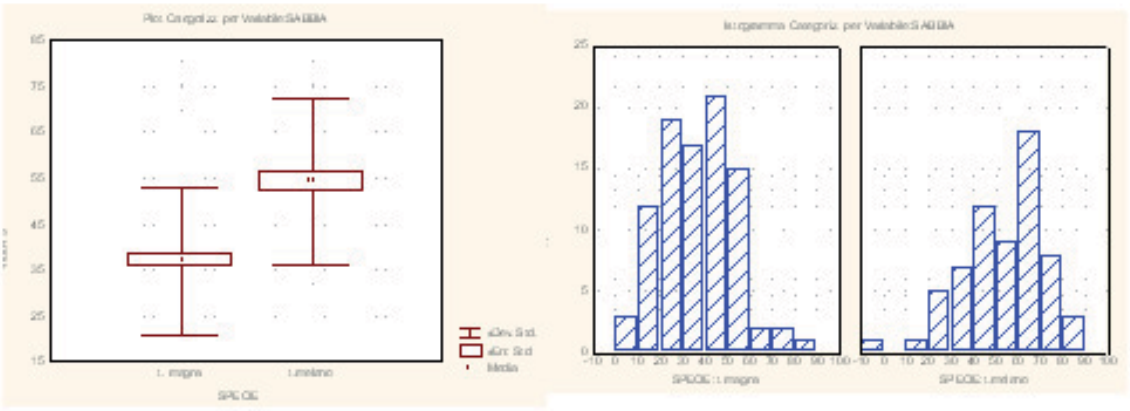

Fig. 7 Contenuto in sabbia nei suoli delle due specie di tartufo.

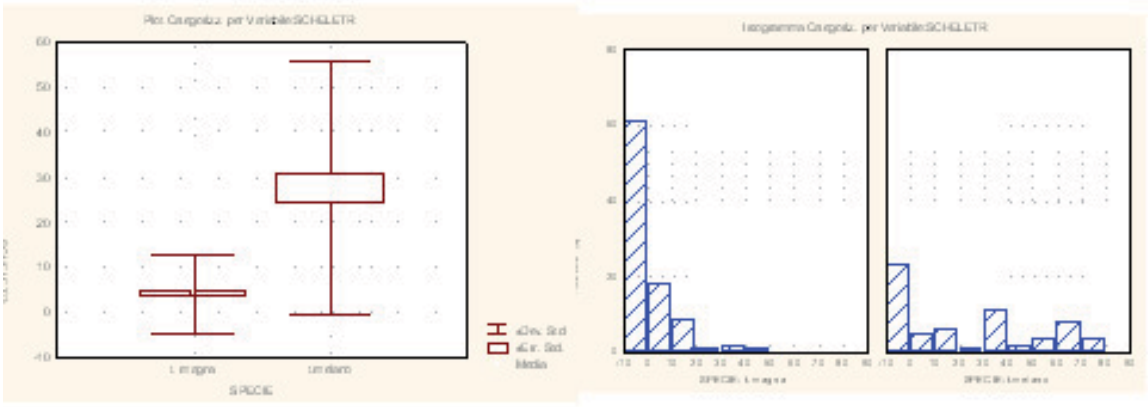

Fig. 8 Contenuto in scheletro nei suoli delle due specie di tartufo.

Conclusioni

Le indagini eseguite hanno evidenziato una chiara differenziazione ecologica delle due specie di tartufo pregiato (*Tuber magnatum, T. melanosporum*) rispetto ai fattori clima, vegetazione e suolo, confermando, anche nella regione Abruzzo, molte delle caratteristiche autoecologiche riscontrate in altri territori.

Per quanto riguarda il clima, le tartufaie di *T. melanosporum* risultano caratterizzate da inverni più freddi, precipitazioni estive meno abbondanti e stress idrico più intenso e più prolungato.

Mentre la vegetazione delle tartufaie di *T. melanosporum* è piuttosto omogenea e riferibile a due associazioni note di bosco di roverella, quella relativa ai siti di *T. magnatum* è molto varia e spesso non caratterizzabile in quanto relativa ad aspetti di transizione tra tipologie differenti. Ad eccezione dei boschi di versante a roverella o a carpino nero e/o cerro, si tratta comunque sempre di formazioni meso-igrofile che si sviluppano alla base dei versanti o sui terrazzi fluviali

I suoli delle tartufaie naturali di *Tuber melanosporum* sono risultati avere, rispetto a quelli di *T. magnatum*, contenuti più elevati di sostanza organica, di carbonati totali e di scheletro, maggiori percentuali di sabbia e minori di limo e di argilla.

La ricerca, che comunque abbisogna di ulteriori approfondimenti in ordine ad una più puntuale caratterizzazione bioclimatica da eseguirsi sugli stessi siti di rilevamento, alla collocazione sintassonomica di alcune tipologie vegetazionali, ad una più omogenea copertura territoriale dei rilevamenti nell'ambito della regione, costituisce un primo importante contributo alla caratterizzazione ecologica delle tartufaie naturali di tartufo bianco e nero pregiati, che potrebbe, se ulteriormente sviluppata ed approfondita, condurre alla costruzione di modelli cui riferirsi, su scala regionale, per l'impianto di tartufaie coltivate.

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STUDIES ON *TUBER MACROSPORUM* VITTAD. NATURAL TRUFFLE HABITATS IN THE CARPATHO-PANNON REGION

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Abstract
Literature considers *Tuber macrosporum* as a truffle distributed between 41° and 51° latitude north in Europe on soils generally limy and heavy in habitats similar to *Tuber magnatum* concerning pedoclimatic characteristics. In Central Europe it is a common species reported from Czech Republic, Hungary, Romania, Serbia and Ukraine. This species is mainly occurs on water affected river banks, temporarily flooded river valleys or in deep and shaded valleys. During our study soil samples were collected in truffle beds and detailed analysis was carried out. Phytoindication method of Borhidi and Zólyomi were used for habitat characterization. Studies carried out on natural habitats of Hungary, Slovakia and Transylvania (Romania), revealed that *Tuber macrosporum* soils are mainly luvisols from subacidophilus to slightly basic concerning pH with variable lime content and high organic matter level. Nitrogen, phosphorous and potassium content is also very variable from very low to very high. Phytocoenology revealed majority of mesophyl and submontane broad-leaved mesophyl forests concerning temperature, semi-humid and intermediate moisture conditions with high plant cover with the majority of plants of neutral soils but tolerant to pH. Coenological research resulted that from the 45 investigated habitats 23 belong to *Melampyro bihariensi-Carpinetum* association, 6 belong to *Carici pilosae-Carpinetum* association, and 5 to *Carici brizoidis-Quercetum* association.
Key words: ecology, *Tuber macrosporum*, soil characteristics, phytocoenology.

Introduction

Tuber melanosporum Vittad., *Tuber magnatum* Pico and *Tuber aestivum* Vittad. are the truffle species considered to be the most common and collected and/or produced in the greatest amounts. Research on the ecology, genetics and cultivation of the abovementioned fungi has a history of centuries, while other species as *Tuber macrosporum*, despite its excellent gastronomic value, are less recognized. This species is considered to be less valuable by some experts (Mannozzi Torini, 1991; Ravazzi, 2003), others blame for its small size (Pacioni, 1985), while some researchers definitely regard it as one of the best truffle species (Ceruti, 1968; Montecchi et Lazzari, 1993; Zambonelli et Iotti, 2005).
Fruitbodies are generally irregular, blackish-brown coloured and easily distinguishable from other black truffle species due to its smoother surface made of black and brownish warts (Figure 1). The size of the carpophores is mainly of hazel-walnut, sometimes reaching the size of an egg (Pacioni, 1985). The gleba has a colour of grey-brown, brown-lilac, finally purple-brown with white-yellowish veins. Asci are ellipsoid containing 1, rarely to 3-4 brown-yellow, big (40-80 x 30-55 µm) spores of reticulate-alveolate surface (Hollós, 1911, Astier, 1998; Montecchi et Sarasini, 2000; Riousset *et al*., 2001; Ceruti *et al*, 2003). The garlicky flavour of the fruitbody resembles to the *Tuber magnatum* (Gregori et Stocchi, 2000; Zambonelli et Iotti, 2005).
The species has a variable occurrence in Europe, considered to be common in Serbia, less frequent in Italy, Slovenia, rare in France, Great-Britain, but also occurs in Germany, Czech Republic, Swiss, Ukraine, Croatia, Slovakia and Romania (Pázmány, 1990; Astier, 1998; Milenkovic et Marjanovic, 1999; Ceruti, 2003; Piltaver *et al,* 2008). Fruiting period has been reported from June (Vezzola, 2008), more often from September to December (Bernini, 1990; Gregori et Stocchi, 2000). According to Milenkovic and Marjanovic (2001) main season of the

species lasts from September to January. In Hungary it is considered to be a common species between August and December (Bagi et Fekete, 2007).

Fig. 1 *Tuber macrosporum* fruit body

Tuber macrosporum mainly grows under deciduous trees, host tree species include oaks (*Quercus pubescens* Willd., *Q. robur* L., *Q. petraea* Liebl., *Q. cerris* L., *Q. suber* L.), hazelnut (*Corylus avellana* L.), hornbeams (*Ostrya carpinifolia* Scop., *Carpinus betulus* L.), alder (*Alnus cordata* (Loisel.) Desf.), willows (*Salix viminalis* L., *S. alba* L., *S. vitellina* L., *S. caprea* L.), lindens (*Tilia cordata* Miller, *T. platyphyllos* Scop.) and poplars (*Populus nigra* L., *P. tremula* L., *P. alba* L.) (Ceruti, 2003). Literature reports the species from humid, loamy habitats either of north-oriented slopes, valley bottoms (Vezzola, 2004) or alluvial soils close to watercourses (Milenkovic et Marjanovic, 1999). Vezzola (2004) also found it in reddish, iron oxide rich, and slightly acidic soils containing lime only in traces.

However, most of the authors agree that *Tuber macrosporum* occurs only in habitats very similar to or the same like *Tuber magnatum* (Stecchi, 1994; Mazzei, 1998; Gregori et Stocchi, 2000; Milenkovic et Marjanovic, 2001; Riousset *et al*, 2001; Vezzola, 2003) mentioning that *Tuber macrosporum* can also have a wider tolerance to drought (Gregori et Stocchi, 2000). In some cases characteristics of its habitats are close to those of *Tuber aestivum* (Rossi, 1990; Vezzola, 2004).

According to Vezzola (2004) *Tuber macrosporum* habitats in Italy are mainly characterized by mixed forests of *Corylus avellana* and *Ostrya carpinifolia*. Bratek *et al.,* (2001) found it in *Melitti-Fagetum* and *Querco-Ulmetum* forests in the Carpathian basin. Pázmány (1990) reported from Transylvania from *Carpino-Quercetum petreae* association. In this territory *Tuber macrosporum* habitats were mainly characterized by basic soils in stream valleys or river banks temporarily covered by water (Gógán *et al.*, 2007).

Material and methods

During our study soil samples were collected in truffle beds and detailed analysis was carried out. To characterize truffle habitats by phytoindication methods of Borhidi (1993) adapted to Carpathian flora (based on Ellenberg 1974 and 1991) and method of Zólyomi (1992) were used. Presence and coverage of herb layer plant species were both estimated.

Results and discussion

In the Carpatho-Pannon region *Tuber macrosporum* occurs mainly on luvisols in the mountains, however, tolerance of the species toward soil characteristics would explain presence on other soil types. Soil analysis results showed that *Tuber macrosporum* soil pH varies from slightly acidic to slightly basic with very variably lime content; organic matter considered to be high. Concentration of macroelements shows high variation from very low to very high (Table 1.).

Tab. 1 Results of soil analysis of 61 *Tuber macrosporum* nests

Soil parameter	Average (SD)	Min.	Max .
pH(H_2O)	6,6(±0,4)	5,8	7,3
pH(KCl)	6,5 (±0,5)	5,2	7,3
EC (%)	0,04 (±0,03)	0,0	0,1
$CaCO_3$ (%)	3,5 (±5,4)	0,0	23,0
Soil organic matter (%)	6,8 (±1,7)	3,4	9,9
NO_3NO_2N (ppm)	12,8 (±12,7)	1,2	66,3
P_2O_5 (ppm)	142 (±131)	20	512
K_2O (ppm)	523 (±318)	88	1.790
Ca (ppm)	12 999 (±14967)	2.620	49.000
Mg (ppm)	383 (±207)	80	1.170
Mn (ppm)	347 (±207)	34	906
Na (ppm)	48,0 (±36,9)	13,0	220,0
Zn (ppm)	11,2 (±6,6)	2,8	38,0
Cu (ppm)	5,7 (±2,6)	0,1	11,5
SO_4S (ppm)	26,7 (±19,1)	0,1	87,4

Coenological investigation shows that examined habitats of *Tuber macrosporum* in the Carpatho-Pannon region belong mainly to *Melampyro bihariensi-Carpinetum* association (23 sites of 45), *Carici pilosae-Carpinetum* association and *Carici brizoidis-Quercetum* association (6 and 5 of 45, respectively) (Figure 2 and 3). Woody plants of the canopy and the shrub level indicate the dominance of *Carpinus betulus* L., *Acer campestre* L., *Quercus robur* L. *Cornus sanguinea* L., *Corylus avellana* L. *Euonymus europaeus* L. and *Fagus sylvatica* L. In the herb layer the most common herbaceous plants are *Viola sylvestris* Lam., *Brachypodium sylvaticum* (Huds.) R. et Sch., *Geum urbanum* L. and *Asarum europaeum* L..

Fig. 2 *Tuber macrosporum* Carpathian habitat, Romania

Fig. 3 *Tuber macrosporum* Pannon habitat, Hungary

Phytoindication results revealed that habitats of *Tuber macrosporum* belong to submontane broad-leaved mesophyl forests concerning temperature. Phytoindication proves the importance of water for the fungus as in both associations semi-humid and intermediate moisture conditions are dominant.

Soil reaction indicates slight differences between the two associations as pH of *Melampyro bihariensi-Carpinetum* association proved to be moderately basic while plants of *Carpinion betuli* associations are more indifferent to pH or live in neutral soils (Figure 4 and 5).

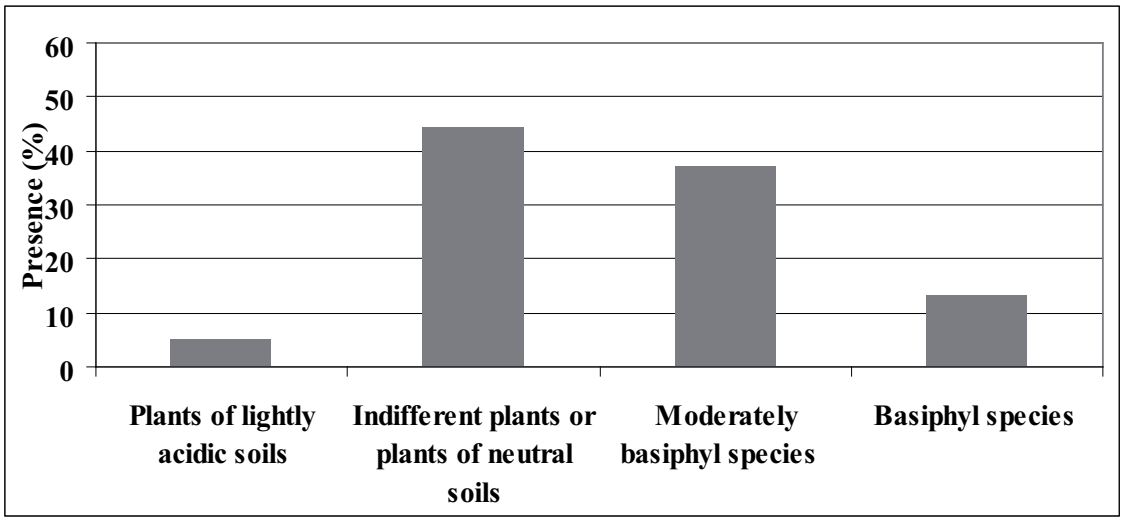

Fig. 4 Phytoindication results of *Carpinion betuli* association concerning soil reaction (pH)

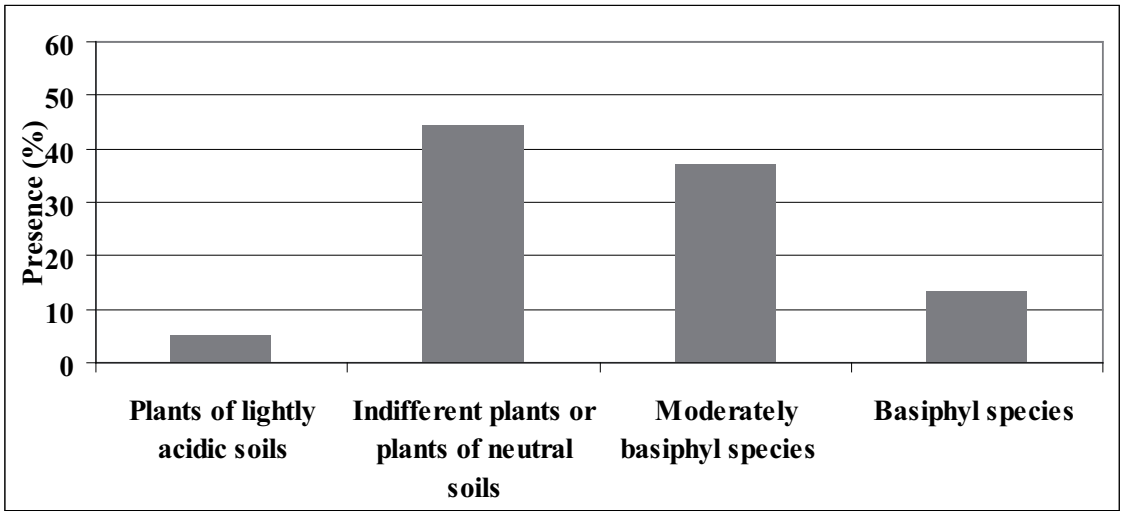

Fig. 5 Phytoindication results of *Melampyro bihariensi-Carpinetum* association concerning soil reaction (pH)

Further difference was found as dominance of shadow—halfshadow plants like *Galium odoratum* (L.) Scop., *Oxalis acetosella* L., *Asarum europaeum* L. and *Carex sylvatica* Huds. which indicated higher canopy closure in *Melampyro bihariensi-Carpinetum* habitats while *Carpinion betuli* associations moved towards the presence halfshadow-halflight plants (Figure 6 and 7).

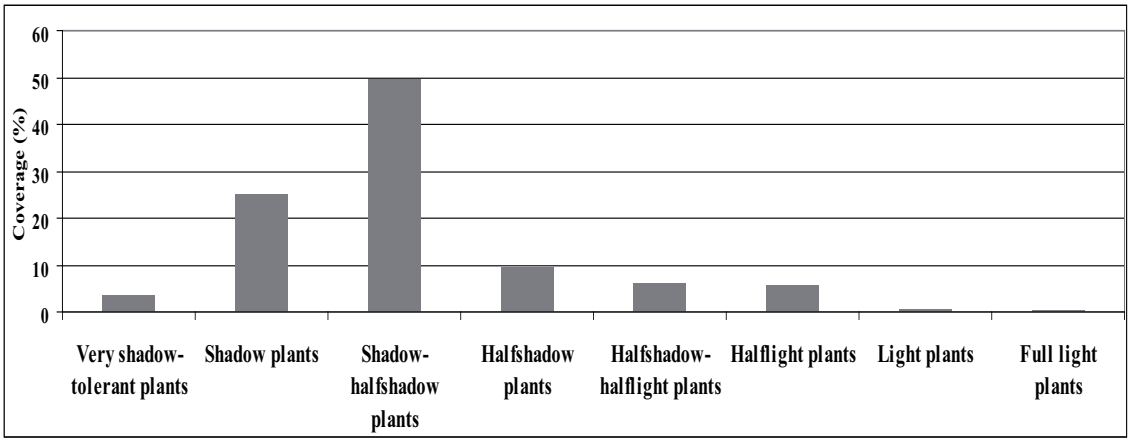

Fig. 6 Phytoindication results of *Melampyro bihariensi-Carpinetum* association concerning light intensity on herb layer

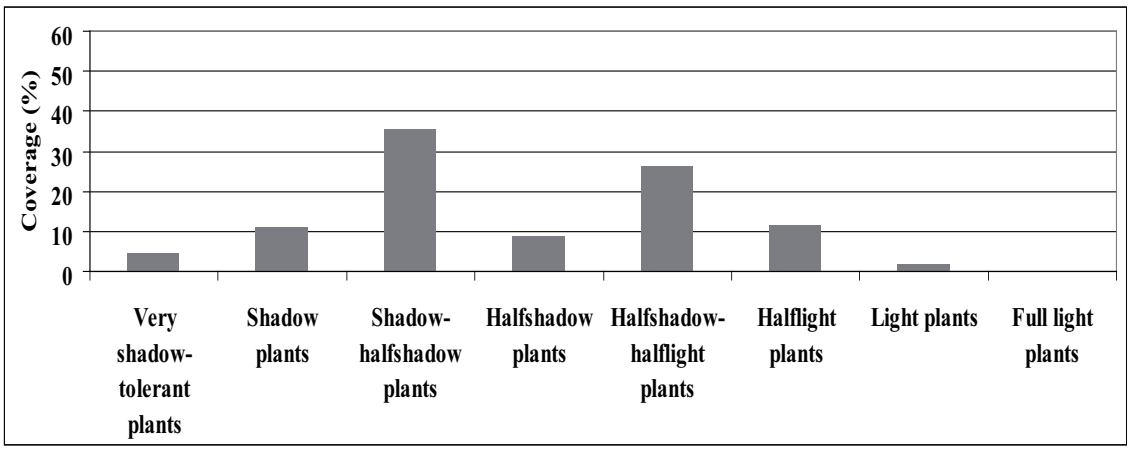

Fig. 7 Phytoindication results of *Carpinion betuli* association concerning light intensity on herb layer

Conclusions

Our studies reported some similarities to literature data of *Tuber macrosporum* ecological demand. We agree on previous findings (Vezzola, 2004; Milenkovic et Marjanovic, 2001) that the species has a strong dependence on water presence, being collected mainly from habitats of valleys and river banks, sometimes temporarily covered by water. *Tuber magnatum* habitats are also reported to be fresh and humid soils (Stecchi, 1994; Mazzei, 1998; Gregori et Stocchi, 2000; Riousset *et al.*, 2001; Vezzola, 2003; Gógán *et al.*, 2005).

Considering soil reaction figures *Tuber macrosporum* seems to have narrow tolerance to pH, it prefers around neutral soils, however, Vezzola (2004) mentioned some occurrences in slightly acidic environment. Lime content probably has no significant role in *Tuber macrosporum* soils being detected in a high variation (0-23%), including absence of $CaCO_3$ in half (30) of the samples. Variable lime content in truffle beds was also traced in *Tuber aestivum* Carpatho-Pannnonic ecotypes (Bratek, 2008).

Narrow range of high humus level (~7%) indicates *Tuber macrosporum* soils to be similar to those of *Tuber rapaeodorum* and *Tuber rufum* (Bratek *et al.,* 2001).

Plant community research could complete our soil analysis revealing the results that *Tuber macrosporum* habitats in the Carpatho-Pannon area are humid-semihumid submontane broad-leaved mesophyl, closed forests with plants of neutral, moderately basic or pH-indifferent plants.

For the future, further basic and applied research would be reasonable to establish comprehensive cultivation technology for this less appreciated but outstanding truffle.

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LOCATION AND ECOLOGICAL CHARACTERIZATION OF NATURAL TRUFFLE GROUNDS IN THE REGION OF MURCIA (SOUTH EAST OF SPAIN)

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Abstract
The Region of Murcia is located in the South East of the Iberian Peninsula, with a typical semiarid Mediterranean climate that determinates the presence of a Termo- or Mesomediterranean vegetation. Only in the highest mountains, such as "Sierra Espuña" and "Revolcadores complex mountains", the precipitation reaches averages over 350 mm/year.
Both, the Regional Park of Sierra Espuña and Revolcadores mountains, are considered as ASCI (Areas of Special Conservation Interest) and are included in the Natura 2000 network. These areas present calcareous soils with Meso-humid, Supra- or even Oromediterranean vegetation. *Quercus ilex* subsp. *rotundifolia* natural woody formations predominate alone or mixed with *Pinus halepensis* plantations up to 800-900 m altitude or with *Pinus nigra* subsp. *salzmannii* from 900 to 1600 m at the sea level.
On the basis of the Spanish Forest Map and using the ARCMAP 9.2 and ARCVIEW-GIS 3.3 modelling and mapping software tools, we developed a cartography on the potential distribution of the black truffle (*Tuber melanosporum* Vittad.) in the Region of Murcia. By the help of trained truffle-hunter-dogs we located several ascomata of this appreciated truffle in three natural truffle grounds in Sierra Espuña Regional Park and one in Revolcadores mountains.
The flora and the mycobiota of the truffle grounds in Murcia were studied and soil analyses were carried out. The most significant results show that the vegetation coverage, sand percentage, organic matter and C/N relation are higher in these zones than in other truffle grounds or orchards from other parts of Spain, France or Italy. Smell and taste of Murcian black truffle ascomata also vary with respect to those from black truffles growing in other latitudes.
Molecular studies, based on the total soil DNA isolation followed by PCR amplification with *T. melanosporum*-specific primers are currently carried out to detect the mycelium of the black truffle in soil.
We can consider the Murcian populations of black truffle as ones of the most Southerly located in Spain, living at the ecological edge for this *Tuber* species. This, together with its phylogenetic importance, according to the glacial refugia hypothesis, make it necessary to develop a conservation program for the black truffle.
Key words: ecology, vegetation, mycelium, *Tuber melanosporum*.

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ECOLOGICAL FEATURES OF *TUBER MAGNATUM* PICO IN THE CONDITIONS OF WEST BALKANS – SOIL CHARACTERIZATION

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Abstract
The precious white autumn truffle (often called Piedmont truffle), *Tuber magnatum* Pico has been reported from Serbia as late as 1998, much later then in Italy or Croatia (Istria). Since then it has been recognized as a significant economic potential, but there has been no detailed ecological analysis of its growing and fruiting characteristics in the conditions of Balkan Peninsula. Quite recently, this truffle was scientifically confirmed from Slovenia as well, and the ambitious project was settled between these two countries in order to describe *T. magnatum* habitats in the Balkans. As the simple habitat overview revealed significant differences between north Istria and Karst habitats (Slovenia) habitats and Serbian habitats, vegetation and soil analysis were set as the first goal of the project.
Here we report surprising differences between Slovenian (that can be assumed as Istria due to geographical closeness) and Serbian soils that support white truffle production. Results clearly indicate wider ecological limits of *T. magnatum* then previously reported.
Analysis of rDNA ITS sequences of samples from the experimental sites in both countries revealed 100% sequence identity, as well as high similarity to other sequences deposited in Gene Bank. Although the differentiation between Balkan and Istria truffles could exist at the ecological (adaptation) level, at the level of commonly assumed eukaryotic genetic diversity (ITS regions) there were no differences. General discussion will be on soils supporting *T. magnatum* in West Balkans in comparison to Italian soils.
Key words: *Tuber magnatum*, ecology, soil.

Almost entire life cycle of truffles takes place in the soil. Therefore, it is correct to state that the truffle environment is basically characterized by soil parameters and presence of the suitable host plant. On the other hand, soil parameters are closely related to the nature of basic rock, climate and vegetation cover. Majority of truffle species live in the soils laying on the calcareous rocks and sediments and in the regions with more or less pronounced Mediterranean climate. The most valuable truffle in the world, *Tuber magnatum* Pico, is widespread in Apennine and Balkan peninsulas (Ceruti *et al.* 2003). Due to the closeness, these peninsulas have been undergoing very similar and simultaneous geological history and practically share geological origin (Karamata, 2006). Common geographical latitude caused similar vegetation history during the ice ages as well (Ray & Adams, 2001), but one significant difference have been causing climate differentiation - the formation of Alps on the north of nowadays Italy and, later on, Pannonian basin on the north of nowadays Balkan region. Alps were the natural barrier that has been preventing moist Mediterranean climate from spreading further north into the central Europe. For this reason Italy has been getting significant and continuous annual amount of rainfall from both west (Mediterranean) and east (Adriatic) directions.
On the other side, in the west of Balkan Peninsula Dinaric Alps have been developed directly on the western coast, forming therefore wide and high barrier to the wet air from the Adriatic sea. Ancient Pannonian sea was creating quite a wet climate on the terrestrial part of what will become a Balkan Peninsula in Pliocene, (Ticleanu *et al.* 2006), and therefore for significant period (9×10^6 years) climate was probably quite similar to that of Apennine Peninsula. After the sea retreated in Pleistocene 600 000 y.a. (Ticleanu *et al*. 2006), Balkan Peninsula was formed as wide dry land,

with no barrier on the northern border, and therefore with much more pronounced influence of the modern continental climate from the north east. Nowadays, continental part of the Peninsula gets much less precipitation then Italy. One small exception is the westernmost peninsula of the Balkan region that is situated in Adriatic sea, south of Alps, and west of Dinaric Alps, and with climate similar to that in northern Italy - Istria.

In the Istria background, Alps are forming the barrier between Apennine and Balkan peninsulas as well. Even though *Tuber magnatum* is one of the connecting factors of biodiversity between two peninsulas, its habitats in Italy, joining area between two peninsulas (Istria), and central Balkan regions (Serbia) are significantly different. Since the presence of this species on the Balkan Peninsula was scientifically confirmed and reported (Marjanović & Milenković, 1998, Piltaver & Ratoša, 2006) it has been clear that the descriptions of the Italian habitats have not matched situation in the Balkan habitats (Lulli & Primavera, 2001). Main differences were host plants, habitat topography, and, the most pronounced, - soil characteristics (Milenković & Marjanović, 2001). Habitats described from Italy were mostly characterized from hilly regions, while in Serbia *T. magnatum* is inhabitant of wide open, flat lands of river valleys and lake surroundings. In Slovenia (Istria) it can be found in smaller rivers beds in the sub Mediterranean region. In order to clarify main differences between soils supporting fructification of *Tuber magnatum* Pico in Istria Peninsula (Slovenia) and continental part of the Balkan Peninsula (Serbia) soil samples from the representative truffle beds of both regions were taken and analyzed, and the results are reported here.

Material and methods

Soil samples were taken from six sites in Slovenian part of Istria, and ten sites throughout Serbia. Representative sites were chosen by the experienced truffle hunters that were following the white truffle production over few seasons. Samples were taken from the depth of 20-30cm, as this was an average most probable depth of the ascocarp formation. They were further subjected to the texture and chemical analysis in the Institute for Soil Science in Belgrade. A number of parameters were measured, and only significant features were further compared and discussed: texture, total N, carbonates, amounts of Ca, Mg, K, pH (in distilled water), mineral nitrogen and available phosphorous. All methods used for chemical analysis were done as described in Nelson & Sommers (1996). Texture analysis was done according to Green (1981).

Results

While investigating *T. magnatum* ecology, many Italian researchers gave major significance to the soil texture. Therefore this was the starting point of investigation. Texture of different soil samples from Serbia and Slovenia are shown in Table 1.

Tab. 1 Percentage of total sand, silt and clay, as well as textural classes of analyzed soils measured in samples from Serbia (Ser) and Slovenia (Slo); C-clay, L - loam, CL - clay-loam, SCL - sandy clay-loam

total sand >0.02 mm		silt 0.02-0.002 mm		clay <0.002 mm		textural class	
Ser	Slo	Ser	Slo	Ser	Slo	Ser	Slo
24.0	42.7	34.6	32.8	41.4	25.2	C	L
20.8	29.0	27.5	43.7	51.7	27.4	C	L
51.6	37.8	23.1	28.1	25.3	34.1	SCL	CL
16.7	48.4	43.5	29.4	39.8	22.2	CL	L
26.0	48.3	37.8	31.34	36.2	20.4	CL	L
42.0	28.3	20.0	41.7	38.0	30.0	SCL	CL
22.8		29.2		48.0		C	
16.4		27.0		56.6		C	
39.1		24.8		36.1		CL	
28.3		28.6		43.1		C	
average	average	average	average	average	average		
27.7+/-11.7	39.1 +/- 9.0	29.6 +/-7.11	34.5 +/ - 6.6	41.6 +/- 8.9	26.5 +/- 5.1		

Soils originating from Serbia, mostly have clayey (or heavier) texture, while Slovenian soils are characterized by dominance of sandy and silty fractions (Table 1). Considering fact that soil samples were from European temperate forests, chemical analysis resulted in surprising soil parameters common for all investigated forests: high content of total nitrogen, good supply of available potassium but very low amount of available phosphorous (Table 2).

Tab. 2 Content of total nitrogen (%), ammonium and nitrates/nitrites nitrogen species (mg/kg of dry soils), available phosphate and potassium (mg/100g of dry soil)

N %		$(NH_4)^+$ mg/kg		$(NO_3)^- + (NO_2)^-$		P_2O_5 mg/100g		K_2O mg/100g	
Ser	Slo	Ser	Slo	Ser	Slo	Ser	Slo	Ser	Slo
0.22	0.26	9.53	20.00	4.19	3.50	0.76	0.41	18.60	18.30
0.27	0.23	10.41	18.20	5.76	4.90	0.57	1.06	40.72	27.45
0.43	0.19	9.48	12.60	5.22	4.20	0.65	1.00	18.86	19.87
0.20	0.31	7.00	17.50	11.43	3.50	1.07	1.55	26.85	24.10
0.38	0.26	2.10	18.80	19.25	4.00	1.56	1.20	26.65	21.21
0.28	0.23	19.14	18.80	6.00	4.10	0.56	1.44	17.86	32.38
0.28		11.52		6.45		1.34		9.87	
0.26		11.50		3.77		0.77		21.95	
0.30		21.04		5.65		0.17		26.86	
0.23		28.00		7.00		1.43		23.04	
average	average	average	average	average	average	average	average	average	average
0.30 +/-0.07	0.30 =/- 0.04	13.00 +/- 7.60	18.00 +/- 2.60	7.50 +/- 4.60	4.00+/- 0.50	0.90 +/- 0.40	1.10 +/- 0.40	23.10 +/- 8.10	23.90+/- 5.30

It is noticeable that macronutrients content in the soils of Serbia and Slovenia do not differ significantly, and show similar pattern. On the other hand significantly different parameters were: soil pH (Figure 1), carbonate content (Figure 2) and distribution of exchangeable cations (Ca^{2+} and Mg^{2+}) (Figure 3).

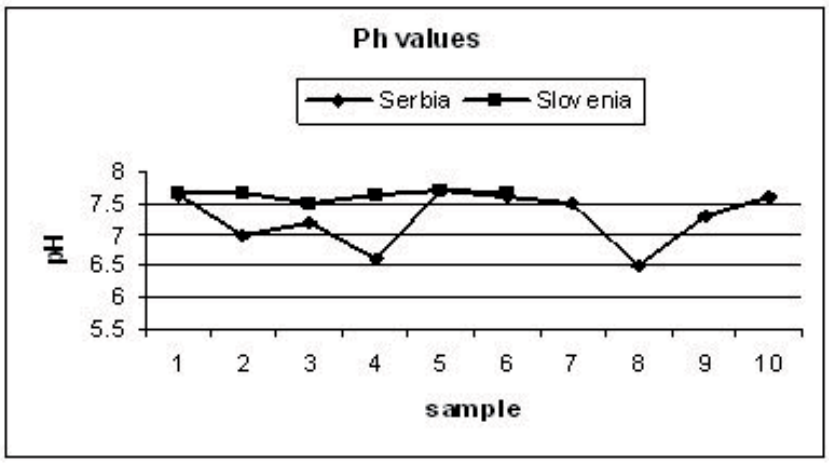

Fig. 1 Variation of pH values of soil samples from Serbia and Slovenia

pH of Slovenian soil samples was uniform, all values above 7.5, while samples from Serbia showed significant variation from 6.5 - 7.7. In general, pH of Serbian samples was lower then Slovenian samples.

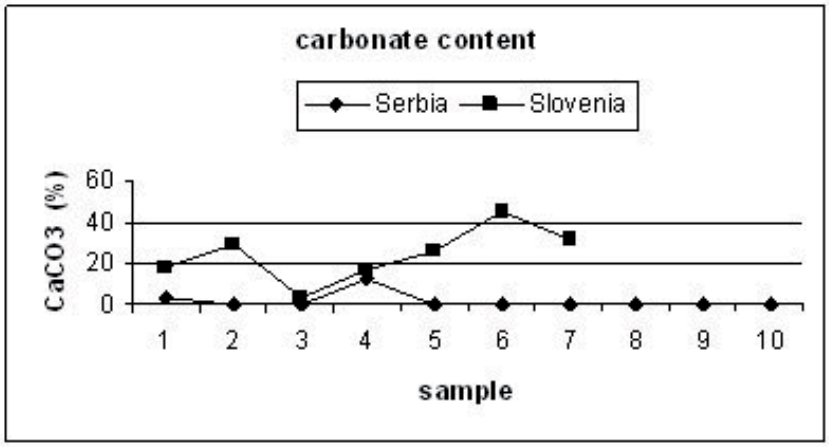

Fig. 2 Variation of $CaCO_3$ content of the soils from Serbia and Slovenia

A variable amount of carbonates was measured in Slovenian samples, while it was recorded in only two samples from Serbia (Figure 2), which could explain lower pH values of Serbian soil samples.

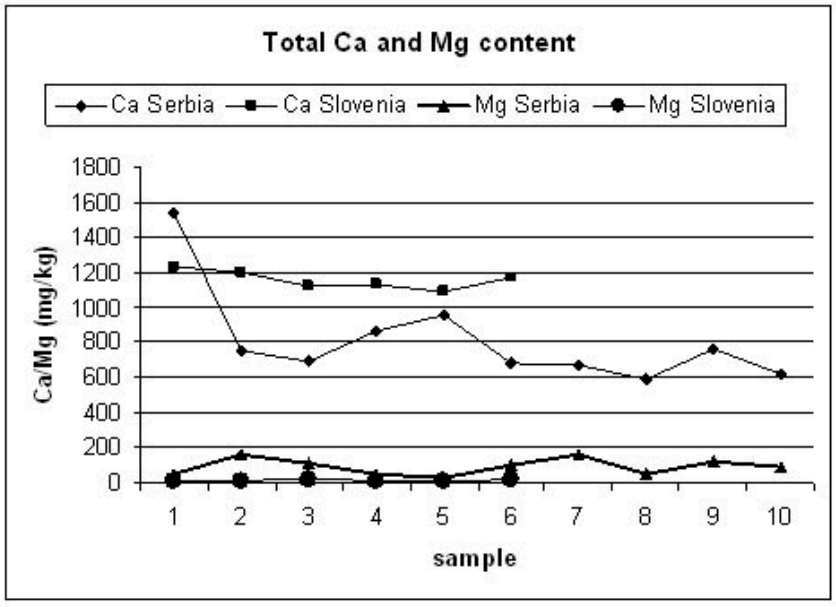

Fig. 3 Variation of basic cations content in the soils of Serbia and Slovenia

Ca^{2+} and Mg^{2+} contents showed similar pattern like previous parameters - they were much equable in Slovenian samples and varied significantly in Serbian samples. It is interesting that content of Ca^{2+} is generally higher in Slovenian, and Mg^{2+} in Serbian samples.

Discussion

Majority of data on *T. magnatum* supporting soils originate from Italy (Montacchini, 1968; Bencivenga & Granetti 1988, 1990; Panini *et al.,* 1991; Bragato *et al*. 1992; Lulli *et al*. 1991, 1992; Bencivenga, 1994; Bencivenga & Donnini, 1995, Lulli & Primavera, 2001). In central Italy, with sub continental climate, white truffle can be found on altitudes of 200 - 600 m asl (Bencivenga & Granetti, 1990) while in south of Italy with the sub Mediterranean climate it lives on 400-1200m asl (Bencivenga & Granetti 1988). Italian authors described soils of *T. magnatum* as: always calcareous, sub alkaline (pH > 7.6) and well aerated, but with good moisture content in all seasons (Lulli & Primavera, 2001). Unfortunately, there were not many data on chemical composition of these soils. Majority of our measurements from Slovenia fit

well with the known Italian data, but data originating from Serbia do not fit into this picture - they have lower pH values (Figure 2), almost no carbonates (Figure 3), and are often not well aerated due to the presence of very high clay content (Table1). This is actually in a strong opposition to previous descriptions of *T. magnatum* soils - carbonate presence, good aeration and pH around 7.5 were strongly defined as necessary for truffle production. High clay content of samples from Serbia is not surprising, since the majority of its habitats in the continental part of Balkan Peninsula are normally wide beds of larger rivers, where the soils are usually thick (aluvial soils) and with the high table of ground water. Majority of them are flooded for a part of the year, usually in spring. As the rainfall in Serbia is much lower then in Italy, clay rich soils with high ground water table would be the only ones capable to retain sufficient moisture in long and hot dry periods during the summer. This is probably the reason of *T. magnatum* existence exactly in these kind of soils in Serbia since it is known that white truffle demands high soil moisture (Lulli & Primavera, 2001). In Slovenia (Istria) rainfall is much higher then in Serbia, and *T. magnatum* is distributed in moist but not flooded soils. Appearance of *T. magnatum* in lighter soils characteristic for Slovenia can be explained by significantly higher precipitation rates in Slovenia as well (1000- 1200mm compared to 800-500 mm in Serbia).
From our data it can be concluded that *T. magnatum* Pico is not associated to high carbonate contents, but rather to high amounts of basic cations - in Slovenia with Ca^{2+}, and in Serbia with a mixture of Ca^{2+} and Mg^{2+}. The previously established rule that pH of *T. magnatum* supported soils must be above 7.5 was proven inaccurate since in Serbia, some soils with pH as low as 6.5 still support white truffle production.
On the other hand, it is quite interesting that analysis of ribosomal RNA (ITS1, 5.8s and ITS2) regions of samples of *T. magnatum* from Slovenia and Serbia showed 100% sequence identity to previously published Italian samples (Grebenc *et al.,* 2008), which rules out genetic differences on any taxonomic level of samples originating from Balkan Peninsula. It would certainly be important to investigate possible genetic differences at lower level between Italian, Slovenian and Serbian populations in order to see if a population differentiation has happened due to the adaptation of *T. magnatum* on different ecological conditions.
We conclude that even though *Tuber magnatum* Pico shows almost no diversity at the level of ribosomal RNA (Grebenc *et al.*, in press, Marjanović *et al*., in press,), it has wider ecological range then it was previously published. In future, we expect that some white truffle natural plantations will be possible to establish in wider and more variable conditions then previously thought.

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ECOLOGIA DI *TUBER MAGNATUM* PICO NELL'ALTA VALLE DEL CHIASCIO (ITALIA CENTRALE)

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Abstract: Ecology of *Tuber magnatum* Pico in the high valley of Chiascio (Central Italy)

Tuber magnatum Pico is the rarest and more expensive species belonging to the *Tuber* genus that fructifies in the central Italy and whose production is decreasing very fast. Typical environments where it is common to collect white truffles are represented by valley areas characterized by particular soil and climatic conditions as described by several authors.

In the "Alto Chiascio" area, white truffles are also harvested on hillsides and hilltops. A complete ecological study on selected productive areas was performed. In particular, soil samples for chemical, physical and molecular analysis, and root samples for mycorrhizal analysis were collected in sites where fruit bodies were harvested. A morphological and molecular characterization of unknown ectomycorrhizae frequently found in fruit bodies collection sites was also carried out. The results show: the presence of *T. magnatum* in associations with different plants; soils with homogeneous chemical and physical features composed by marly-arenaceous formations: the absence of *T. magnatum* ectomycorrhizae; the presence of ectomycorrhizae that belong to other *Tuber* species such as *T. aestivum* Vittad., *T. macrosporum* Vittad*. T. brumale* Vittad*., T. borchii* Vittad. and *T. rufum* Pico. The molecular analysis of soil samples also show the presence of white truffle DNA, but we did not find other *Tuber* spp. DNA despite we found their ectomycorrhizae. The molecular and morphological characterization of unknown ectomycorrhizae showed that they belong to the *Tomentella* genus.

Further researches are needed for a deeper characterization of the ecology and biology of this precious fungus, necessary to safeguard the natural growing environment and plan the future cultivation.

Key words: *Tuber magnatum*, Ecology, ectomycorrhizae, nrDNA, ITS1/ITS4.

Premessa

Il tartufo bianco, *Tuber magnatum* Pico, è tra le specie di tartufo quella più rara e pregiata, ma al contrario del tartufo nero (*Tuber melanosporum* Vittad.) la sua coltivazione è ancora in fase sperimentale (Bencivenga, 2005). *T. magnatum* ha un areale limitato (Italia peninsulare, Croazia, Slovenia, Serbia, Ungheria, Romania) e nell'ambito di questo occupa nicchie ecologiche di piccola estensione, unicamente dove si realizzano particolari condizioni micro-ambientali (Bencivenga & Granetti, 1990; Bencivenga & Donnini, 1995).

Lo studio è mirato alla caratterizzazione dei vari habitat di produzione di *T. magnatum* nell'Alta Valle del Chiascio.

Materiali e metodi

E' stata scelta un'area naturale molto produttiva compresa tra i comuni di Gubbio e di Sigillo (Umbria - Italia).

Caratteri generali dell'area studiata

Si tratta di un'area caratterizzata da vaste zone collinari-montane formate da sedimenti marini del Miocene (Marnoso - Arenacea) il cui movimento tettonico caratteristico è dato da dolci pieghe in direzione NW-SE (Fig. 1).

Fig. 1 Paesaggio dell'area studiata

La formazione marnoso arenacea rappresenta il 56,84% di tutto il territorio e l'83,61% dell'intera area collinare (Servizio geologico italiano, 1980).
Sono presenti fenomeni di erosione che danno origine ai calanchi generalmente presenti ai bordi di piccole valli profondamente incise.
L'area presenta: Macrobioclima Temperato; Bioclima Oceanico, Termotipo Mesotemperato Superiore; Ombrotipo Umido Inferiore (ARPA, 2004).
La piovosità media annua è superiore alle altre medie della regione ed è di 1028,92 mm; essa si va riducendo costantemente infatti la media degli ultimi 10 anni è di 890 mm.
La vegetazione è inquadrata nella Serie *Aceri obtusati – Quercetum cerris* sigmetum, associazione: *Aceri obtusati - Quercetum cerris,* con la variante ad *Ostrya carpinifolia* (Orsomando *et al*., 1999).
Mediante numerosi sopralluoghi con i tartufai della zona**,** sono state individuate 3 tipologie di zone produttive (ZP) caratterizzate da:

- ZP1 - Vegetazione ripariale lungo il fiume Chiascio e i corsi d'acqua che in esso confluiscono (Fig. 2);
- ZP2 - Fasce di vegetazione residue lungo le scoline, i fossi e i canali di drenaggio in prossimità dei coltivi (Fig. 3);
- ZP3 - Boschi cedui a carpino nero misto a cerro e roverella nei versanti collinari (Fig. 4).

Fig. 2 Tartufaie lungo corsi d'acqua

Fig. 3 Tartufaie limitrofe a campi coltivati

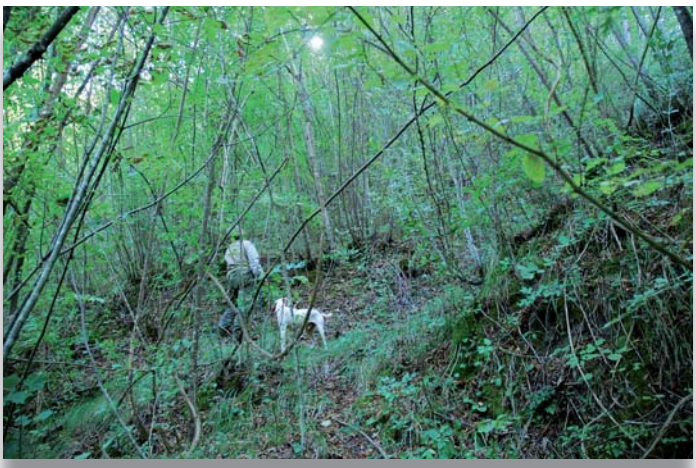

Fig. 4 Tartufaie in bosco

Modalità di campionamento del suolo e degli apparati radicali

Per ciascuna tipologia, sono state scelte 2 tartufaie rappresentative. In ciascuna di esse, individuato il punto di produzione dello sporoforo è stata tracciata una retta passante per il punto di produzione e il simbionte. Su questa retta sono stati effettuati campionamenti di suolo e di radici:

- nel punto di produzione;
- ad un metro dal punto di produzione verso il simbionte;
- ad un metro dal punto di produzione in direzione opposta al simbionte;
- in una area distante al massimo 100 metri e sicuramente non produttiva.

In totale sono stati raccolti 18 campioni di radici (6 per ogni zona produttiva, ovvero 3 per ogni tartufaia), e 48 campioni di suolo: 24 per l'analisi molecolare e 24 per l'analisi chimico-fisica.

Analisi morfologica e biomolecolare delle ectomicorrize

Le micorrize sottoposte al controllo morfologico sono state suddivise in morfotipi in base ai caratteri morfologici e brevemente descritte seguendo Agerer (1987-08) e Zambonelli *et al.* (1993).

Per 3 morfotipi non identificati dai caratteri tassonomici è stata eseguita l'analisi molecolare con l'obiettivo di associarli al relativo *taxa* di appartenenza. Da una singola ectomicorriza la regione dello spaziatore intergenico ribosomale (ITS - Internal Transcribed Spacer) è stata amplificata tramite PCR diretta secondo il protocollo pubblicato da Iotti *&* Zambonelli (2006) e sequenziata. Le reazioni di sequenza sono state effettuate mediante l'utilizzo del "Big Dye Terminator v1.1 Cycle Sequencing Kit" (Applied Biosystem) seguendo il protocollo fornito con il kit. La separazione elettroforetica è stata effettuata con sequenziatore capillare AB3130XL

ed i dati di sequenza ottenuti sono stati utilizzati per analisi di comparazione con le sequenze presenti in banca dati utilizzando il tool BLASTn della NCBI (National Center for Biotechnological Information).

Analisi chimico-fisica e biomolecolare del suolo

Le analisi chimico-fisiche del suolo sono state eseguite con procedure standard adottate presso il laboratorio della Sezione di Chimica Agraria dell'Università degli Studi di Perugia.

Con l'obiettivo di verificare la presenza di eventuali strutture vegetative di *T. magnatum* è stata eseguita l'analisi biomolecolare di campioni di suolo. In corrispondenza di ciascun punto in cui sono state prelevate le radici micorrizate è stato successivamente raccolto, con un contenitore sterile, un campione di suolo di circa 25 gr.. In laboratorio i campioni sono stati conservati in cella fredda a - 4°C fino al momento dell'utilizzo.

Il DNA genomico totale è stato estratto utilizzando il kit "Power Soil DNA Isolation" (MO BIO Laboratories, Inc) a partire da 0,5 *gr.* di terreno per ciascun campione seguendo le istruzioni riportate nel protocollo dalla casa produttrice. Qualità e quantità del DNA estratto sono stati valutati, per ogni campione, mediante lettura allo spettrofotometro e confermata tramite corsa su gel di agarosio con l'impiego del marker *MassRulerTM* (Fermentas) utilizzato come riferimento.

Dopo estrazione i DNA genomici ottenuti sono stati impiegati per:

- l'amplificazione della regione ITS;
- l'amplificazione di porzioni di DNA tramite primer specie-specifici.

Ciascuna PCR è stata eseguita in un volume totale di 25 µl contenti DNA genomico stampo (15 *ng* circa) e una mix contenente: PCR Buffer, 1X; MgCl2, 1.8 mM; dNTPs, 200 pM; Primer 1, 30 pmol, Primer 2, 30 pmol; Taq, 1U, utilizzando un ciclo termico composto da uno step iniziale di denaturazione a 94 °C per 3', seguito da 38 cicli composti da: 94° C 30''; X° C 30'', 72° C 30''. Con X è indicata la temperatura specifica di annealing per ciascuna coppia di primer (Tabella 1).

Tabella 1 Nome dei primer, relative sequenze nucleotidiche, temperatura di annealing e riferimento bibliografico

Primer	**Sequenza di nucleotidi**	**Annealing**	**Riferimento Bibliografico**
ITS1	5'-TCCGTAGGTGAACCTGCGG-3'	59° C	White *et al.* (1990)
ITS4	5'-TCCTCCGCTTATTGATATGC-3'		White *et al.* (1990)
ITSmagn	5'-GTCACTGAAAACCCACTCAC-3'	59° C	Amicucci *et al.* (1998)
ITSback	5'-TGAGGTCAACCCAGTTGGAC-3'		Amicucci *et al.* (1998)
ITSBACKM3	5'-TGAGGTCTACCCAGTTGGGCAGTGG-3'	70° C	Mello *et al.* (1999)
ITSMAGNP7	5'-TCCTACCAGCAGTCTGAGAAAGGGC-3'		Mello *et al.* (1999)
UNC-I	5'-TGGGCCGCCGAAAACTTG-3'	59° C	Mello *et al.* (2002)
UNC-II	5'-CTGACGAGATGCCCCGGA-3'		Mello *et al.* (2002)
TborchA	5'-TGCCCTATCGGACTCCCAAG-3'	60° C	Mello *et al* (1999)
TborchB	5'-GCTCAGAACATGACTTGGAG-3'		Mello *et al* (1999)

I prodotti di PCR ottenuti sono stati analizzati mediante separazione elettroforetica in gel di agarosio con concentrazioni variabili a seconda delle dimensioni dei frammenti attesi.

Risultati

Cenni alla flora presente sulle tartufaie
Le tartufaie sono concentrate in una fascia altimetrica compresa tra i 500 e 600 m s.l.m., su suoli poco evoluti caratterizzati da frequenti eventi franosi.
Le cave si rinvengono sotto una copertura vegetale totale.
Nelle tartufaie in bosco (ZP3) la specie simbionte più frequente è *Ostrya carpinifolia* Scop. seguita da *Quercus cerris* L. e *Q. pubescens* Willd.
La copertura arbustiva è operata prevalentemente da *Hedera helix* L., *Rubus hulmifolius* Schott., *Clematis vitalba* L., *Cornus mas* L.
Nelle tartufaie in prossimità di corsi d'acqua e di coltivi (ZP1, ZP2), le specie simbionti sono ascrivibili soprattutto ai generi *Populus* (*P. alba* L.; *P. nigra* L.; *P. nigra* var. *italica*) e *Salix* (*S. alba* L.). Nello strato arboreo sono presenti anche il ciliegio (*Prunus avium* L.) e l'acero campestre (*Acer campestre* L.); si intensifica la copertura dello strato arbustivo costituito dalle specie dei versanti insieme a salici (*Salix* sp. pl.) e cornioli (*Cornus sanguinea* L., *C. mas* L.).

Analisi delle ectomicorrize
Nei campioni di radici prelevati nei punti di raccolta dei carpofori di *T. magnatum* e alla distanza di un metro da essi sono state individuate forme ectomicorriziche attribuibili ai seguenti *taxa*: *Tuber aestivum* Vittad., *T. brumale* Vittad., *T. borchii* Vittad., *T. macrosporum* Vittad., *T. rufum* Pico, *Cenococcum geophilum* Fr., *Trichophaea woolhopeia* (Cooke & W. Phillips) Arnould e 3 morfotipi non identificabili morfologicamente, contraddistinti con le sigle UM1 (Unknown Mycorrhiza), UM2 e UM3. La percentuale di apici secchi o nudi è risultata sempre molto elevata (in media del 65%).
In nessun caso sono state rinvenute micorrize di *T. magnatum* così come descritte da Rubini *et al*., (2001).
Per quanto riguarda i 3 morfotipi non identificati (UM1, UM2, UM3), la sequenza di tutti gli ampliconi studiati è risultata molto simile (identità compresa tra il 93% ed il 98%) a sequenze di funghi appartenenti alla famiglia delle Thelephoraceae e più precisamente al genere *Tomentella*. Una breve descrizione morfologica è fornita in Fig. 7.

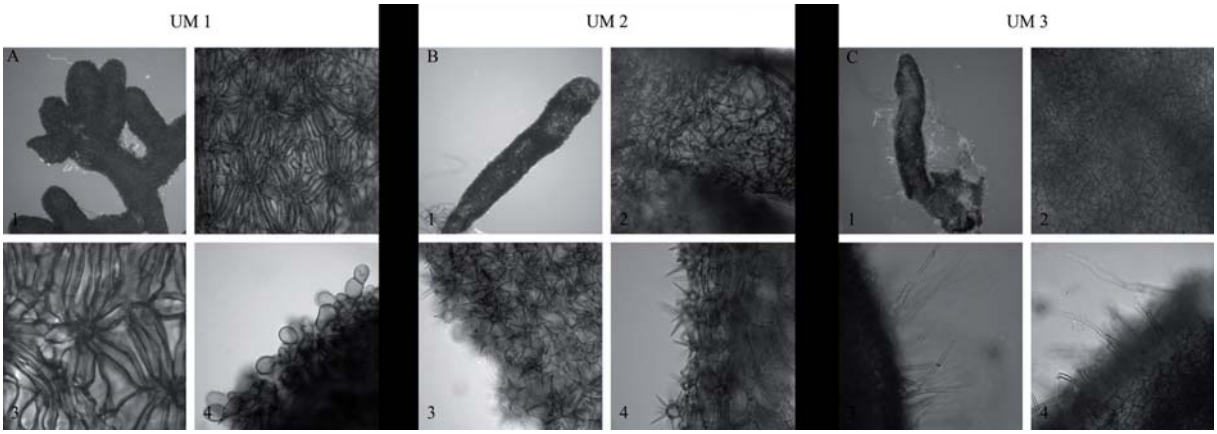

Fig. 5 Breve caratterizzazione morfologica dei morfotipi individuati in accordo con Agerer l.c.: **UM1** morphotype: A1 - ramified mycorrhizal tips (32x, Wild MZ8 stereomicroscope); A2 - outer mantel layer (200x, Leica – Leitz DMRB); A3- star-like hyphal cells (400x); A4 - globose cystidia (200x). **UM2** morphotype6: B1 - simple mycorrhizal tip (32x), B2 - irregular polygonal-like hyphal mantle layer (200x), B3 - 4 globose star-like cystidia (200x). **UM3** morphotype: simple mycorrhizal tip (32x), polygonal-shaped cells of outer mantle lyer (200x), B3 - 4 needle-like cystidia with middle clamp (400x).

Caratteri pedologici delle aree studiate
Le analisi chimiche e fisiche dei suoli evidenziano caratteri simili tra i punti di produzione delle

tre tipologie di tartufaie prese in considerazione. Considerando i dati medi riferiti a 18 campioni prelevati in aree produttive e 6 in aree non produttive si evidenzia:

- un maggiore contenuto medio in sabbia ed un minore contenuto di elementi fini (limo e argilla) nelle aree produttive rispetto a quelle non produttive;
- un contenuto medio in calcio scambiabile più abbondante nei punti di produzione, pur essendo più bassa la percentuale media di carbonati e di calcare attivo; lo stesso elemento valutato in percentuale sul complesso di scambio ha valori simili nelle due zone;
- valori del magnesio e del potassio scambiabile, della capacità di scambio cationico e il contenuto in fosforo assimilabile mediamente più elevati nelle aree produttive.

I caratteri rilevati concordano con quanto riportato in letteratura (Lulli *et a*l., 1991; Lulli *et al*., 2001; Raglione e Owczarek, 2005) a conferma della grande omogeneità dei suoli delle tartufaie di bianco italiane (Tabella 2).

Tabella 2 Analisi chimico-fisiche dei campioni di suolo relativi alle 3 tipologie di zone produttive studiate: ZP1, ZP2, ZP3. I dati relativi ai campioni sono valori medi (n=numero campioni).

	ZPI		ZP2		ZP3		MEDIE	
Caratteri analitici	Prod. (n=6)	Non Prod. (n=2)	Prod. (n=6)	Non Prod. (n=2)	Prod. (n=6)	Non Prod. (n=2)	Prod. (n=18)	Non Prod (n=6).
Sabbia grossa %	3,00	2,22	1,67	1,64	2,02	1,00	2,23	1,62
Sabbia fine %	22,11	13,04	13,26	8,55	14,44	4,03	16,60	8,54
Limo %	53,85	60,76	57,61	59,64	64,29	60,74	58,58	60,38
Argilla %	21,04	22,98	27,46	30,17	19,24	34,23	22,58	29,13
pH	8,00	8,20	7,98	8,20	8,07	8,30	8,02	8,23
Calcare totale %	18,67	20,00	18,50	22,00	26,00	42,00	21,06	28,00
Calcare attivo %	11,07	12,60	12,10	13,10	12,23	13,30	11,80	13,00
Carbonio organico totale %	2,00	3,60	2,15	1,90	2,00	2,40	2,05	2,63
Sostanza organica %	3,50	6,20	3,70	3,30	3,43	2,60	3,54	4,03
P assimilabile mg /kg	3,73	2,20	6,10	2,60	3,70	1,50	4,51	2,10
CSC meq/100 gr	23,93	16,10	29,03	19,00	22,17	15,50	25,04	16,87
Ca scambiabile mg/kg	3656,67	2618,00	4224,50	2905,00	3627,67	2313,00	3836,28	2612,00
Ca scambiabile % su CSC	76,17	78,20	79,48	76,30	81,57	74,70	79,07	76,40
Mg scambiabile mg/kg	577,67	340,00	565,25	424,00	389,33	413,00	510,75	392,33
Mg scambiabile % su CSC	20,37	17,60	16,25	18,50	14,83	22,20	17,15	19,43
K scambiabile mg/kg	186,67	170,00	327,50	200,00	176,67	63,00	230,28	144,33
K scambiabile % su CSC	2,00	2,70	2,90	2,70	2,07	1,00	2,32	2,13
Na scambiabile mg/kg	80,67	56,00	94,00	106,00	74,00	72,00	82,89	78,00
Na scambiabile % su CSC	1,47	1,50	1,38	1,40	1,53	2,00	1,46	1,63

Analisi molecolare dei campioni di suolo

L'amplificazione della regione ITS è stata eseguita su tutti i campioni di terreno estratti ed inoltre sono stati aggiunti, due campioni di controllo rappresentati dal DNA genomico di *Poa pratensis* L. per il positivo (+) ed H_20 ultrapura per il controllo negativo (-) (Fig. 6).

Per quanto riguarda i campioni di DNA estratti dal terreno l'amplificazione produce in tutti un bandeggio complesso costituito da un minimo di poche bande minori (per intensità) visibile nel campione 7, fino a 4 bande maggiori come evidente nel campione 12. La lunghezza degli amplificati è compresa tra le 900 paia di basi (pb) dell'amplificato del campione 6 fino alle 400 pb circa dell'amplicone più corto generato nell'amplificazione del campione 12. Infine non si riscontrano amplificati nel controllo negativo.

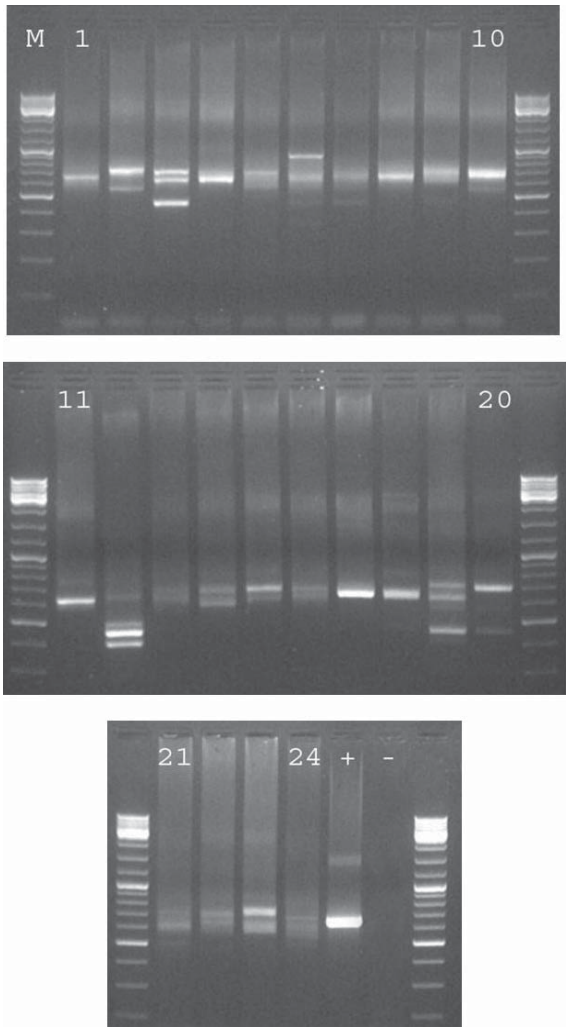

Fig. 6 Risultato di corsa su gel di agarosio (2% v/v) di ampliconi ottenuti con primer generici ITS1/ ITS4 su DNA estratto da suolo. Per la totalità dei campioni analizzati si evidenzia la presenza di un bandeggio complesso (campioni da 1 a 24). + controllo positivo, **-** controllo negativo e M marker.

L'impiego in PCR dei primer ITSbackM3-ITSmagnP7 ha permesso di testare la presenza di DNA di *T. magnatum* Pico nei campioni di terreno studiati. Dopo corsa elettroforetica su gel, per i campioni 1,2,4,5 e 9 è stata riscontrata la presenza dell'amplicone della lunghezza attesa di 280 pb circa corrispondente a *T. magnatum* (Fig. 7). Il risultato ottenuto è stato confermato dall'amplificazione con i primer specifici ITSmagn/ITSback sugli stessi campioni la cui immagine non viene riportata in questo lavoro. L'amplificazione con i primer specifici TborchA/TborchB ed UncI/UncII ha evidenziato l'assenza in tutti i campioni del DNA di *T. borchii* e *T. aestivum*. Considerata l'assenza di amplificato le immagini non sono state riportate in questo lavoro.

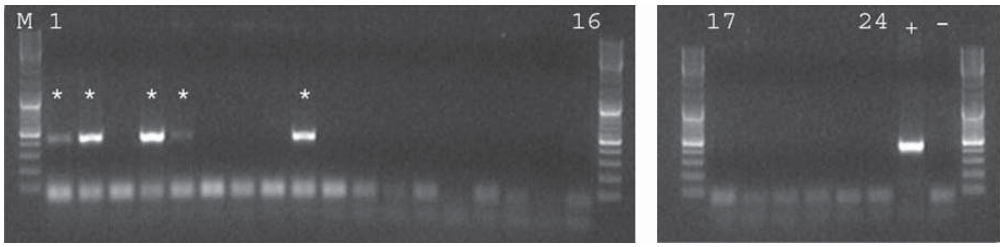

Fig. 7 Visualizzazione dopo corsa su gel di agarosio (2% v/v) di ampliconi ottenuti con primer specifici ITSbackM3-ITSmagnP7 su DNA estratto da suolo. Nei campioni 1,2,4,5 e 9 si riscontra la presenza di amplificato di *T. magnatum* (*); + controllo positivo; **-** controllo negativo e M marker.

Conclusioni

Le ricerche sull'ecologia del tartufo bianco nell'alta Valle del Chiascio hanno consentito di confermare le conoscenze relative al clima, suolo, e vegetazione richieste da *T. magnatum* per poter completare il suo ciclo biologico.

Sono emersi nuovi interrogativi circa le modalità di vita del tartufo bianco in quanto non sono state trovate le sue micorrize nei punti di raccolta degli sporofori ed in quelli immediatamente vicini.

Il DNA di *T. magnatum* è invece presente in 4 campioni corrispondenti alla zona ZP1 (campioni 1, 2, 4, 5, Fig. 7) e un campione nella zona ZP2 (campione 9, Fig. 7). In particolare è interessante notare che si è ottenuto amplificato nei punti di raccolta del carpoforo (campioni 2, 4) e anche nei punti ad un metro di distanza (campioni 1, 5, 9).

Dai dati ottenuti si presuppone un comportamento diverso rispetto alle altre specie di tartufo dove le micorrize da essi prodotte, sono esclusive o nettamente prevalenti nei punti di produzione (Bencivenga *et al*., 1992).

Nell'area studiata *T. magnatum* condivide l'habitat soprattutto con *T. aestivum*, *T. borchii:* l'indagine condotta ha permesso di verificare la presenza delle ectomicorrize di queste specie e l'assenza di amplificato quando con i relativi primer specie-specifici.

Questa constatazione rafforza l'idea che il ciclo biologico di *T. magnatum* abbia fasi diverse da quelle della altre specie di *Tuber* e ancora da chiarire.

La ricerca continua per individuare le strutture responsabili della presenza di DNA di tartufo bianco nel suolo ed i rapporti che si instaurano con le piante simbionti.

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RAPPORTO FRA LA GESTIONE DEI SOPRASSUOLI FORESTALI E PRODUZIONE DI *TUBER AESTIVUM* VITTAD. IN UNA TARTUFAIA NATURALE DEL MONTE AMIATA (TOSCANA-ITALIA): PRIMI RISULTATI

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Abstract: Relationship between the management of forest vegetation and the production of *Tuber aestivum* Vittad. in a natural truffle ground on Monte Amiata (Tuscany, Italy): preliminary results. We report the preliminary results of an experimental project aimed at clarifying the relationship between tree and shrub cover and the production of *Tuber aestivum* fruit-bodies. The thinning carried out in a high forest of *Quercus cerris* in the municipality of Castell'Azzara (Grosseto) was preceded by classification of the area from the points of view of climate, pedology, forestry and vegetation, exploring the possibility of applying the BACI statistical method (Before-After-Control-Impact). Following this, 10 plots of a surface area of 1,000m^2 each were selected, in half of which approximately 30% of the tree and shrub cover had been removed by thinning. In each plot a qualitative and quantitative survey of truffles was performed, using a specially designed data registration form. Collections were made every 10 days in the period indicated on the harvesting calendar. The fructification processes of *Tuber aestivum* were compared to the climatic and pedological data, shedding more light on the ecology of the species: in particular, it emerged that the production of fruit-bodies does not seem to be hampered by suboptimal climatic conditions, considering that the greatest productivity was recorded in 2007, a year in which temperatures were relatively high and rainfall low. An increase in both the number and weight of fruit-bodies was observed following thinning. Interestingly, fruit-bodies were also found in plots that had been recorded as unproductive at the beginning of the project. Although preliminary, this result could be connected to the silvicultural operations carried out.
Key words: ecology, silviculture, *Tuber aestivum*, *Quercus cerris*

Introduzione

Il Monte Amiata si presenta come il più importante rilievo della Toscana meridionale e accoglie, a partire dalle sue pendici, tutte le fasce vegetazionali, dalla macchia mediterranea (piano basale) fino alla faggeta (piano montano), raggiungendo una copertura forestale complessiva di 30000 ha. Nel tempo questi boschi hanno assolto alle necessità e ai bisogni dell'uomo. Fino ad alcuni anni fa le funzioni prevalenti erano quelle economiche derivate dallo sfruttamento diretto del legname e del pascolo oltre che, naturalmente, al prelievo della selvaggina e dei vari prodotti del sottobosco. Inoltre sul Monte Amiata il legname ha trovato largo impiego nell'attività mineraria sia per la costruzione delle gallerie delle miniere che per l'alimentazione dei forni utilizzati per l'estrazione del cinabro.
Oggi i funghi, non solo quelli epigei, ma anche quelli ipogei come i tartufi, sono uno dei più importanti prodotti del sottobosco e, in alcuni casi, il loro valore commerciale può superare il valore del legname ricavabile da una foresta. Questo è particolarmente vero per quei boschi con suolo povero di nutrienti a bassa produttività.
La Toscana è senza dubbio una regione in cui è presente una consolidata tradizione tartuficola che si è da sempre rivolta quasi esclusivamente alla presenza del tartufo bianco pregiato (*Tuber magnatum* Pico), ma negli ultimi anni gli operatori del settore hanno dimostrato un notevole interesse anche per le specie di tartufo minori. Le specie comunemente note come

il tartufo bianchetto (*Tuber borchii* Vittad.), lo scorzone (*Tuber aestivum* Vittad.), il tartufo uncinato (*Tuber uncinatum* Chatin) sono oggi interpretate autonomamente come vere e proprie risorse territoriali da promuovere o da recuperare, anche in termini di ambienti naturali di produzione.
Sulla base di queste considerazioni nasce il presente lavoro volto a valutare l'impatto della copertura arborea sulla produzione di ascomi di *Tuber aestivum* Vittad. che vede la collaborazione dell'Agenzia Regionale per lo Sviluppo e l'Innovazione nel settore Agricolo-forestale, della Comunità Montana Grossetana e dell'Università degli Studi di Siena.

Materiali e Metodi
All'interno della tartufaia è stato realizzato un disegno sperimentale verificando la possibilità di applicare il metodo statistico BACI (Before-After-Control-Impact). In particolare è stato testato se l'area fosse sufficientemente omogenea per applicare una scelta randomizzata dei plot su cui effettuare il successivo intervento selvicolturale di diradamento. In seguito a questa verifica sono stati individuati e delimitati sul terreno, mediante pali e picchetti tinteggiati, 10 plot di superficie unitaria pari a 1.000 m^2 (Fig. 1).

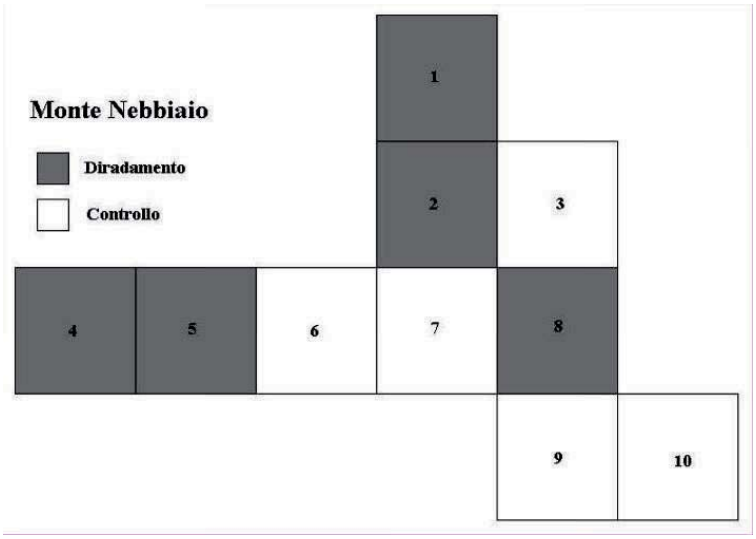


Fig. 1 Disegno sperimentale realizzato nell'area di indagine

Per l'analisi climatica, i dati relativi al periodo 1994-2007 sono stati desunti dalla stazione meteorologica di rilevamento n. 55 posta in località Cortevecchia-Semproniano, a circa 7 km in direzione Sud-Ovest ad un'altitudine di 499 m.s.l.m. Dai dati termo-pluviometrici sono stati calcolati l'indice di concentrazione stagionale delle piogge (rapporto tra il totale di ogni stagione ed un quarto totale medio annuo), l'indice di Fournier modificato da Arnoldus (1977) e noto anche come indice F_{FAO} (che esprime il grado di concentrazione mensile delle piogge, dato dalla somma dei quadrati delle precipitazioni mensili diviso per il totale annuo), nonché la serie di indici climatici tradizionalmente utilizzati per l'analisi climatica (Tab. 1) (Pinna, 1977; Blasi, 1998).
Con riferimento alla carta litologica della Regione Toscana l'area di studio ricade su substrati geologici diversi: marne argilliti, argilloscisti e alternanze di calcari, calcareniti, calcari marnosi. La descrizione del suolo è stata realizzata nel mese di settembre 2007, facendo al centro di ogni plot un carotaggio prelevando un campione nella profondità compresa fra 0-15 cm, avendo avuto cura di escludere dal campionamento la lettiera e altri materiali organici grossolani eventualmente presenti. I principali parametri pedologici importanti per la comprensione dei processi pedogenetici e per la loro intima relazione con la crescita e fruttificazione del tartufo sono stati rilevati facendo uso delle specifiche contenute nella "Guida alla Descrizione dei Suoli in Campagna e alla Definizione delle loro Qualità", messa a punto dall'Istituto Sperimentale per lo Studio del Suolo di Firenze e dalla Regione Toscana.

La caratterizzazione forestale è stata effettuata sulla base di una metodica che prevede una valutazione della frequenza delle coperture rilevata su 4 transect per ciascun plot (uno orizzontale, una verticale e due diagonali).
Il monitoraggio della produzione di tartufo è stato sia qualitativo che quantitativo ogni 10 giorni nel periodo previsto dal calendario di raccolta.

Risultati e Discussione
La tartufaia naturale oggetto di studio - Monte Nebbiaio- ricade nel Comune di Castell'Azzara (GR) a pochi km dal centro abitato, nei pressi della sorgente del Monte Penna, ad un'altitudine di circa 1000 m s.l.m.
Nell'area indagata la specie arborea più diffusa è *Quercus cerris* L., altra specie ritrovata in tutti plot esaminati fatta eccezione per il 5 è *Pinus nigra* Arnold. La presenza di questo taxon negli altri plot è comunque da considerare sporadica. Nel sottobosco trovano spazio specie arbustive come *Crataegus monogyna* Jacq., *Euonymus europaeus* L. e *Cornus mas* L. Il piano erbaceo è costituito prevalentemente da *Alliaria petiolata* (Bieb.) Cavara et Grande, *Geranium robertianum* L. e *Smyrnium perfoliatum* L.
In tabella 1 si riportano i principali caratteri meteorologici registrati durante il periodo di studio (2005-2007) nell'area oggetto di indagine.

Tab. 1 Principali caratteri meteorologici registrati durante il periodo di studio (2005-2007) nell' area oggetto di indagine.

EUTM [m]	712527
NUTM [m]	4734225
Altitudine [m s.l.m.]	494
Distanza dalla costa [Km]	54
Temperatura media annua (°C)	12,7
Temp. media mese + caldo (°C)*	24,3
Temp. media max mese + caldo (°C)*	30,3
Temp. media mese + freddo (°C)**	4,3
Temp. media min mese + freddo (°C)**	1,9
Precipitazione media annua (mm)	747,7
N° giorni con temp. media ≥ 10°C	227
N° giorni piovosi	105
Indice concentrazione stagionale delle pioggie (inverno)	0,77
Indice concentrazione stagionale delle pioggie (primavera)	0,7
Indice concentrazione stagionale delle pioggie (estate)	0,9
Indice concentrazione stagionale delle pioggie (autunno)	1,45
F-FAO	93
Pluviofattore di Lang	56
Indice di De Martonne (IA)	34
Quoziente pluviometrico di Emberger (Q)	83

*: mese + caldo di ciascuna annata del periodo
**: mese + freddo di ciascuna annata del periodo

La temperatura media annua si aggira intorno ai 12.7°C. Il mese più caldo è luglio, con una temperatura media che supera i 30°C. Febbraio (subordinatamente gennaio e dicembre) è solitamente il mese più freddo con media 7.2°C da notare come questo valore sia aumentato di

circa 3°C negli ultimi tre anni (2005: 5.8°C; 2007: 8.9°C). Il numero medio di giorni con temperatura media maggiore o uguale a 10°C (Tab.1), considerato un indice della durata potenziale della stagione vegetativa (Gregori *et al.,* 2004), è risultato abbastanza elevato (Tab. 1).
La precipitazione media annua si attesta intorno ai 747.7 mm e contrariamente alle temperature questo valore è andato diminuendo nel corso dei tre anni di studio, passando dai 1069 mm misurati nel 2005 ai 491 del 2007. Da evidenziare come i valori relativi alle precipitazioni risultino molto più bassi rispetto a quelli riportati da Barazzuoli & Salleolini (1993) che indicano per l'area di studio precipitazioni medie annue comprese fra gli 1400 e 1450 mm. Per i primi due anni i mesi in cui si sono registrate le maggiori precipitazioni sono quelli tipicamente autunnali, mentre nel 2007 è stato nel mese di febbraio che si sono verificati i maggiori fenomeni. La ripartizione stagionale media delle precipitazioni risulta abbastanza omogenea: gli indici di concentrazione stagionale delle piogge sono infatti compresi nell'intervallo 0.70-1.45 (Tab. 1). Il minor numero di giorni piovosi (1-5 giorni) si registra sempre nei mesi tipicamente estivi (luglio e agosto), mentre ottobre, novembre e dicembre ne presentano mediamente il triplo.
L'indice di Fournier modificato (F_{FAO}) evidenzia un modesto grado di concentrazione mensile delle piogge, risultando inferiore a 100 (Tab.1),
Fra gli indici tradizionalmente utilizzati per l'analisi climatica e riportati in Tab. 1, il pluviofattore di Lang individua condizioni climatiche abbastanza piovose e non eccessivamente calde. L'indice di De Martonne testimonia condizioni climatiche temperate di ambienti prettamente forestali come sottolineato anche dal quoziente pluviometrico di Emberger (Tab. 1). Questi valori sono perfettamente in linea con quelli rilevati in altre tartufaie in Toscana e più in generale nell'Italia centrale (ARSIA, 1995; Tocci, 1985; Tocci *et al*., 1995).
In tabella 2 si riportano i dati relativi ai principali parametri pedologici rilevati.

Tab. 2 principali parametri pedologici rilevati nell'area di studio.

	SABBIA (%)	LIMO (%)	ARGILLA (%)	classe USDA	pH	CONDUCIB (mS/cm)	CALCARE (%)	SOST.ORG (%)	Densità apparente (gr/cm3)	pietrosità sup. (%)	grado struttura	scheletro (%)	PROFONDITA' (cm)
Plot 1	42	30	28	FA	4,8	0,065	0	6,8	1,11	1	3	0	M
Plot 2	41	31	28	FA	5,8	0,409	0	21,86	0,8	10	4	2	M
Plot 3	42	28	30	FA	6,7	0,239	0	11,11	1,19	20	4	5	S
Plot 4	32	36	32	FA	5,7	0,119	0	13,7	0,9	5	5	5	M
Plot 5	34	36	30	FA	5,5	0,174	0	18,89	0,83	5	4	2	M
Plot 6	35	36	29	FA	5,1	0,083	0	14,21	0,77	5	5	1	M
Plot 7	37	33	30	FA	5,7	0,112	0	16,79	0,79	1	5	1	M
Plot 8	38	29	33	FA	5,5	0,11	0	18,86	0,63	3	5	5	M
Plot 9	39	27	34	FA	6,1	0,313	0	16,78	0,77	5	4	5	M
Plot 10	35	32	33	FA	5,2	0,061	0	6,99	1,28	1	4	2	M

Legenda - grado di struttura: 2 massivo (assenza di struttura); 3 debolmente strutturato; 4 moderatamente strutturato; 5 fortemente strutturato. Profondità: S - suoli sottili (roccia entro 50 cm); M - suoli moderatamente profondi (roccia fra 50 e 100 cm); P - suoli profondi (roccia oltre 100 cm). Classe tessiturale: F – franca; FS - franco sabbiosa; FA - franco argillosa; A – argillosa; FAS - franco sabbioso argillosa.

I suoli rilevati nella tartufaia presa in esame sono moderatamente profondi poiché si ritrovano intorno a 80-100 cm di profondità e significativi impedimenti all'approfondimento radicale sono dovuti al substrato roccioso, costituito in prevalenza da calcari marnosi. I suoli sono in genere formati da un orizzonte organico costituito dalla lettiera di cerro, acero e pino nero, spessa

intorno ad un centimetro, a cui fa seguito un orizzonte A di accumulo di sostanza organica, di colore bruno grigio molto scuro, con strutture grumose associate a strutture poliedriche di piccolissime dimensioni, molto sviluppate, spesso circa 3-5 cm. Al di sotto è presente un orizzonte B cambico, bruno o bruno scuro, ancora in parte interessato dalla presenza di sostanza organica ma in misura assai minore, e con strutturazione poliedrica moderatamente sviluppata. Questo orizzonte si estende fino a 70-100 cm ove si ritrova il substrato roccioso inalterato. In tutti i suoli non sono stati mai osservati fenomeni di ossidoriduzione causati da ristagni idrici anche temporanei; tutti i suoli, negli orizzonti superficiali sono privi di calcare ed hanno una tessitura franco argillosa, con contenuti in argilla (Tab.2) variabili da 28 a 30% e di sabbia (Tab. 2) da 32 a 37%; lo scheletro di tipo calcareo è generalmente scarso (2-5%).
La densità apparente (Tab. 2) è in genere molto bassa da 0,63 a 1,28 (mediamente 0,91) e ciò è dovuto al forte grado di strutturazione degli aggregati di suolo che formano un sistema assai poroso. In particolare ciò sembra manifestarsi per i plot ubicati sulla spalla del versante (n. 4, 5, 6, 7, 8 e 9) ove il fenomeno è più evidente. La reazione del suolo (Tab. 2) presenta in quest'area valori di pH assai bassi, da 4,8 a 6,7 (mediamente 5,6); in particolare i suoli del plot 1 hanno un pH di 4,8, ma ciò sembra essenzialmente dovuto al differente substrato litologico su cui i suoli si sono originati, di tipo scistoso, non calcareo, e ciò è confermato anche dal colore più olivastro dei suoli. Nel plot 3 il pH di 6,7, il più alto dell'area, è dovuto alla presenza di abbondante pietrosità e rocciosità superficiale (Tab. 2) che riescono a tamponare gli effetti del processo di acidificazione del suolo.
Nell'estate del 2005 sono state effettuate le misurazioni delle coperture arboree e arbustive nei 10 plot oggetto di indagine (Tab. 3) Tali misurazioni si sono poi ripetute anche nel 2006 in seguito all'intervento di diradamento.

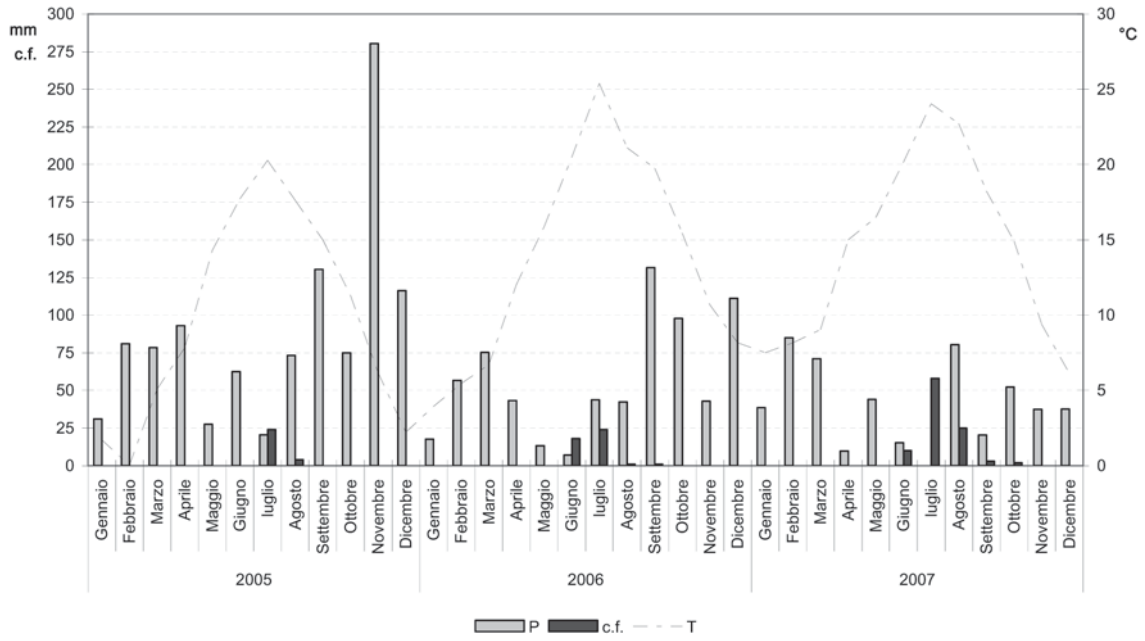
Tab. 3 Sintesi delle percentuali di copertura arborea e arbustiva rilevate nei 10 plot

		% copertura arborea	% copertura arbustiva
Plot 1	**pre diradamento**	78,85	23,08
	post diradamento	70,00	5,00
Plot 2	**pre diradamento**	85,90	21,48
	post diradamento	56,50	3,30
Plot 3	**controllo**	97,51	9,94
Plot 4	**pre diradamento**	73,08	27,56
	post diradamento	54,40	29,70
Plot 5	**pre diradamento**	82,70	21,15
	post diradamento	63,90	15,50
Plot 6	**controllo**	90,51	0,63
Plot 7	**controllo**	97,48	13,84
Plot 8	**pre diradamento**	84,61	42,30
	post diradamento	48,70	39,00
Plot 9	**controllo**	93,08	5,03
Plot 10	**controllo**	93,83	10,43

Nei plot in cui è stato eseguito l'intervento forestale in media le specie arboree coprivano l'81.03% e quelle arbustive il 27.11%. La maggiore percentuale di copertura era stata misurata nel plot 2 (85.90%), mentre il plot 4 era quello più aperto (73.08%). Il plot in cui era maggiormente sviluppato lo strato arbustivo (42.30%) era il plot 8.
In seguito all'intervento di diradamento la percentuale di copertura arborea è diminuita di circa il 30% (Tab. 3). Il plot in cui è stato asportato il maggior numero di piante è stato il plot 8 in cui da una copertura superiore all'80% si è passati ad una copertura inferiore al 50%.
I plot di controllo sono quelli in cui non è stato fatto alcun tipo di diradamento. Il controllo è necessario in qualsiasi tipo di sperimentazione affinché sia possibile evidenziare gli effetti che dipendono dai parametri esterni a quelli che si intende valutare (Dytham, 1999).

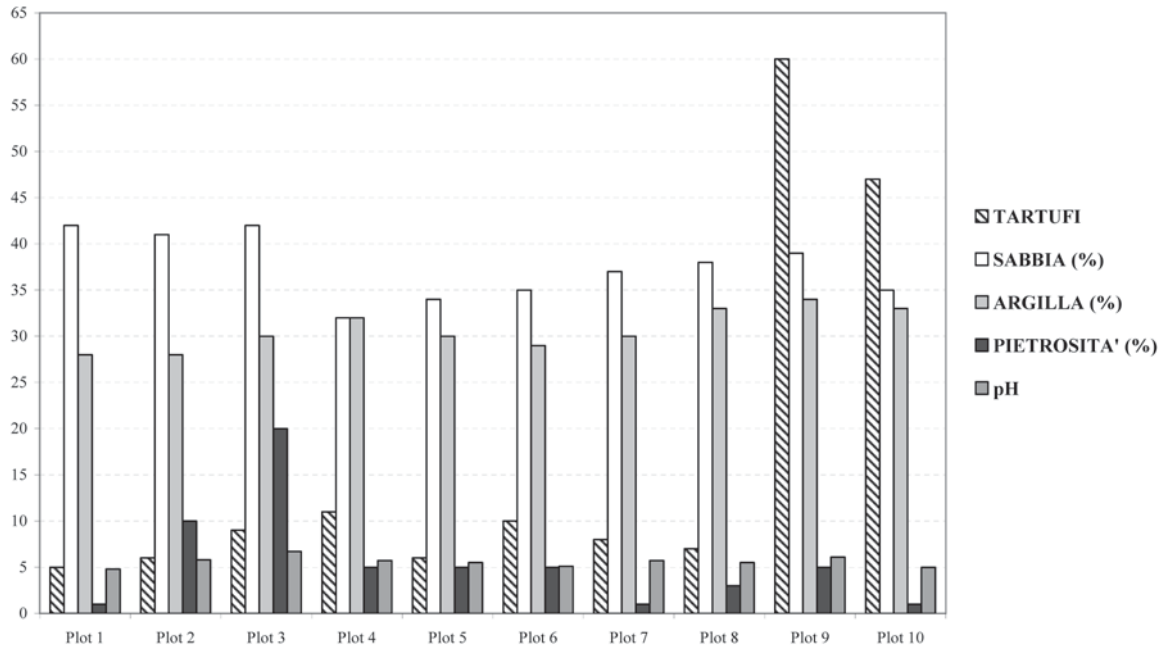
Durante i primi tre anni di indagine (2005-2007) in totale sono stati contati 169 ascomi di *Tuber aestivum*. In Fig. 2 viene riportato graficamente l'andamento termopluviometrico dei 3 anni registrato nell'area di studio, messo in relazione con la produzione di tartufi. Il maggior numero di corpi fruttiferi è stato osservato nel luglio del 2007 (58 ascomi). È interessante notare come questo aumento produttivo abbia fatto seguito ad una stagione invernale non particolarmente fredda e abbastanza siccitosa (Fig. 2). Tali condizioni sembrerebbero non aver impedito l'accrescimento miceliare necessario affinché avvenga la produzione di corpi fruttiferi (Witkamp,1960; Nagel-De Boois & Jansen, 1971).

Figura 2 - Temperatura media , precipitazioni e n° di tartufi raccolti nei primi tre anni di studio



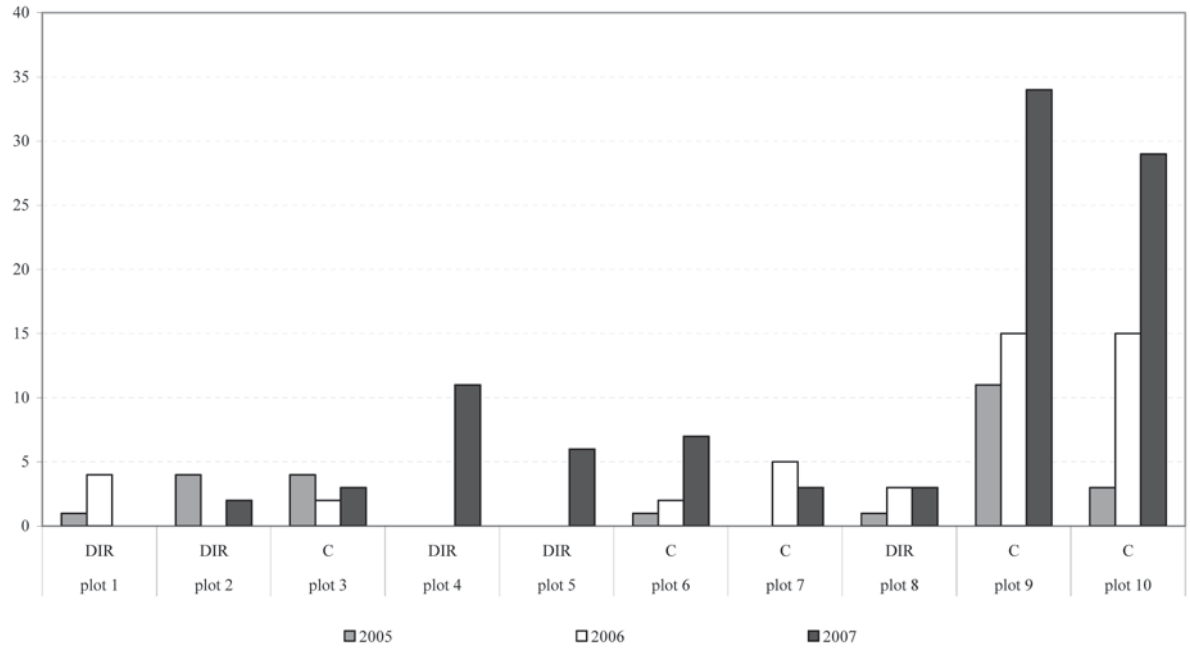
Mettendo in relazione il numero di ascomi di *Tuber aestivum* osservato con alcuni dei parametri pedologici disponibili (% di argilla, pietrosità, sabbia e pH) emerge che il maggior numero di tartufi è stato contato nel plot 9 (Fig. 3) in cui la % di argilla è la più elevata (34%). Anche nel plot 10 il numero di ascomi raccolto è stato ragguardevole (47 ascomi) sebbene il pH misurato in quest'area sia inferiore a 5 (Fig. 3). Il minor numero di corpi fruttiferi è stato osservato nel plot 1 (5 ascomi) in cui è stata misurata la più elevata percentuale di sabbia (42%) e in cui il pH è risultato relativamente acido (4.8). Tale dato confermerebbe la predilezione dei tartufi per suoli basici o al massimo neutri.

Figura 3 - pH, percentuale di argilla, pietrosità, sabbia e numero di tartufi osservato nella tartufaia



In figura 4 si riporta l'andamento della fruttificazione nei 10 plot durante i tre anni di studio, mettendo a confronto i dati rilevati nei 5 plot diradati (plot 1, 2, 4, 5, 8) con quelli di controllo. Il maggior numero di tartufi è stato contato nei plot di controllo 9 e10 (34 e 29 rispettivamente), mentre nel plot 1 non è stato trovato nessun ascoma.
Rispetto alla situazione iniziale (2005) si è verificato un incremento di produttività nei tre anni di indagine. Tale dato per il 2006, anno in cui è stato operativamente fatto il diradamento, è probabilmente da mettere in relazione con la recinzione dell'area che ha impedito l'accesso della fauna selvatica. L'aumento di produzione dell'anno successivo, invece, potrebbe essere effettivamente imputabile agli interventi selvicolturali come testimoniato anche dalla comparsa di ascomi nei plot 4 e 5.

Figura 4 - Numero di ascomi di *Tuber aestivum* rilevato in ogni plot durante i tre anni di studio (DIR - plot sottopodti a diradamento; C - plot di controllo)



Conclusioni

Scopo del presente progetto sperimentale era quello di chiarire i rapporti fra copertura arborea e la produzione di ascomi di tartufo scorzone e sebbene, quelli riportati in questa sede, siano dati preliminari è comunque possibile trarre alcune indicazioni: le analisi climatologiche, pedologiche e forestali hanno confermato l'idoneità dell'area scelta.

L'andamento anomalo del clima registrato nell'ultimo anno di indagine sembra aver favorito i processi di fruttificazione, visto che è stato proprio durante il 2007 che si è osservata la maggiore produttività.

L'analisi pedologica ha evidenziato delle differenze fra i singoli plot che sembrano influire sui processi di fruttificazione e conferma come la conoscenza dei principali parametri pedologici sia importante per la crescita e sviluppo dei tartufi.

L'ipotesi che la recinzione, impedendo l'accesso all'area agli animali selvatici, abbia favorito una ripresa di produttività, conferma come l'eccessiva pressione della fauna abbia un effetto negativo su tutti i prodotti del sottobosco.

Inoltre dopo l'intervento di diradamento sono stati ritrovati sporomi di *Tuber aestivum* anche in plot improduttivi, tale risultato, sebbene preliminare, potrebbe essere messo in relazione proprio con gli interventi selvicolturali effettuati.

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THE SWEDISH POPULATIONS OF *TUBER AESTIVUM*

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Abstract

The Burgundy truffle *Tuber aestivum* (syn. *T. uncinatum*) grows naturally on the Swedish islands of Gotland and Öland in the Baltic Sea. The climate is drier and colder there than in Central Europe (*e.g.* Burgundy) and Gotland is the northmost reported locality for *T. aestivum*. As yet *T. aestivum* has not been found on the Swedish mainland. In previous studies the truffle populations on Gotland have been shown to consist of closely related genotypes, implicating a single introduction or repeated introductions from the same origin. Despite the genetic homogeneity, local truffle hunters have reported differences in the organoleptic properties of the truffles between different sites. In order to test if chemical differences could be site specific or correlated to genetic traits, we have initiated an ongoing study analyzing correlations between chemical and genetic variation, using chemical fingerprinting by liquid chromatography – mass spectrometry (LC-MS) and ITS sequencing respectively. The hypothesis that *T. aestivum* was introduced to the island shortly after the establishment of *Corylus avellana* after the last glaciation some 9000 years ago, is in accordance with the estimated date of divergence for the samples from Gotland investigated in this study.

Key words: Ecology, population biology, *Tuber aestivum*, biogeography, chemical profiling.

Introduction

Sweden has a funga of more than 65 different reported hypogeous ascomycete and basidiomycete species (Danell, 1996, Wedén *et al.,* 2001). Following a research project initiated in 1997, the gastronomically appreciated Burgundy truffle *T. aestivum,* was found to be widely distributed on the Swedish islands of Gotland and Öland (Fig. 1, Wedén 2004). Previous to this, only three records of the species had been made and there had been no historical local knowledge or use of this truffle from the Swedish islands. Another edible species, the Bagnoli truffle *T. mesentericum*, was only recorded recently from the island of Gotland (Wedén *et al*. 2001). In the genus *Tuber*, five species has thus far been recorded: *T. aestivum*, *T. mesentericum*, *T. rufum*, *T. maculatum* and *T. puberulum*. In addition, there is one possible observation of *T. rapaeodorum* (Landvik & Eriksson 1994). Since *T. aestivum* and *T. uncinatum* are synonyms for the same species and *T. aestivum* is the older of the two names, only *T. aestivum* is referred to here (Wedén *et al*. 2005).

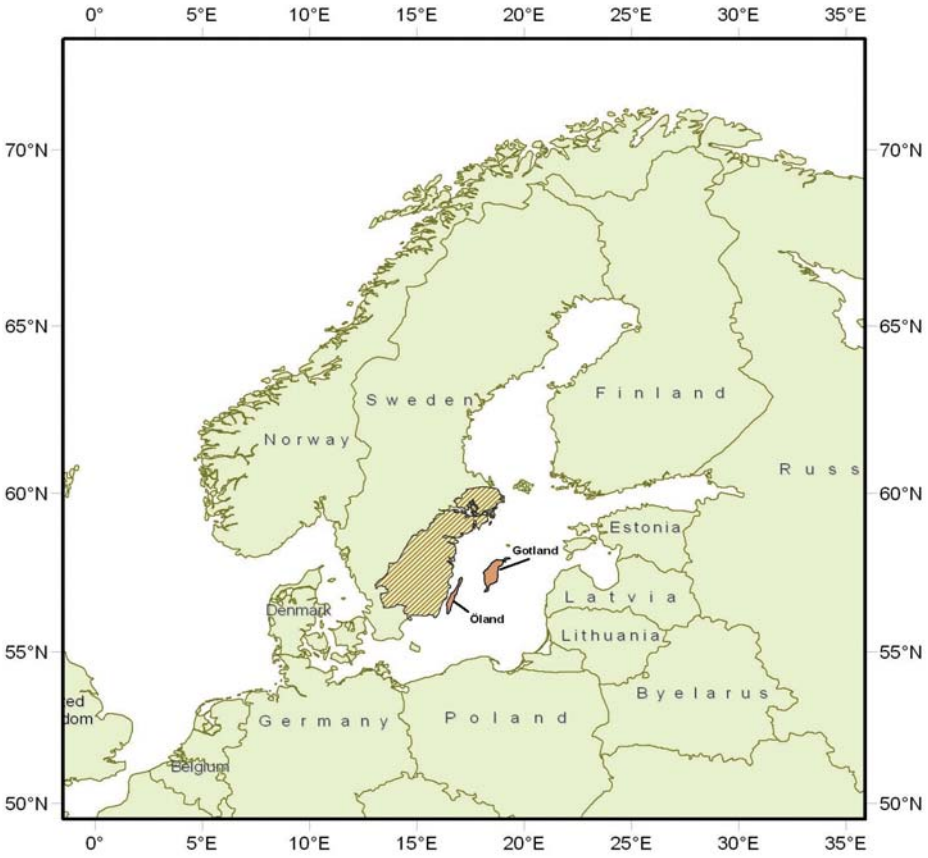

Fig. 1 Map of northern Europe with the Swedish regions of naturally occurring edible truffles: *Choiromyces venosus* (lined, orange); *Tuber aestivum* (filled, orange); *T. mesentericum* (only on the island of Gotland). *Choiromyces venosus* has only been found on mainland Sweden, while *Tuber aestivum* has only been found on the Swedish islands of Gotland and Öland. Illustration: Anders Larsson.

In Sweden *T. aestivum* commonly grows with *Quercus robur* and *Corylus avellana*, but it has also been found in a few pure *Fagus sylvatica* and *Tilia cordata* stands. A typical Swedish locality is characterised by an open forest with tree crowns shading the ground and sparse ground vegetation of vascular plants such as *Hepatica nobilis* (Wedén *et al.* 2004a). Soil analyzes from natural populations of *T. aestivum* on Gotland have shown great variations in soil chemistry and soil particle size, from sand to silt soils, with ranges from 80% sand to 65% silt respectively (Wedén *et al.* 2004a). At several occasions during the last few hundred millennia have large proportions of the northern hemisphere been covered by ice due to climate induced glaciations, the last one known as the Würm glaciation and ending ca 10000 BC. The effect on the geology and geomorphology of these repeated glaciations were tremendous. Not only was the land covered by a more than 1000 m thick ice shelf, which scraped of virtually all loose material and reshaped protruding parts of the bedrock, but the shear weight of the ice shelf pressed down the Earth crust immersing major parts of present day Sweden below sea level. Once the ice retreated and melted away, the crust started to reflex, resulting in a land-uplift, which still today is approximately 4 mm per annum in the Stockholm-Uppsala region. All land up to approximately 75 meters above present day sea level was during this period first the bottom of a gigantic fresh water lake fed by the melting sea, and later as the connection to the Atlantic ocean opened sea bottom, and then eventually becoming today's Baltic Sea with a brackish water. As the ice shelf started

to retreat and melt, large amounts of sand, gravel and stones were deposited, later partly covered with bottom sediments from the covering waters. The highest parts of the island of Gotland started to rise again above what was then a fresh water lake approximately 11 600 years ago. *C. avellana* was the first possible *T. aestivum* host tree to establish on Gotland some 9000 years ago. This detailed and well-established history of the island gives unique parameters for the calculation of *e.g.* date of divergence of *T. aestivum* from these data, and possible island biogeographic hypotheses for the mode of introduction, population dynamics and dispersal patterns on newly introduced land and also over large bodies of water might be tested.

Truffle cultivation was first initiated in Sweden by planting *T. aestivum*-inoculated *Q. robur* and *C. avellana* seedlings in 1999 (Wedén *et al.,* 2001; Wedén, 2004). The first cultivated truffle was found in 2005 and today around 6000 seedlings inoculated with Swedish *T. aestivum* have been planted (Wedén & Danell, 2007; Wedén *et al.,* 2009). In year 2009, 500 kg of top quality *T. aestivum* was sold from Gotland and contributed to BNP with approximately 3 MSEK.

Material & Methods

Ethanol extracts of 14 *T. aestivum* and one *T. mesentericum* fresh fruit bodies with mature spores from 15 different collection sites on the island of Gotland, Sweden, were analysed. LC-MS analyses were performed on a LCQ electrospray Ion Trap MS (Thermo Finnigan, San Jose, CA, USA), operated in positive-ion mode and fed by a Shimadzu LC10 system (Shimadzu, Kyoto, Japan). The capillary temperature was set at 220°C and the spray voltage at 4 kV. A portion of the extracts was injected into the LC-MS system, equipped with a Grace Vydac Everest C18 column (100 × 2.1 mm i.d., 5 µm, 300 Å), using a linear gradient from 10% ACN in 0.05% HCO_2H to 60% ACN in 0.045% HCO_2H operating at a flow rate of 0.3 ml/min and set to detect molecular masses of 100-2000 m/z. Marker ions were selected based on peak intensity and retention time.

Results & Discussion

In a previous molecular population study, *T. aestivum* from Gotland was found to consist of a closely related population possibly indicating a single introduction or several introductions from a similar source (Wedén *et al*., 2004b). Genotypes showed a normal distribution indicating sexual reproduction (Wedén, 2004). Despite the genetic homogeneity, local truffle hunters have reported differences in the organoleptic properties of the truffles between different sites. In the ongoing study of the Gotland *T. aestivum* population, we have aimed to investigate if chemical differences could be site specific or correlated to genetic traits. The molecular data set consists of sequences of the internal transcribed spacer region (ITS) from 14 fresh fruit bodies of *T. aestivum* from 14 different localities on Gotland.

Ethanol extracts of the same fruit bodies were analysed using LC-MS to create a corresponding data set of chemical profiles. The results from that analysis show that there exist distinct differences in their chemistry, as judged by different retention times and m/z values, but also in ion intensity and the mere number of peaks. There were also similarities: several peaks were found in more than one sample (Table 1, Fig. 2). One fruit body (sample T11) had a divergent smell and taste characteristic for fruit bodies from that specific collecting site. This sample is lacking *e.g.* the 318 m/z peak, which could be a reflection of its different organoleptic properties (Fig. 2). *T. mesentericum* is the closest known related species to *T. aestivum*. A *T. mesentericum* fruit body (sample T12) was therefore included in our study. It shows distinct differences from the *T. aestivum* samples, but also share peaks at *e.g.* 318 m/z (Fig. 2). In a study by Ekenäs *et al.* (2009) congruencies between DNA sequence data and the secondary chemistry of the vascular plant genus *Arnica* were demonstrated and investigated. This type of combined analysis has to our knowledge not previously been applied in studies of fungi.

Tab. 1 Occurrence of LC-MS chromatogram peaks in the 15 analysed *Tuber* samples.

m/z and (RT)	279 (4)	202 (6)	832 (9)	833 (10)	202 (11)	318 (15)	635 (19)	635 (19)	207 (21)
T1	X	-	-	-	-	-	-	-	-
T2	X	X	X	X	X	X	-	-	-
T3	X	X	X	X	-	X	-	-	-
T4	-	-	-	-	-	X	X	X	X
T5	X	-	-	-	-	X	-	-	X
T6	X	X	X	X	-	X	-	-	X
T7	-	X	X	X	X	X	X	X	X
T8	-	-	-	-	X	X	X	X	-
T9	-	X	X	X	X	-	X	X	-
T10	-	-	X	X	-	X	X	-	-
T11	-	X	X	X	X	-	X	-	-
T12	-	-	-	-	-	X	-	-	-
T13	-	X	X	X	-	X	X	X	X
T14	-	X	X	X	X	X	X	X	-
T15	-	-	X	X	-	X	X	X	X

Molecular mass dived by charge (m/z) and approximate retention time in minutes (RT) of peaks occurring in three or more of the 15 analysed *Tuber* samples. Peaks are recorded as present (X) or absent (-). Samples T1-T11 and T13-T15 are *T. aestivum* collected at 14 different sites on the island of Gotland, Sweden. Sample T12 is *T. mesentericum* collected on the island of Gotland, Sweden.

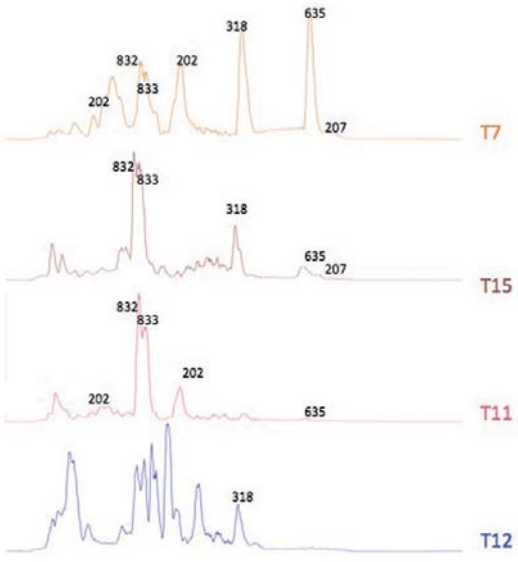

Fig. 2 Comparison of chemical profiles of LC-MS chromatograms from four different fruit body samples. Chromatograms show relative abundance in each sample (y-axis) and retention time (x-axis). Samples T7 and T15 are representatives of chemical profiles typical of *Tuber aestivum* in this study. Sample T11 represents a fruit body with a smell and taste specific for its collection site and divergent from typical *T. aestivum* organoleptic properties. Sample T12 represents a *T. mesentericum* fruit body. This species often co-exist with *T. aestivum* in Gotland habitats and is its closest known relative. The m/z values are given for peaks recorded in Table 1.

Acknowledgements
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ECOLOGYCAL ASPECTS OF TRUFFLE POPULATIONS IN SALENTO (SOUTHERN ITALY)

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Abstract
In this work are reported preliminary data on the ecology of *Tuber borchii* Vittad. e *T. aestivum* Vittad., that form beneficial symbiosis (ectomycorrhizas) with some of the most representative local wood vegetation, providing also an economic values, thanks to the production of edible hypogeous fruit bodies. The results regard some areas of Salento, where the natural truffiere correspond to vegetal balanced ecosystems (climax): *Quercus ilex* L. woods, *Pinus halepensis* Miller woods, woods were *Q. ilex* and *P. halepensis* co-grow.
Key words: *Tuber borchii* Vittad., *Tuber aestivum* Vittad., *Quercus ilex* L., *Pinus halepensis* Miller, Salento.

Introduction

For many years the Botanical Garden of the University of Salento has been employed in the environmental restoration with the aim to reconstruct the plant associations destroyed from the anthropic activities, by using local tree and shrubby species (Accogli *et al*., 2007). Much effort has been spent on the identification of ecological conditions such as the presence of mycorrhizal fungi (MF) which are ideal candidates for the introduction of structuring plant species. MF are able to form symbiosis with roots of both trees and shrub species, with the ability to increase plant growth and nutrient uptake (Smith and Read, 1997).
The presence of natural truffière is the testimony of a symbiotic relationship that is present only in good environmental conditions, both for plant and fungus (Granetti *et al.,* 2005). Moreover, herbaceous plants, shrubs and trees are indicators of well-defined pedo-climatic conditions. The truffière distribution, the floristic-vegetational aspects and the proximity to EU Community Interest Sites (pSIC) are essential information for the establishment of ecological networks and artificial truffière that will ensure the survival and spread of the mycorrhized structuring plant species, propagated ex situ before to be introduced in natural environments. In a first stage, has been made efforts to deepen knowledge about the ecology and distribution of the more representative hypogeous MF of the Salento area, *T. borchii* Vittad. and *T. aestivum* Vittad., of great interest also for organoleptic and nutraceutical aspects (Cornelli, 2004).

Matherials and methods

The sampling areas were chosen in Salento (Southern Italy) in relation to the future establishment of ecological networks. Fruit bodies, periodically collected (2 years, two times for each seasonal period) from natural truffière were located with the help of dogs (dogs were trained from Giuseppe Lolli) and identified with morphological and molecular analyses, as reported in Russo *et al*. (2008).
Samples were employed for the mycorrhizal inoculation of local selected plants (Bencivenga, 1982) propagated ex situ (Botanical Garden of University of Salento) and then introduced in natural environments.

Results

The results regard some areas of Salento, where the natural truffiere correspond to vegetal balanced ecosystems (climax): *Quercus ilex* L. woods, *Pinus halepensis* Miller woods, woods were *Q. ilex* and *P. halepensis* co-grow. The average age of holm oak woods in the natural truffière investigated was about 90 years and *Cyclamino hederifolii-Quercetum ilicis* Biondi,

Casavecchia & Gigante 2003 represent the more diffuse association confirming the data reported by Biondi *et al*. (2004). The average age of pine woods in the studied areas, mostly constituted of Aleppo pine wood, was about 60 years. Pines represent structuring species that were introduced in Salento by the Italian "Corpo Forestale dello Stato" during multiple local vegetal reconstruction processes.
In this study, the individuation of the plants involved in natural mycorrhizal symbiosis has been considered important for the introduction of mycorrhized plants in degraded areas, considering also the homogeneous climatic conditions and the ecological adaptability of *T. aestivum* and *T. borchii* (Baglioni et Mazzei, 1998; Hall *et al*., 2007). *Q. ilex* (Fig. 1) and *P. halepensis* (Fig. 2) represent the plants found in symbiotic association with the *Tuber* species collected (Russo et al., 2008).

Fig. 1 Quercus wood of Salento

Fig. 2 Pines wood of Salento

With the aim to verify if *T. aestivum* (Fig. 3A) and *T. borchii* (Fig. 3B) are able to for mycorrhiza with the most diffuse plant species of the studied area, sample of *T. aestivum* and *T. borchii* were employed for mycorrhizal inoculation of local seeds and seedlings belonging to the following species: *Quercus ilex* L., *Pistacia lentiscus* L., *Cistus creticus* L., *Quercus ithaburensis* Decaisne subsp. *macrolepis* (Kotscvhy) Hedge.

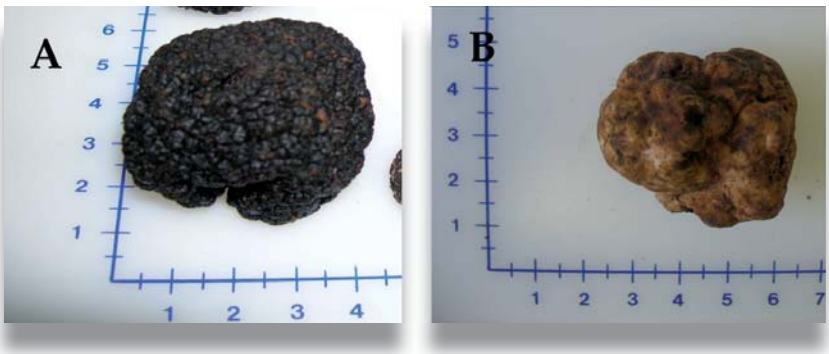

Fig. 3 Fruit bodies of *T. aestivum* Vittad. (A) and *T. borchii* Vittad. (B) collected in Salento.

Six months after inoculum, the plants mycorrhized with *T. aestivum* and *T. borchii* (data not reported) showed a normal development.
Preliminarily data regarding *Q. ilex* and *Q. ithaburensis* mycorrhized with *T. aestivum* indicate a plant mycorrhization of approximately 10%, six months after inoculum (Fig. 4). After about 18 months the percentage of mycorrhization became about 20% for both kind of plants, which will be employed for reforestation.

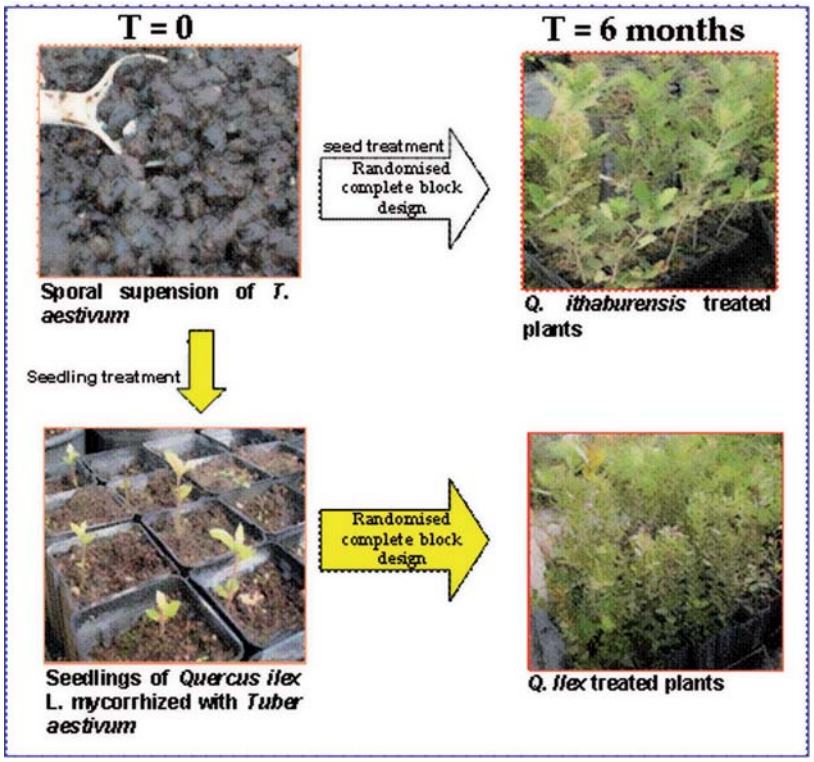

Fig. 4 *Q. ithaburensis* seeds and *Q. ilex* seedelings inoculated with sporal suspension of *T. aestivum* collected in Salento

Conclusion

Erbaceous and arboureus plant species of the natural truffière are important indicator of specifc pedo-climatic conditions; the evaluation of the more suitable truffière, their floristic-vegetational aspects as well as their position in relation to pSIC could provide useful information in the planning of reconstruction of degraded areas. Finally, from an economic point of view, the study has the goal to investigate the production breakdown of the natural truffière analysed.

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ECOLOGY AND DISTRIBUTION OF HYPOGEOUS FUNGI IN ARMENIA

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Abstract
This study reports on the distribution and biodiversity of hypogeous fungi in Armenia. Our collections were identified with morphological and/or molecular methods using rDNA-ITS sequence data. Twelve species were found belonging to Glomeromycota (*Glomus macrocarpum*), Ascomycota (*Elaphomyces muricatus, E. granulatus, Hydnotrya tulasnei, Tuber rapaeodorum, T. rufum, T. scruposum, Tirmania pinoyi, Picoa juniperi*) and Basidiomycota (*Hymenogaster griseus, H. olivaceus, Lactarius stephensii*). *L. stephensii* and *E. granulatus* have previously been found in Armenia and the South Caucasus region but the remaining 10 species are new records. The ecological characteristics of each of the hypogeous fungi are described. Our research more than doubles the number of hypogeous fungi now known in Armenia.
Key words: ecology, Armenia, hypogeous fungi, rDNA-ITS sequences.

Introduction

Studies on hypogeous fungi have both scientific and practical significance because of their role as the fungal partner in ectomycorrhizal associations with forest plants and the economical importance of some species belonging to the genus *Tuber* (Hall *et al.,* 2007). Some species such us *Tuber mesentericum* Vittad., *Tuber brumale* Vittad., *Tuber aestivum* Vittad., *Choiromyces meandriformis* Vittad. and *Terfezia arenaria* (Moris) Trappe, are reputed to have medicinal properties (Denisova, 1998, Tardif, 2000).
Armenia occupies an area of 29.800 km^2 in the mountainous region of the South Caucasus with an average altitude of 1700 m above sea level. The climate in Armenia is humid in the north and dry in south and has temperature extremes from +42 °C to –40 °C. The main soil types are mountainous meadows, grey sylvan, turf – carbonate sylvan, brown sylvan, black, brown, and saline-alkaline (Map of Soil of the republic of Armenia, 1990; Badalyan *et al.,* 2005). The principal forest-forming trees are *Quercus, Fagus*, *Carpinus*, *Pinus*, *Fraxinus* and *Acer* (Takhtajyan, 1936).
Few species of hypogeous fungi have been reported in Armenia: *T. aestivum* (Taslakhchyan & Nanagulyan, 1988), *Elaphomyces granulatus* Fr. (Nanagulyan & Taslakhchyan, 1991), *Lactarius (Octaviania) stephensii* (Berk.) Verbeken & Walleyn, *Rhizopogon roseolus* (Corda) Th. Fr., *Rhizopogon luteolus* Fr. & Nordholm, *Cremeogaster klikae* Mattir. (Melik-Khachatryan & Nanagulyan, 1983; Melik-Khachatryan & Martirosyan, 1971; Nanagulyan & Taslakhchyan, 1991) . Recently Badalyan *et al.* (2005) described three *Tuber* sp. *(T. rapaeodorum* Tul. & C. Tul., *T. scruposum* R. Hesse and *T. rufum* Pico) new species for Armenia. In this paper a more complete collection of Armenian hypogeous fungi is reported.

Material and Methods

Fruiting bodies of hypogeous fungi were collected in Armenian forests between 2002 and 2005 near Dilijan, Vanadzor, Kapan, Ijevan and Meghri (Fig. 1). The hypogeous fruiting bodies were found using a garden cultivator as described by Trappe and Castellano (2007). The fruiting bodies were morphologically identified (Pegler *et al.*, 1993, Montecchi & Sarasini, 2000, Ceruti *et al.*, 2003) and the dried specimens stored in the herbarium of the Dipartimento di Protezione e Valorizzazione Agroalimentare (CMI Unibo), University of Bologna (Italy).

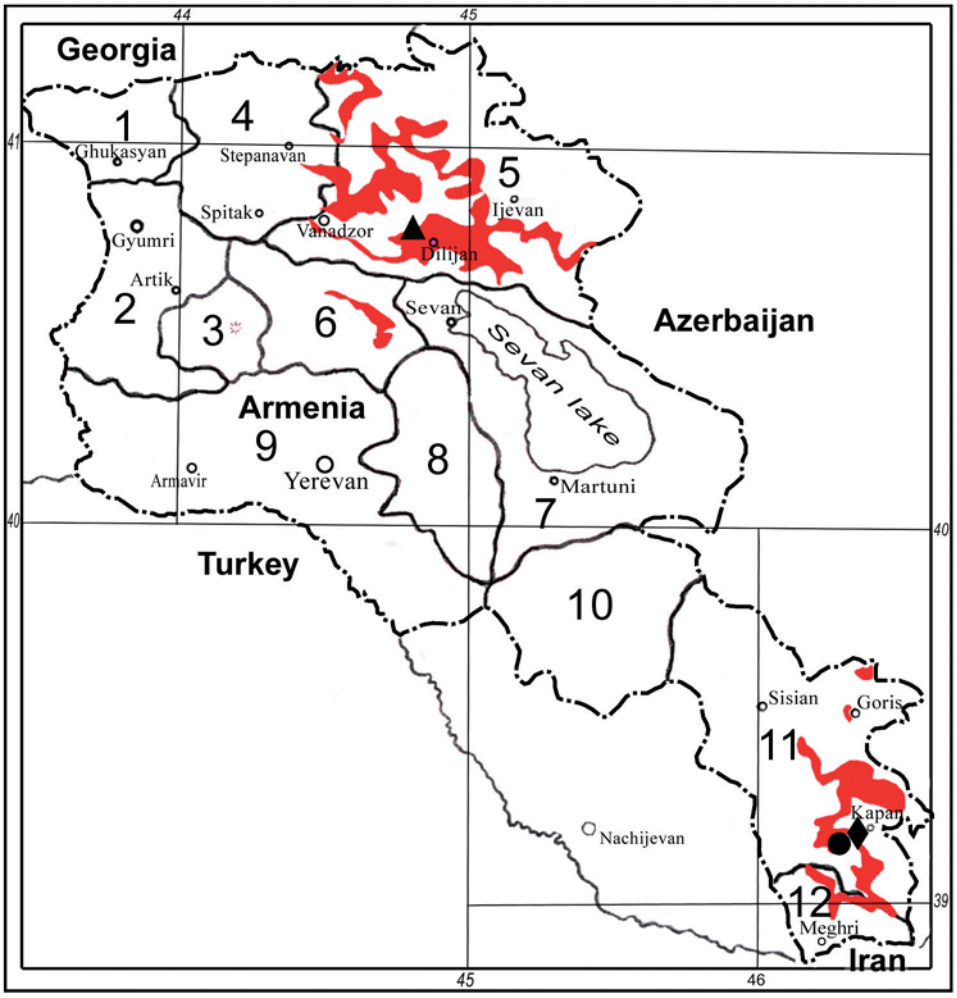

Fig. 1 Map of flora regions of Armenia (Takhtajyan 1978): 1 – Upper-Akhuryan, 2 – Shirak, 3 – Aragats, 4 – Lori, 5 – Ijevan, 6 – Aparan, 7 – Sevan, 8 – Geghama, 9 – Yerevan, 10 – Daralagez, 11 – Zangezour and 12 - Meghri. The regions that are likely to favour the growth and cultivation of truffles in Armenia are marked in red.

Molecular analysis of samples of fruiting bodies were performed using sequence data of the ITS regions of the ribosomal DNA as reported previously (Badalyan *et al.,* 2005). Ru1f species-specific primer and ITS4 were used to identify *T. rufum* ascomas (Iotti *et al.,* 2007). The ITS sequences of *T. scruposum* and *T. rapaeodorum* were deposited on GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/) with the accession numbers DQ011845, DQ011846, DQ011847, DQ011848 and DQ011849, DQ011850 respectively (Badalyan *et al.*, 2005).

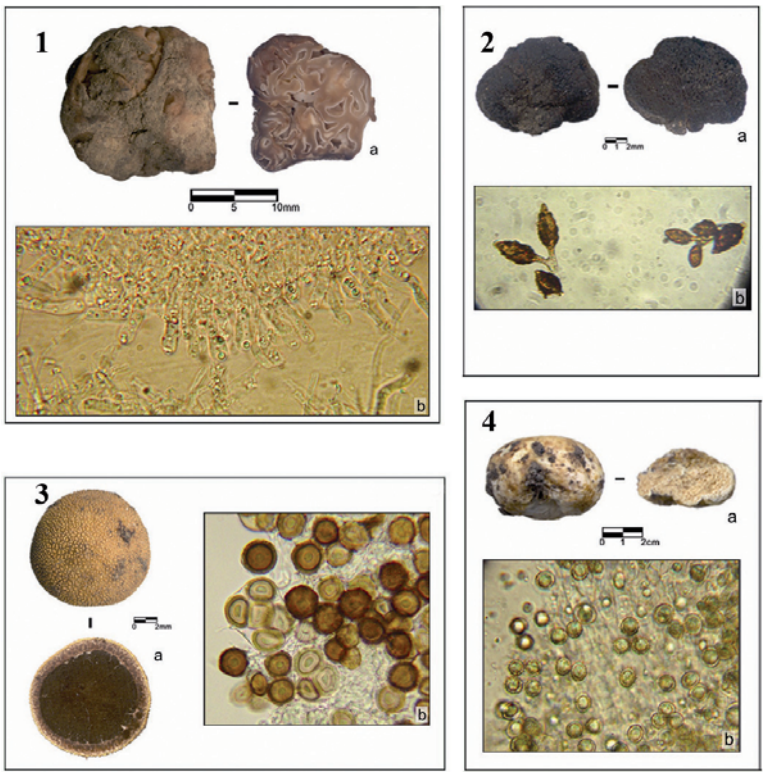

Fig. 2: 1 - *Hydnotrya tulasnei*; 2 - *Hymenogaster olivaceus* 3 - *Elaphomyces muricatus*; 4 - *Lactarius stephensii*; a - fruiting bodies, b - asci/basidia and/or spores.

Results and Discussion

The fruiting bodies were found in northern and in southern part of Armenia (850-2300 m asl) in the humus layer of sandy-clayish forest soils under *Fagus orientalis*, *Carpinus betulus, Tilia cordata, Corylus avellana* and *Pinus* sp. (Fig. 1).

Twelve taxa were found: *Tuber scruposum, T. rapaeodorum* and *T. rufum* (Tuberaceae, Pezizales), *Tirmania pinoyi* (Maire) Malençon (Pezizaceae), *Picoa juniperi* Vittad. and *Hydnotrya tulasnei* (Berk.) Berk. & Broome (Discinaceae, Pezizales), *Elaphomyces muricatus* Fr., *E. granulatus* (Elaphomycetaceae, Eurotiales), *Hymenogaster griseus* Vittad. and *Hymenogaster olivaceus* Vittad. (Strophariaceae, Agaricales, Basidiomycota), *L. stephensii* (Russulaceae, Russulales) and *Glomus macrocarpum* Tul. & C. Tul. (Glomeraceae, Glomerales, Glomeromycota). Ten species, all those listed above except *L. stephensii* and *E. granulatus*, were first records for Armenia and the South Caucasus region.

T. scruposum and *T. rapaeodorum* are generally found only in the central and northern parts of Europe (Ceruti *et al.*, 2003) and so finding them in the South Caucasus region extended the distribution of these species considerably. The *T. rapaeodorum* sequences from the Armenian collections seemed to be more closely related to *T. rapaeodorum* from Transylvania (Romania) and are somewhat different from other *T. rapaeodorum* sequences deposited in GeneBank (Bratek personal communication). *Tuber aestivum*, which was previously reported by Taslakhchyan & Nanagulyan (1988) for Armenia was not found in our three year survey. Trained dogs will be used in future in order to systematically explore the areas having ecological characteristics suitable for this truffle.

A rough comparison of the climatic characteristics of areas of Europe where *Tuber aestivum* and the other commercial species of truffle fruit have been tabulated by Hall *et al.* (2008). If we compare these data with the general climate in Armenia (Plant Genetic Resources in Central Asia and Caucasus, 2003, Bagdasaryan, 1962) and by superimposing our map of truffle distribution (Fig. 1) on the map of the precipitation in Armenia (Plant Genetic Resources in Central Asia and Caucasus, 2003) we can make some comparisons. In Armenia hypogeous

fruiting bodies were often found where annual precipitation was 500-800 mm, there were 2700 hours of sunshine and the mean daily temperature in summer are similar to those in Spain, whereas the mean daily winter temperatures (under 0 °C) are more similar to those on Gotland (58°N), the most northern location where *T. aestivum* has been found and cultivated (Table 1).

Tab. 1 Climate conditions in regions of Armenia where hypogeous fruiting bodies were collected (Bagdasaryan, 1962)

Location	Annual precipitation (mm/year)[1]	Air temperature		Monthly precipitation in summer (mm/ month)
		Winter[1] (°C)	Summer[2] (°C)	
Alaverdi	583	–2.7 - +1.2	15.0 - 20.3	71.0
Vanadzor	571	–5.0 - -0.4	14.7 - 19.3	72.0
Dilijan	576	–2.1 - + 1.2	15.3 - 19.6	68.0
Ijevan	552	–0.7 - +3.1	17.6 - 22.6	66.3
Kapan	531	–0.5 - +3.8	19.6 - 24.7	43.3

[1]In the highest altitude where the hypogeous fungi where collected in Armenia (2000-2300 m asl) mean winter temperatures can range from -4 to -6 °C, sometimes up to –20°C and annual precipitation is 700-800 mm (Bagdasaryan, 1962).
[2]In some productive areas mean summer air temperatures can be up to +33°C.

Wide seasonal variations are also found in Hungary where *T. aestivum* is particularly abundant (István & Oszkár, 2007; Hong Kong Observatory, 2003). Consequently, despite the harsh winters and very hot summers, which characterise the climate, we believe that it should be possible to cultivate *T. aestivum* and perhaps other commercial species in Armenia.

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CARTE DI ATTITUDINE DEI SUOLI AI TARTUFI IN PIEMONTE METODOLOGIE E RISULTATI

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Abstract: Soil suitability to truffle in Piedmont region: methodology and results
This work, financed by Piedmont Region, concerned *Tuber magnatum, T. melanosporum and T. aestivum*.
Suitability maps to truffle were realised firstly at 1:250.000 scale for the overall region, secondly at 50:000 scale for a hilly area (500.000 hectares) where truffle harvest is practised.
The methodology used to produce suitability maps can be summarised as it follows.

- Bibliographical research.
- Interpretation of aerial photograph and remote sensing images on surveyed land.
- Land Units characterisation and drawing on topographic maps, according to main features (geomorphology, lithology, land use).
- Pedological survey by soil profiles (120) and cores (700); chemical-physical analyses on 400 samples; data were integrated by pre-existing data from the regional soil database.
- Definition of the main Soil Types and classification according to Soil Taxonomy (USDA-1999).
- Field verification of Land Units and Soil Types.
- Realisation of an evaluation table for the attribution of FAO Land Suitability Classes.
- Printing of the Land Units map.
- Printing of the Suitability Maps to the three species of truffle, according to the evaluation table of their specific environmental requirements, created by a synthetic work from bibliography and field experiences both.

In this evaluation table variability of dominant soils characteristics was classified according to the different pedo-environmental requirements, in order to attribute every Land Unit to FAO suitability classes (FAO Land Evaluation, 1976 and 1983).
Land was consequently divided into 4 suitability classes (high, moderate, low, very low), which were attributed to the three different truffle species, so that 3 specific maps were created, at 1:250.000 and 1:50.000 scale both.
The two maps show a complete frame of the truffle productive potential of the overall region, according to the resolution limitations of the reference scales.
It must be underlined that these maps show the truffle productive potential, and not the present use and management, which has a strong influence beside a land suitability evaluation.
Key words: Land evaluation; soil maps; cultivation.

Premessa
I tartufi sono prodotti tipici regionali che vantano una notevole diffusione in tutta l'area collinare piemontese, che risulta essere la regione maggiormente vocata a livello nazionale. Essa corrisponde al cosiddetto "Bacino Terziario Piemontese", ed è costituita da antichi depositi marini – in prevalenza limosi e sabbiosi - che in epoche geologiche lontane si sono sollevati ed hanno subito importanti processi erosivi (ancora oggi attivi), principali agenti della genesi dell'attuale paesaggio tipico dei rilievi delle Langhe, del Monferrato, del Roero, dei Colli Tortonesi, della Collina di Torino.
Il tartufo bianco, in particolare, è un prodotto spontaneo del territorio di elevatissimo valore unitario, in ragione della sua rarità, ed è il motore di un ampio indotto economico che coinvolge il settore turistico e quello enogastronomico. Le numerose fiere e sagre del tartufo hanno, infatti, la prerogativa di attrarre i consumatori nelle zone tipiche di produzione, valorizzando anche tutti gli altri prodotti di qualità del territorio collinare regionale.

Da anni la Regione Piemonte incentiva lo sviluppo della tartuficoltura sia attraverso la conservazione di tartufaie naturali (in particolare per il bianco) sia per la costituzione di nuovi impianti tartufigeni di tartufo nero, coltura estremamente interessante per lo sviluppo economico di aree collinari e pedemontane marginali.
Essa è intervenuta con importanti strumenti legislativi a normare la raccolta, la coltivazione, la conservazione e la commercializzazione di questa peculiare risorsa del territorio. In questo senso la "Carta di attitudine alla produzione del tartufo bianco pregiato" rappresenta un esempio di concreta applicazione poiché è stata utilizzata, a livello legislativo regionale, per l'attualizzazione della superficie territoriale massima delle aree da destinare a tartufaie e nelle quali riservare la raccolta.

Metodologia
Le Carte d'Attitudine dei suoli per i tre tartufi (*Tuber magnatum* Pico, *Tuber melanosporum* Vittad. e *Tuber aestivum* Vittad.) sono state realizzate con il finanziamento della Regione Piemonte, dapprima a scala regionale (1:250.000) per l'intero territorio piemontese e, successivamente, sviluppate alla scala di semidettaglio (1:50.000) su oltre 500.000 ha che comprendono le superfici sulle quali viene tradizionalmente effettuata la raccolta dei tartufi.
L'impostazione metodologica del lavoro ha previsto, per entrambe le scale di realizzazione delle cartografie, una successione di attività che possono essere sinteticamente riassunte come segue.

Esame dei dati e degli studi disponibili
Le cartografie a scala regionale sono state realizzate a partire dai dati della Carta dei Suoli del Piemonte in scala 1:250.000; da questa base di conoscenze si è quindi proceduto direttamente alla realizzazione della *land suitability*, come specificato di seguito. Per le cartografie a scala di semidettaglio sono state invece individuate, attraverso la consultazione del Sistema Informativo Pedologico, le aree coperte dalle Carte dei Suoli a scala 1:50.000 già realizzate dall'IPLA ed estratti i dati pedologici disponibili. Si è, quindi, proceduto all'identificazione delle superfici ancora da rilevare del tutto e di quelle ove era necessario effettuare alcuni approfondimenti. Su queste porzioni di territorio sono state realizzate le Carte delle Unità di Terre a scala 1:50.000. Questo metodo di suddivisione del territorio fa riferimento alla "Classificazione delle terre" degli autori australiani (C.S.I.R.O.) che prevede un rilevamento del territorio di "tipo integrato".
La Carta delle Unità di Terre individua, a partire dagli elementi visibili del paesaggio (morfologia, geologia, uso del suolo), aree omogenee alle quali risultano associate, a seguito del rilevamento pedologico, determinate tipologie di suoli; mette cioè in relazione suoli e paesaggi.

Fotointerpretazione
Questa fase preliminare del rilevamento pedologico ha riguardato: l'esame stereoscopico di fotografie aeree, l'osservazione di immagini satellitari e l'elaborazione di dati relativi al modello digitale del terreno.
La visione stereoscopica dei fotogrammi disponibili ha permesso la stratificazione dell'area di studio: il risultato è stato l'inquadramento del territorio in Unità di Terre e la loro delimitazione sulla carta di rilevamento.
L'elaborazione a video di immagini da satellite disponibili è stata utilizzata per controllare e convalidare le Unità definite. Inoltre, a partire dalla matrice del modello numerico dell'altimetria (DEM - Digital Elevation Model), sono stati elaborati i livelli informativi relativi a quota, pendenza ed esposizione. Queste informazioni sono state utilizzate per affinare ulteriormente il documento cartografico preliminare.

Indagine di campagna
Ad una prima verifica in campo delle Unità di Terre individuate è seguita la fase di rilievo di campagna che è consistita nell'esecuzione di trivellate manuali (circa 700) e di profili pedologici (circa 120), volta alla definizione delle principali Tipologie Pedologiche presenti.

Per ogni profilo sono state descritte le caratteristiche stazionali e degli orizzonti minerali che sono stati tutti campionati.
Tali dati si sono aggiunti alle migliaia di osservazioni già archiviate nel Sistema Informativo Pedologico.

Foto 1e 2 Profilo pedologico in tartufaia naturale di *T. magnatum* Pico in impianto di arboricoltura a Montechiaro d'Asti.

Analisi di laboratorio
I campioni prelevati sono stati analizzati a cura del laboratorio dell'IPLA al fine della determinazione della granulometria (U.S.D.A., 1993) e dei principali parametri chimici dei suoli.

Unità di Terre e Tipologie Pedologiche definitive
Si è proceduto alla classificazione delle principali Tipologie Pedologiche individuate secondo la tassonomia americana (Soil Taxonomy, 2006) al livello del Sottogruppo.
Si è quindi giunti all'individuazione delle Unità di Terre definitive e delle Tipologie Pedologiche definitive ad esse collegate: per ogni Unità sono state attribuite le percentuali areali delle differenti Tipologie Pedologiche riconosciute.
E' estremamente importante sottolineare per la successiva attribuzione delle attitudini, che ad ogni Unità di Terre sono collegate diverse Tipologie Pedologiche delle quali una sola risulta prevalente per distribuzione areale.

Redazione delle carte di attitudine dei suoli
La redazione delle carte di attitudine alla produzione del tartufo rientra nella più ampia casistica della valutazione delle attitudini delle terre per usi specifici (*land suitability for specific uses*), che comporta il confronto tra i requisiti specifici dell'uso prescelto con le caratteristiche delle qualità delle terre.
In particolare si è fatto riferimento alla metodologia della Land Suitability adottata della FAO e descritta nello schema da essa pubblicato (FAO, 1976); tale sistema di classificazione delle terre è articolato in quattro livelli che vengono identificati come classi: alta, media, bassa, nulla. Sulla base di questo schema, l'I.P.L.A. ha individuato le qualità e le caratteristiche delle

terre che identificano le indispensabili esigenze pedo-ecologiche per lo sviluppo della singola specie di tartufo. Questi caratteri, che sono leggermente differenti per le tre specie, possono essere raggruppati nei seguenti sottoinsiemi:
- Caratteri stazionali (substrato, morfologia, quota, pendenza, uso del suolo)
- Caratteri intriseci del suolo (profondità, idromorfia, umidità, evoluzione pedogenetica)
- Caratteri fisici del suolo: tessitura, scheletro
- Caratteri chimici del suolo (pH, $CaCO_3$ totale, rapporto C/N, sostanza organica)

Alla gamma dei possibili valori di ogni carattere, opportunamente suddivisi in diversi intervalli, è stata fatta corrispondere una differente classe.
Il risultato si esplicita nelle tabelle che seguono che hanno costituito gli strumenti di valutazione per l'attribuzione delle attitudini.

Tab. 1 Attitudine delle terre per la produzione del *Tuber magnatum* Pico – Tartufo bianco pregiato.

Caratteri	**Attitudine delle terre**			
	Alta	**Media**	**Bassa**	**Nulla**
Tessitura	F-FL-FS-FA-FLA	A-FSA-AL-L-AS	S-SF	-
$CaCO_3$ totale	>10%	>10%	<10%	Assente
Profondità	>50cm	<50cm	-	-
Scheletro	Assente nei 50cm	Presente nei 50cm	-	-
Idromorfia	Assente nei primi 40cm	Assente nei primi 40cm	Presente nei primi 40 cm	-
Umidità	Costante	Non costante	Non costante	Assente
Evoluzione pedogenetica	Assenza di orizzonti di alterazione	Presenza di orizzonti di alterazione	Presenza di orizzonti di alterazione	Presenza di illuviazione di argilla (suoli antichi)
Rapporto C/N	<10	>10 e < 15	>15	-
Sostanza organica	Non in accumulo	In accumulo	-	-
PH	7.6-8.4	7.0-7.6 e >8.4	7.0-7.6 e >8.4	<7.0
Substrato	Calcareo	Calcareo	Calcareo	Non calcareo
Morfologia	Fondivalle	Versante	-	-
Quota	<400	400-800	400-800	>800
Pendenza	<50%	<50%	<50%	>50%
Uso del suolo	La presenza del bosco denso diminuisce di una classe l'attitudine			

Tab. 2 Attitudine delle terre per la produzione del *Tuber melanosporum* Vittad. – Tartufo nero pregiato.

Caratteri	Attitudine delle terre			
	Alta	**Media**	**Bassa**	**Nulla**
Tessitura	F- FS	SF-FA- FL- FSA	S-AS-A- AL-FLA-L	-
$CaCO_3$ totale	>10%	>10%	<10%	Assenti
Profondità	>30cm	<30cm	-	-
Idromorfia	Assente	Assente nei primi 40 cm	Assente nei primi 40 cm	Presente nei primi 40 cm
Umidità	Costante	Non costante	-	-
Rapporto C/N	<10	>10 e < 15	>15	-
Sostanza organica	Non in accumulo	In accumulo	-	-
PH	7.6-8.4	7.0-7.6 e >8.4	7.0-7.6 e >8.4	<7.0
Substrato	Altre litologie calcaree	Altre litologie calcaree	Calcescisti, dolomie	Non calcareo
Morfologia	Versante	Versante	Basso versante	Fondovalle, pianura
Quota	<600	600-1000	600-1000	>1000
Pendenza	>15%	>15%	>15%	<15%
Esposizione	Sud	Sud-ovest, sud-est	Altre esposizioni	-
Uso del suolo	La presenza del bosco diminuisce di una classe l'attitudine.			

Tab. 3 Attitudine delle terre per la produzione del *Tuber aestivum* Vittad. – Tartufo nero estivo o scorzone.

Caratteri	Attitudine delle terre			
	Alta	**Media**	**Bassa**	**Nulla**
Tessitura	Altre tessiture	Altre tessiture	A-SF	S
$CaCO_3$ totale	Presente	Presente	Assente nel profilo ma substrato calcareo	Assente nel profilo e substrato non calcareo
Idromorfia	Assente nei primi 40 cm	Assente nei primi 40 cm	Assente nei primi 40 cm	Presente nei primi 40 cm
Umidità	Media e costante	Medie e costante	Media e costante	Elevata
Rapporto C/N	<15	15-20	>20	-
PH	7.6-8.4	7.0-7.6 e >8.4	7.0-7.6 e >8.4	<7.0
Substrato	Altre litologie calcaree	Altre litologie calcaree	Calcescisti, dolomie, morene calcaree	Non calcareo
Morfologia	Versante	Versante	Fondivalle	-
Quota	<600	600-1000	600-1000	>1000
Pendenza	>15%	>15%	<15%	-
Uso del suolo	La presenza del bosco denso diminuisce di una classe l'attitudine			

Operativamente si è quindi effettuato l'incrocio tra i caratteri della Tipologia Pedologica dominante in ogni Unità di Terre con le sopraccitate classi.
Ogni carattere (parametro) considerato viene, infatti, a collocarsi, in base ai valori indicati in tabella in una delle quattro classi: alta, media, bassa, nulla.
L'attitudine è definita dal parametro che ricade nella classe inferiore; è infatti sufficiente che uno solo dei parametri considerati rientri in una classe più bassa rispetto agli altri per attribuire quella classe a quelle terre. In questo modo l'attribuzione di una classe di attitudine "alta" significa che tutti i parametri rientrano nell'intervallo di valori individuato dalla prima colonna.
Questi schemi interpretativi per l'attribuzione della classe di attitudine sono il risultato di un pluriennale lavoro che poggia i suoi fondamenti sulle sperimentazioni svolte dall'I.P.L.A. nell'impianto, nel monitoraggio e nella gestione di numerose tartufaie ed è stato accompagnato da approfondite ricerche bibliografiche ed arricchito dalle preziose informazioni raccolte durante interviste con ricercatori ed esperti locali. Questa lunga attività ha rappresentato nel tempo un indispensabile strumento di affinamento e di continuo perfezionamento della metodologia di valutazione.
Notevoli sono stati i riscontri positivi all'applicazione di questo strumento anche se non si è mai potuta effettuare una vera e propria validazione dal momento che i dati sulla raccolta dei tartufi sono difficilmente reperibili e sempre contraddistinti da una scarsa trasparenza.

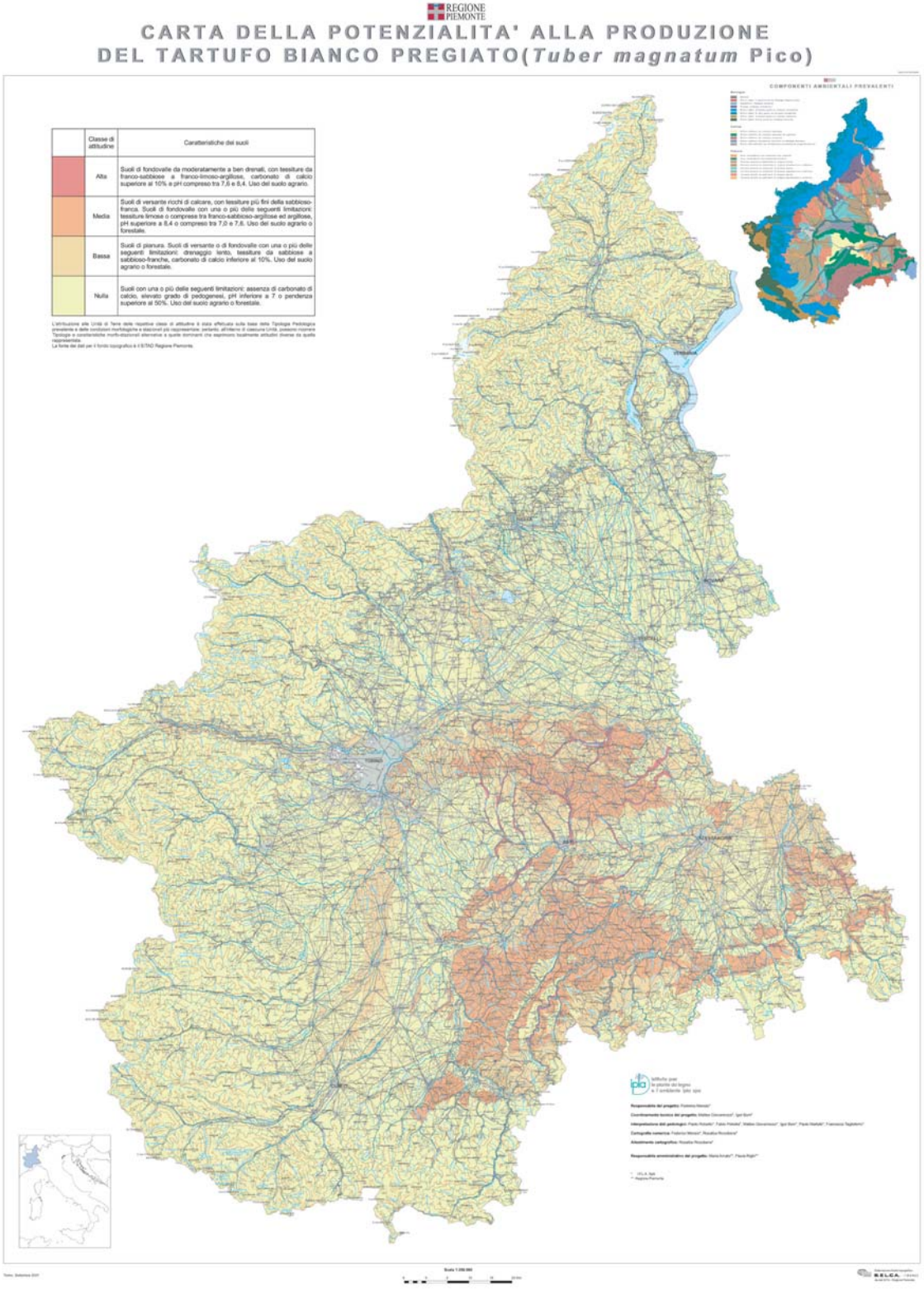

Immagine 1 Carta dell'attitudine dei suoli alla produzione di *Tuber magnatum* Pico a scala 1:250.000.

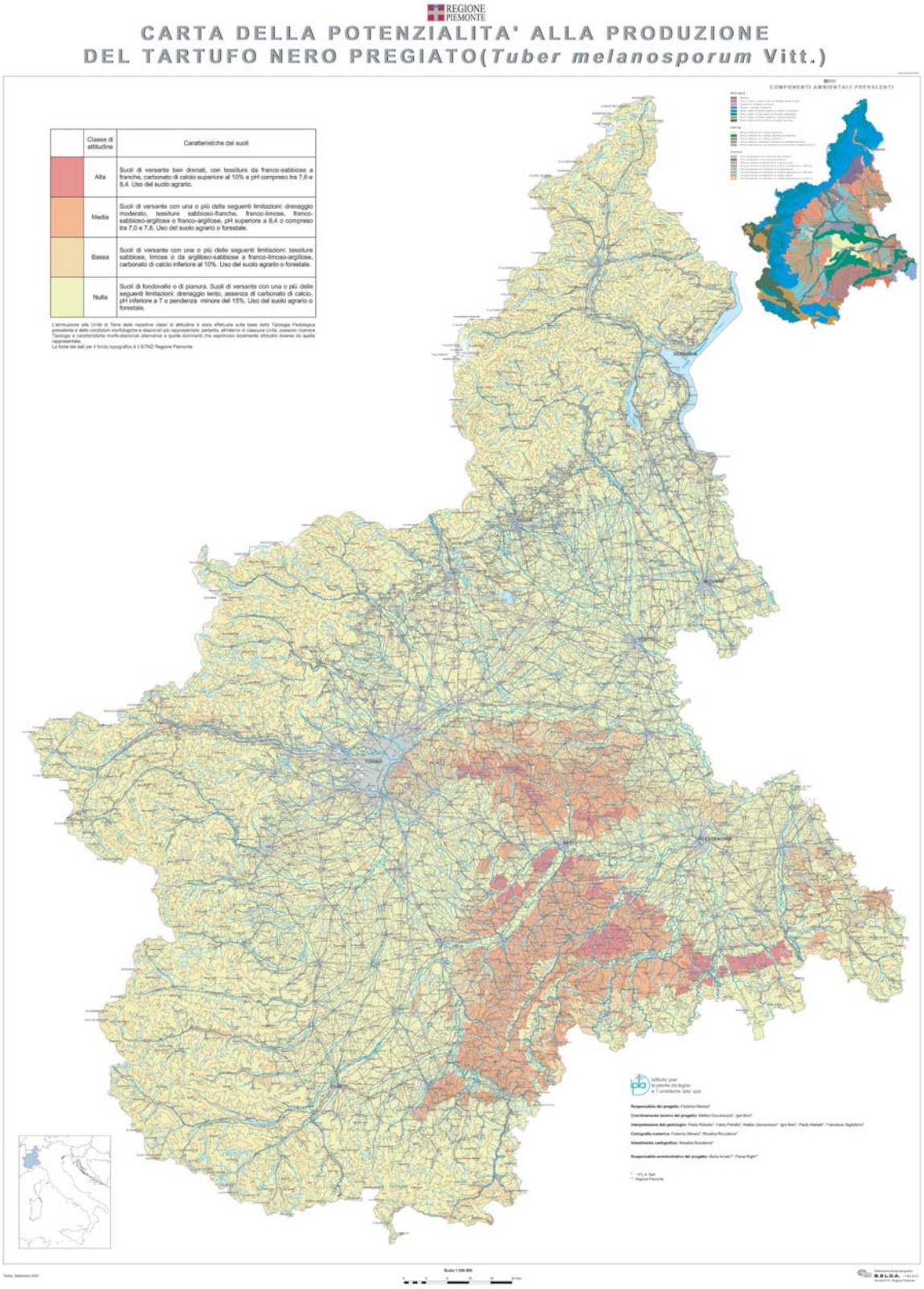

Immagine 2 Carta dell'attitudine dei suoli alla produzione di *Tuber melanosporum* Vittad. a scala 1:250.000.

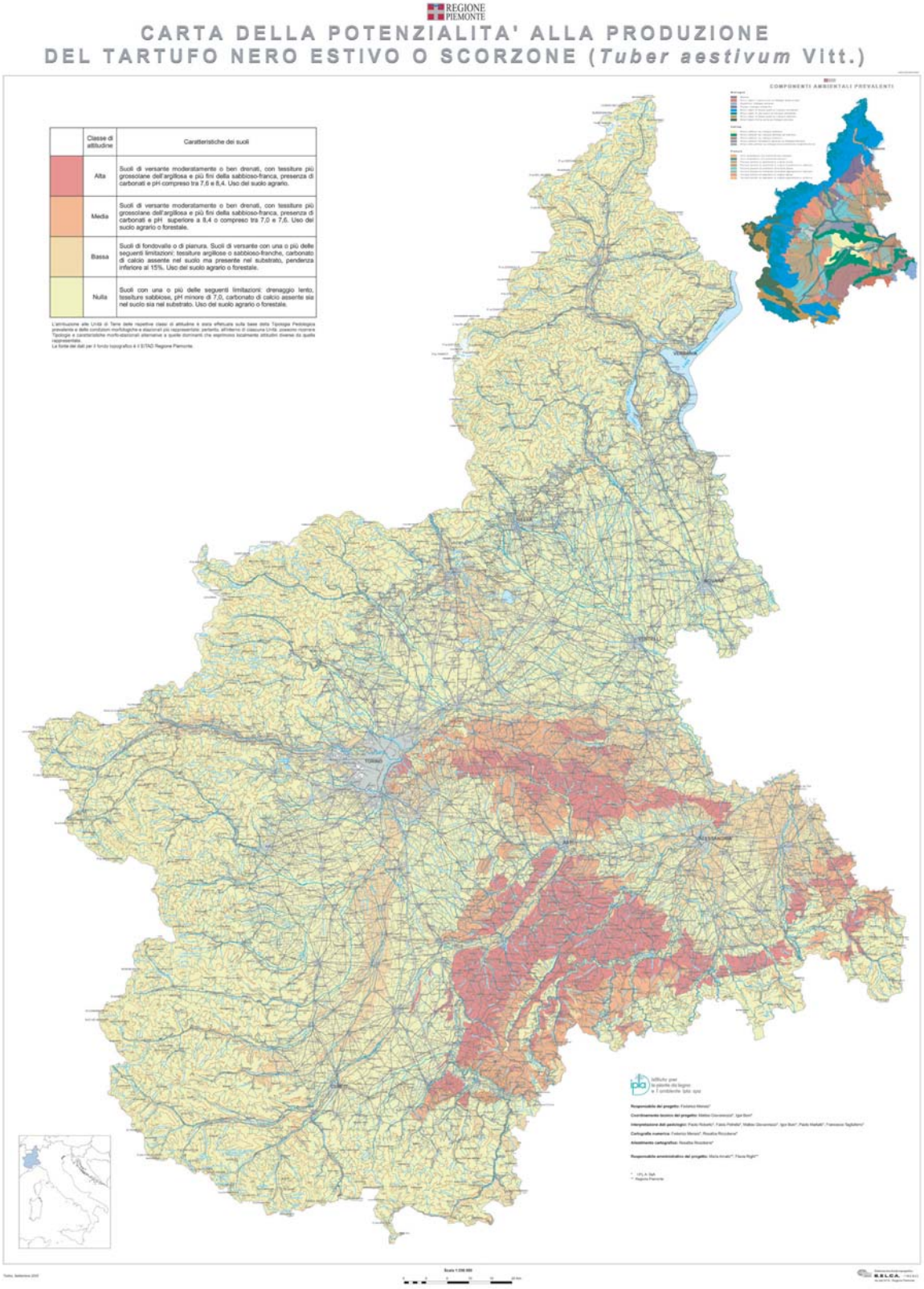

Immagine 3 Carta dell'attitudine dei suoli alla produzione di *Tuber aestivum* Vittad. a scala 1:250.000.

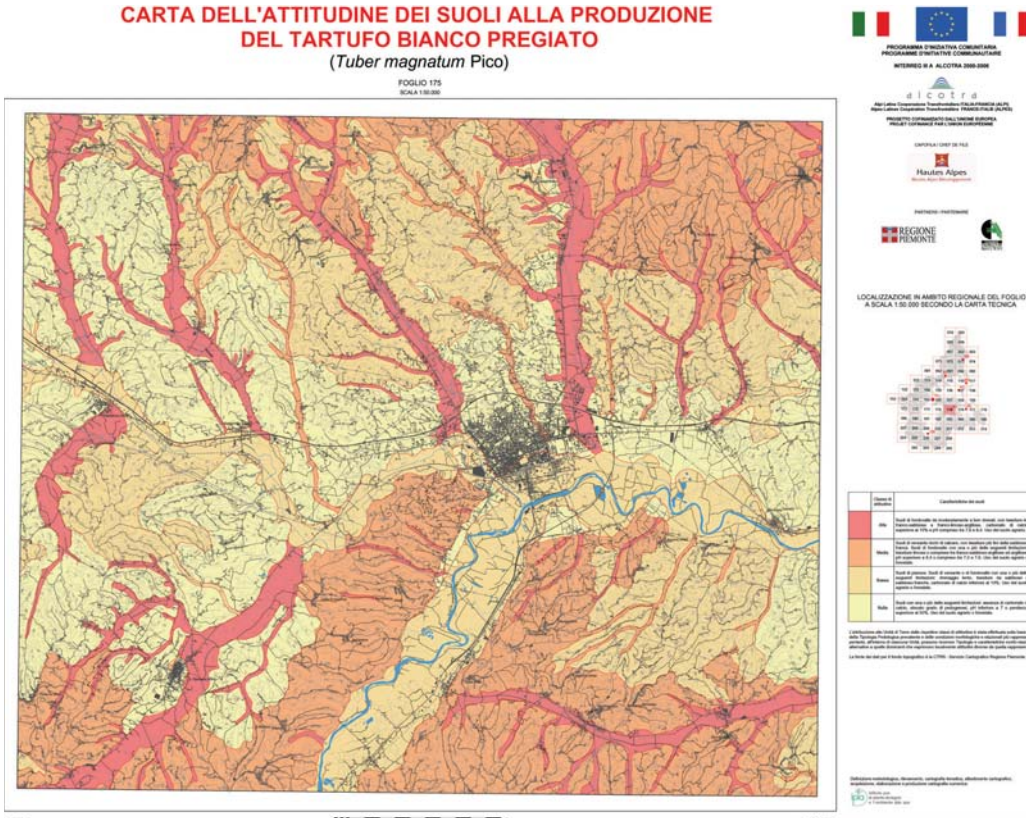

Immagine 4 Carta dell'attitudine dei suoli alla produzione di *Tuber magnatum* Pico a scala 1:50.000. Foglio 175 (Asti).

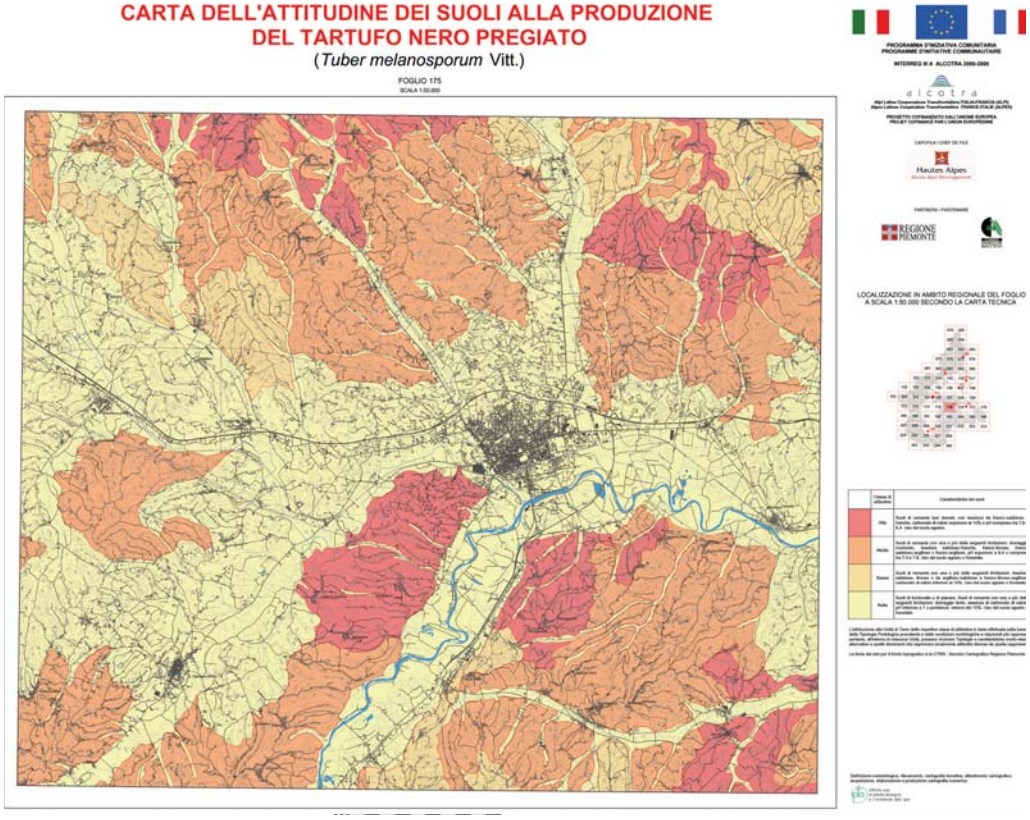

Immagine 5: Carta dell'attitudine dei suoli alla produzione di *Tuber melanosporum* Vittad. a scala 1:50.000. Foglio 175 (Asti).

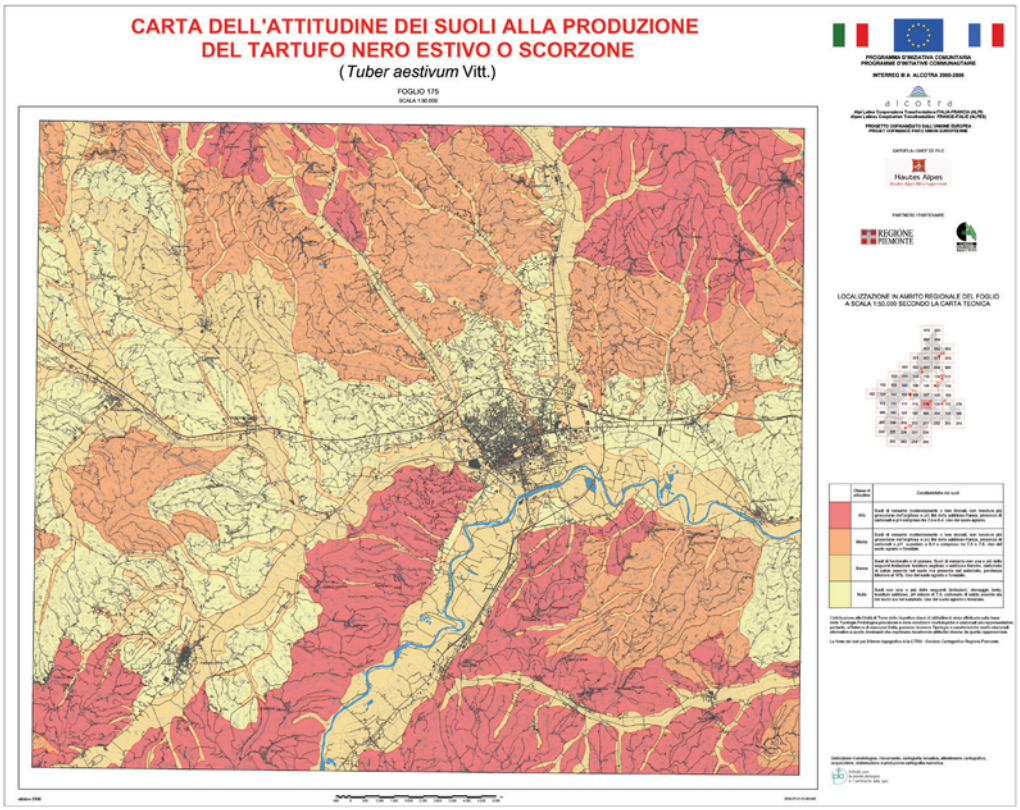

Immagine 6 Carta dell'attitudine dei suoli alla produzione di *Tuber aestivum* Vittad. a scala 1:50.000. Foglio 175 (Asti).

Conclusioni

In conclusione è opportuno ribadire il concetto fondamentale che sta alla base di queste produzioni cartografiche: l'attitudine di un suolo alla produzione dei tartufi rappresenta la sua potenzialità intrinseca, in base alle sue caratteristiche fisiche, chimiche e stazionali e prescinde dalla presenza o meno di piante tartufigene, dalla copertura o dall'uso del suolo.

Le Carte di attitudine realizzate per il Piemonte non individuano pertanto le zone in cui i tartufi "crescono", distinguendole da quelle in cui i tartufi "non crescono" bensì delineano sul territorio le aree ove si trovano suoli che sono intrinsecamente più o meno vocati ad ospitare piante tartufigene.

Il confronto dei dati cartografici sulle attitudini ottenuti a scala 1:50.000 con quelli a scala regionale 1:250.000, ha reso possibili numerose elaborazioni: risulta, ad esempio, che i dati di superficie suddivisi per classe di attitudine, ottenuti alle due diverse scale a livello provinciale, presentano scostamenti significativi in ragione della diversa scala di rilevamento.

A scala di semidettaglio, infatti, è stato possibile suddividere il territorio in un maggior numero di Unità Cartografiche al cui interno sono state individuate Tipologie Pedologiche con maggiore precisione ed un livello tassonomico più accurato. Per contro a scala regionale la definizione delle Unità Cartografiche e delle relative Tipologie Pedologiche sono definite con un livello di maggior semplificazione, che si traduce in un contenuto informativo più generico. Ben si delinea, quindi, come questi due strumenti cartografici possano fornire precise risposte ai diversi livelli di governo del territorio, in ragione della loro scala.

Queste cartografie, di cui la Regione Piemonte si è dotata per prima in Italia, rappresentano oggi uno strumento indispensabile per la pianificazione territoriale, in un'ottica di salvaguardia e di recupero del patrimonio tartufigeno regionale, ed un fondamentale documento di indirizzo per la realizzazione di nuovi impianti tartufigeni e per il ripristino di tartufaie naturali attualmente non gestite.

Per l'immediato futuro si auspica il completamento delle cartografie di attitudine alla scala

di semidettaglio per le aree che presentano una buona vocazione tartufigena, in particolare nell'ottica della creazione di nuovi impianti produttivi in aree pedemontane marginali da valorizzare.
Inoltre si è già avviata, a livello sperimentale, la predisposizione di strumenti cartografici di dettaglio (scala 1:10.000) per alcune zone ad elevata attitudine tartufigena. Tali cartografie divengono uno strumento fondamentale per mettere in atto azioni mirate alla tutela e alla valorizzazione di questa risorsa; rappresentano al contempo per le amministrazioni locali una possibilità concreta di fornire un notevole valore aggiunto al territorio.

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TRUFFLES IN THE ENVIRONMENT: THE MOLECULAR ECOLOGY OF *TUBER*

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Abstract
Truffles belonging to the genus *Tuber* are mycorrhizal fungi characterized by hypogeous fruitbodies. Molecular-based studies of mycorrhizal community composition in various habitats have documented *Tuber* mycorrhizae in many environments including floodplains, clearcut regeneration, tree nurseries, and orchards. However, it is often difficult or impossible to identify the particular species of interest because of a lack of (or biased) sampling of genetic diversity within this genus and taxonomic confusion residing in public databases. Work in our lab to resolve the phylogenetic relationships within *Tuber* has resulted in DNA sequencing from a large number of identified *Tuber* species representing a most of the known phylogenetic diversity of this genus. In this study we use a phylogenetic framework to place and identify a large number of "unidentified" *Tuber* sporocarps and mycorrhizae using ITS sequences from our own studies, Genbank, and colleagues. Our results show that the most commonly found mycorrhizae from *Tuber* species are from non-economic species in the Rufum, Puberulum, and Maculatum clades. Using molecular-based identification, we were able to taxonomically place 39 unrepresented phylotypes of *Tuber* including 9 known only from mycorrhiza.
Key words: ecology, mycorrhizal community, *Tuber*

TYPOLOGY OF ECOLOGICAL TRUFFLE SITES AND MAPPING OF THE PRODUCTION POTENTIAL

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Abstract
The black truffle, *Tuber melanosporum,* is an emblematic product from Mont Ventoux (Vaucluse, France), where it is hunted since early 19th century in natural field. However, as in many other production sites in France, production has substantially decreased in the past century.
Therefore it is all the more an important challenge for the rural and forest activity of this Mediterranean mountain to re-establish truffle production in the Mont Ventoux. However, prior to developing any schedule or management project a thorough assessment of the production potential of *Tuber melanosporum* was mandatory, and at forest scale. We elaborated an efficient tool: a specific map based on the typology of ecological truffle sites.
The method was applied to 13.000ha of private forest. It is based on the crossing of geographic data (using GIS ArcGis 9.2):

- elevation and topography
- bioclimate (18 types)
- soils from pedologic maps at 1/20:000 (37 types)

We obtained 600 ecological units after crossing. 21 were significant on the study area (over 30 ha).
The theoretical production potential of each ecological unit was characterized through an expertise based on the knowledge in truffle ecology and on the participation of Gabriel Callot (INRA former Research Director).
This characterization was validated on the study area. 100 data points were obtained/taken, depending of their representativity (in surface) on the study area. The variation factors of the production potential on each unit were determined using field validation.
The map obtained (at a 1:25 000 scale) classifies the production potential into 5 levels modulated by factors that could induce variation of the production level. These factors have to be checked on the field.
Key words: *Tuber melanosporum*, suitable soil, production potential, mapping, ecological truffle site typology, cultivation, Mont Ventoux.

I SUOLI DELLE TARTUFAIE NATURALI IN ABRUZZO.

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Abstract: The soils of natural truffle-grounds in Abruzzo.
The goal of this work is to construct the Map of the Potential truffle producing earth in the Abruzzo Region to 1:250.000 scale[1].
In order to do so, over 1000 sites of naturally occurring truffle-grounds were explored to study their ecological characteristics.
Of these, 200 sites of *Tuber melanosporum* Vittad. and *Tuber magnatum* Pico were selected to study the environmental characteristics and the types of soil. The study of soils was realized through the excavation of 190 mini pits and 10 profiles chosen among the most representative sites.
The soil in every mini pit and profile was described and classified according to the methodologies used in the Map of Soils in Italy in a 1:250.000 scale. A second step determined the principal chemical-physical properties of the soils. Micro-morphological analysis was done on the soil profiles by means of interpretation of thin sections. The soils were then subdivided and analyzed by the types of truffles studied. This led to the examination of some peculiarities of the soils responsible for the growth of the two species of truffles. From the study it was discovered that common soils features exist and that these belong to a limited number of typical subunits of the soils present in the Abruzzo region.
Key words: ecology and population biology, tuber, soil, soil evaluation.

Introduzione

Per elaborare la Carta delle Potenzialità Tartuficole della Regione Abruzzo in scala 1:250.000 sono stati censiti oltre 1000 siti di tartufaie naturali per studiarne le caratteristiche ecologiche. Tra questi sono stati selezionati 200 siti di tartufaie di *Tuber melanosporum* Vittad. e *Tuber magnatum* Pico in cui, oltre ai caratteri ambientali e vegetazionali, sono state rilevate le tipologie di suolo. La conoscenza del suolo, infatti, riveste una particolare importanza per individuare i diversi ambiti territoriali.

Materiali e Metodi

Il rilevamento nei siti prescelti delle tartufaie è consistito nello scavo di profili di suolo e "minipits", cioè profili a profondità limitata, compatibilmente con le condizioni di accessibilità.
L'intorno del sito di scavo, definito stazione, è stato descritto relativamente a: morfologia, pendenza, esposizione, quota, processi geo-morfologici in atto, substrato geologico e materiale parentale, vegetazione e uso del suolo, pietrosità e rocciosità.
Gli orizzonti individuati in ogni profilo o minipit sono stati descritti secondo il "Manuale per la descrizione del suolo" (Centro SAPA- ARSSA- Regione Abruzzo, 2002) rilevandone lo spessore, limiti, umidità, colore, scheletro, struttura, porosità, consistenza, reazione, densità apparente, conducibilità idraulica, profondità utile alle radici.
Di ogni orizzonte è stato prelevato un campione destinato ad analisi chimico-fisiche di routine e per alcuni orizzonti sono stati prelevati campioni per la preparazione e lo studio di sezioni sottili.
Dai dati di campo e dai risultati analitici si è pervenuti alla classificazione delle diverse tipologie di suolo sulla base del sistema di classificazione USDA-NRCS "Keys to Soil Taxonomy" - Tenth Edition (2006). Tutti i dati sono stati inseriti nel "Soil Database" del Centro SAPA.

1 Research conducted as part of the "Map of the truffle vocation of the Abruzzo Region" project, cosponsored by the Abruzzo Region, within the triennial programs "Interventions of forestation and environmental development", 2001-2003 and 2004-2006 of the ARSSA through the Technical Assistance Programs of 2001-2002-2004-2005

Risultati

I profili e minipits così classificati si dividono nei "sottogruppi" di suolo:

- Per il *Tuber melanosporum*: Pachic Hapludolls, Typic Haprendolls, Pachic Argiudolls, Typic Eutrudepts, Typic Udorthents.
- Per il *Tuber magnatum*: Typic Eutrudepts, Typic Udorthents, Typic Udifluvents, Lithic Udorthents, Fluventic Eutrudepts.

In particolare risulta che per il *Tuber melanosporum* i Pachic Hapludolls sono stati rinvenuti nel 66,3% dei siti investigati e per il *Tuber magnatum* i Typic Eutrudepts addirittura nell'83,0% dei casi.

Di seguito riportiamo, tra i sottogruppi, solo le 4 tipologie di suolo più rappresentate in percentuale:

Per il *Tuber melanosporum*:

- Pachic Hapudolls loamy skeletal 37,5%
- Pachic Hapludolls fine loamy 10,0%
- Typic Haprendolls sandy skeletal 10,0%
- Pachic Hapludolls coarse loamy 8,8%

Per il *Tuber magnatum* :

- Typic Eutredepts fine loamy 50,9%
- Typic Eutrudepts fine 15,2%
- Typic Eutrudepts coarse loamy 13,4%
- Typic Udorthents coarse loamy 7,1%

Caratteristiche dei suoli del *Tuber melanosporum*

I Pachic Hapludolls, che sono da ritenere i suoli più importanti per il tartufo nero pregiato, con il 66,3% dei siti rilevati, sono Mollisuoli, cioè suoli normalmente di colore scuro, ricchi di basi di scambio e di sostanza organica. Si sviluppano prevalentemente sotto le praterie o in aree originariamente boscate. Nelle aree montuose si originano da materiale parentale fortemente calcareo generalmente in aree boscate.

I Pachic Hapludolls hanno in genere un orizzonte cambico ricco di carbonati sotto l'epipedon mollico, che deve essere più spesso di 50 cm. Si trovano a pendenze moderate, in condizioni di accumulo, quindi principalmente nelle falde di detrito dei rilievi calcarei.

I Pachic Hapludolls loamy skeletal, che sono la tipologia più rappresentata nei siti di *Tuber melanosporum* studiati, hanno, in virtù della stessa definizione di loamy skeletal (ovvero classe tessiturale scheletrico franca) la sezione di controllo con argilla <35%, sabbia <70% e scheletro sempre superiore al 35%.

Il Calcare Totale varia da un minimo di 9,6 ad un massimo del 79,6%. Va specificato che trattandosi di ambienti calcarei spesso la frazione sabbiosa è costituita di particelle calcaree che entrano nella somma del calcare totale.

Il Calcare Attivo varia tra 1,93 e 14,3%, il pH in H_2O varia tra 7,2 e 8,3, il Carbonio Organico tra 1,23 e 6,54%, l'Azoto (N) tra 0,14 e 1,57% e il Fosforo (P) tra 4 e 72 ppm.

Tutti i suoli studiati hanno elevata pietrosità superficiale, gli orizzonti superficiali hanno strutture fortemente sviluppate da granulari a poliedriche subangolari fini, sono molto porosi, con scheletro da comune ad abbondante generalmente angolare. L'insieme di tali caratteristiche influisce positivamente sulla permeabilità ed il drenaggio infatti non si riscontrano figure pedogenetiche testimoni di ristagno idrico.

Fanno eccezione i rari casi di Typic Eutrudepts, evolutisi da substrato pedogenetico derivante da torbiditi arenaceo marnose, che comunque hanno strutture simili e porosità elevata, ma si differenziano per lo scheletro che è scarso o assente.

Caratteristiche dei suoli del *Tuber magnatum*

I Typic Eutrudepts sono da ritenere i suoli tipici delle tartufaie di bianco pregiato in quanto su di essi sono stati rinvenuti l'83,0% dei siti rilevati, ma solo quelli originati sulle formazioni flyshoidi

presenti in Abruzzo sono adatti allo sviluppo di tartufaie di *T. magnatum*.
I Typic Eutrudepts sono Inceptisuoli, cioè suoli che hanno un orizzonte cambico e un epipedon ocrico, presenti in aree umide o subumide. Gli Inceptisuoli includono una grande varietà di suoli che si trovano in aree morfologicamente attive, come pendii e versanti dove i processi erosivi rinnovano frequentemente il profilo o in valli fluviali per azione dei depositi alluvionali. Sono formati su depositi Olocenici o Pleistocenici generalmente calcarei e ricchi di basi.
I Typic Eutrudepts fine loamy, che sono la tipologia più rappresentata nei siti di *Tuber magnatum* studiati, hanno, in virtù della stessa definizione di fine loamy (ovvero classe tessiturale franco fine) la sezione di controllo con argilla compresa tra 18 e 35%, sabbia >15% e scheletro < del 35%.
Il Calcare Totale varia da un minimo di 5,6 ad un massimo del 90,7%, il Calcare Attivo tra 0,5 e 13,6%, il pH in H_2O tra 7,3 e 8,4, il Carbonio Organico tra 0,2 e 7,2%, l'Azoto (N) tra 0,01 e 0,93% e il Fosforo (P) tra 2 e 101 ppm.
Tutti i suoli studiati hanno pietrosità superficiale scarsa o assente. Gli orizzonti superficiali hanno strutture fortemente sviluppate da granulari a poliedriche subangolari fini, sono molto porosi e lo scheletro è assente. L'insieme di tali caratteristiche influisce positivamente sulla permeabilità ed il drenaggio, infatti non si riscontrano figure pedogenetiche testimoni di ristagno idrico negli orizzonti di accrescimento del carpoforo. I suoli non presentano periodi di siccità estiva.

Conclusioni
Lo studio effettuato su un numero importante di tartufaie naturali, scelte come rappresentative dei diversi ambienti presenti nel territorio regionale, ci permette di ricavare alcune considerazioni, ma naturalmente la variabilità dei suoli e degli ambienti regionali è tale da non poter considerare esaustive le conclusioni raggiunte.

Tuber melanosporum Vittad.
C'è una tipologia di suolo prevalente, caratteristica delle tartufaie naturali appartenente all'ordine dei Mollisuoli (Pachic Hapludolls loamy skeletal).
Tutti i suoli però presentano alcuni caratteri determinanti per l'accrescimento del carpoforo:

- struttura granulare fine fortemente sviluppata o struttura poliedrica subangolare media moderatamente sviluppata;
- porosità elevata;
- aggregati poco o moderatamente sviluppati con consistenza fragile e modalità di rottura friabile;
- densità apparente (stimata) bassa;
- drenaggio interno da ben drenato a eccessivamente ben drenato;
- assenza di figure di ossidoriduzione;
- assenza di caratteristiche vertiche (fessure, self mulching).

Dai risultati di questo lavoro emerge la difficoltà di valutare l'attitudine di un suolo in base alla tessitura, in quanto, sebbene prevalgano i Pachic Hapludolls loamy skeletal (cioè con classe granulometrica franco scheletrica), sono presenti quasi tutte le altre classi ad eccezione di quelle più fini. A questo si aggiunge la difficoltà della determinazione analitica degli orizzonti che sono ricchi di sostanza organica e calcare, per cui l'analisi della tessitura apparente è spesso poco significativa, d'altronde la distruzione di CO e calcare falserebbe ancor più il risultato.
Il pH e i carbonati non sembrano fornire un criterio di valutazione in quanto la variabilità ottenuta all'interno dei campioni dei siti studiati non li differenzia dalla gran parte dei suoli della regione. Lo stesso dicasi per le altre caratteristiche analizzate, cioè Carbonio Organico, Azoto e Fosforo che hanno oscillazioni tali da ritenersi inutilizzabili al fine di individuare le caratteristiche specifiche dei suoli su cui si sviluppano le tartufaie naturali. Da questo studio appare che le caratteristiche analitiche normalmente determinate non sono utili ad individuare l'idoneità di un sito naturale a sviluppare la presenza del tartufo, ma che invece i caratteri del

suolo, così come individuati nella "*Soil Taxonomy"* hanno una migliore capacità di selezionare le tipologie di suolo idonee.

Tuber magnatum Pico
C'è una tipologia di suolo prevalente, caratteristica delle tartufaie appartenenti all'ordine degli Inceptisuoli (Tipic Eutrudepts fine loamy).
Tutti i suoli però presentano alcuni caratteri determinanti per l'accrescimento del carpoforo:

- struttura poliedrica subangolare media moderatamente o debolmente sviluppata o struttura granulare fine fortemente sviluppata;
- porosità elevata;
- aggregati poco o moderatamente sviluppati con consistenza fragile e modalità di rottura friabile;
- densità apparente (stimata) da bassa a media
- drenaggio interno ben drenato
- umidità da umido a molto umido
- assenza di figure di ossidoriduzione
- assenza di caratteristiche vertiche (fessure, self mulching)

Esiste una variabilità per le caratteristiche granulometriche, ma in questo caso è dominante la classe dei fine loamy (franco fini).
Anche per questi suoli i dati relativi al contenuto di calcare e di pH non sono significativi nella regione Abruzzo che ha quasi sempre suoli calcarei e con pH sub alcalino.
La fisiografia ricorrente è data da colluvi, rotture di pendio, brevi versanti dei corsi d'acqua secondari con presenza di movimenti di massa di piccola entità.
In questo caso c'è un intervallo di tessiture, che rientrano nella classe "fine loamy", che sembrano mostrare una particolare attitudine a sostenere lo sviluppo del tartufo mentre le altre caratteristiche analitiche, normalmente determinate, appaiono poco o niente utili a questo scopo. Anche in questo caso i caratteri del suolo individuati nella "*Soil Taxonomy"* mostrano una migliore capacità di selezionare le tipologie di suolo idonee.

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L'HABITAT DEI TARTUFI IN ABRUZZO: MAPPATURA E BANCA DATI

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Abstract: Maps and data-bank of truffle habitats in Abruzzo (Central Italy).
For a long time Abruzzo has been considered a place of interest concerning with the truffles by a few employed in this sector, in fact, only in the last years, the remarkable vocation of our land is largely perceived and recognized on extraregional field, too.
So it arose a necessity of protecting this important natural patrimony, from the immoderate and irrational exploitation, with a greater awareness of the zones potentially suitable for the production of truffles, evaluating the referring to each species investigated, and the location on the territory.
Joining all the experiences that professional searchers have about their area, we carried out a careful survey, individualizing, through 'GPS' system, over thousands of sites that are concerned with the four most important species: *Tuber magnatum, T. melanosporum, T. aestivum and/or uncinatum, T. borchii.* The researches for the GIS elaboration were done putting on the information of the sites that were indicating the truffle-grounds, with other knowledge concerning soil science, geomorphology, climate and vegetation. The project has been done in 5 years and a thematic map has been drown, in scale 1:250.000, for each of the 4 species investigated. In addiction, for the value that the two most esteemed species have, we have carried out the idening of the inquiry with more detailed cartographies, in scale 1:100.000. The information obtained shows the remarkable extension and the location of the areas where the production of different species of truffle naturally occurs. This knowledge represents the start to defend and improve them. Moreover, the amount of the data recorded in the data-bank is an important reference point in the design of a cultivation plan.
Key words: truffle habitat, Central Italy, *Tuber magnatum*, *Tuber melanosporum*, *Tuber aestivum*, *Tuber uncinatum*, *Tuber borchii*.

Introduzione

L'Abruzzo, territorio particolarmente vocato alla produzione di tartufi, per tanti anni è stato terra di conquista da parte di cercatori extraregionali. Negli ultimi 30 anni vi è stata una crescente presa di coscienza da parte dei residenti che ha portato ad un considerevole incremento del numero dei cercatori locali (oggi stimato in diverse migliaia) e alla nascita di varie attività imprenditoriali legate al commercio, alla trasformazione e alla vendita delle produzioni locali. Una parte ancora consistente della produzione la si ritrova nei mercati di regioni italiane più blasonate anche se quote crescenti di prodotto acquistano una loro identità sui mercati nazionali ed internazionali.
La Regione Abruzzo, attraverso l'Agenzia Regionale per i Servizi di Sviluppo Agricolo (A.R.S.S.A.) ha iniziato da circa 20 anni una serie di attività legate al settore, finalizzate soprattutto alla sperimentazione e assistenza tecnica specialistica nella coltivazione di specie forestali micorrizate con tartufo.
Nell'ambito di queste attività, nel periodo 2003-2008, l'ARSSA ha realizzato il Progetto "Carta della vocazionalità tartuficola della Regione Abruzzo" allo scopo di evidenziare il legame tra distribuzione dei tartufi e caratteristiche ecologiche del territorio per creare uno strumento a

supporto della programmazione del settore e innescare processi di valorizzazione e tutela di questa importante risorsa.
Obiettivi specifici del progetto sono stati:

- creazione di una banca dati contenente i principali parametri caratterizzanti i siti naturali e le tartufaie coltivate;
- realizzazione di cartografie tematiche in scala adeguata relative alla distribuzione delle aree vocate alle principali specie di tartufo presenti nella Regione.

Materiali e metodi

In considerazione dei molteplici fattori ambientali coinvolti nella fisiologia e nell'ecologia dei tartufi, l'impostazione progettuale è stata di tipo multidisciplinare.
Il progetto è stato articolato nelle seguenti attività:

- individuazione e georeferenziazione del maggior numero possibile di tartufaie naturali delle 4 specie di *Tuber* più diffuse nella Regione Abruzzo: *T. magnatum* Pico, *T.melanosporum* Vittad., *T. aestivum* Vittad. *e/o uncinatum* (Chatin) Montecchi et Borelli, *T. borchii* Vittad.;
- studio pedologico, analisi bioclimatica e analisi vegetazionale di una serie di siti rappresentativi per *T. magnatum* e *T. melanosporum;*
- monitoraggio delle tartufaie coltivate realizzate nella regione;
- predisposizione di un database per l'archiviazione e gestione dei dati raccolti;
- elaborazioni GIS e produzione di cartografie tematiche relative alle vocazionalità del territorio alla produzione delle quattro specie di Tartufo più diffuse a livello regionale.

La potenzialità per lo sviluppo di tartufaie spontanee è stata ricavata dall'analisi delle caratteristiche ecologiche territoriali favorevoli. La presenza di siti produttivi spontanei è stata considerata un indice dell'esistenza di condizioni pedo-climatiche idonee. Gli strati informativi territoriali utilizzati sono stati trattati secondo una logica "booleana" e riguardano:

a) Carta dei suoli della Regione Abruzzo in scala 1:250.000 (Chiuchiarelli *et al.*, 2008);
b) Carta geologica dell'Abruzzo 1:100.000 (Vezzani & Ghisetti, 1998);
c) Modello digitale del Terreno;
d) Carta dell'Uso del Suolo della Regione Abruzzo in scala 1:25.000;
e) Carta delle tipologie forestali della Regione Abruzzo (in fase di completamento);
f) Banca dati dei siti tartuficoli d'Abruzzo;
g) Carta fitoclimatica d'Italia in scala 1:250.000 (Blasi & Michetti, 2005);
h) Ortofotocarte digitali del volo Terraitaly 2000.

L'elaborazione cartografica è stata realizzata in ambiente GIS (Sistema Informativo Territoriale), utilizzando i software ArcGis 9.1, ArcView 3.2.
Le cartografie prodotte sono relative ad una potenzialità a medio e lungo termine, in quanto comprendono anche superfici attualmente interessate da usi del suolo non idonei allo sviluppo di tartufi spontanei.
In ragione della scala adottata e delle caratteristiche autoecologiche delle diverse specie di tartufo, all'interno delle aree cartografate è possibile ritrovare siti potenzialmente idonei per le diverse specie indagate.
Il lavoro di individuazione delle tartufaie naturali presenti sul territorio regionale è avvenuto attraverso una intensa "attività in campo" fatta ricorrendo a cercatori esperti e cani addestrati che hanno permesso di monitorare la gran parte del territorio regionale nei rispettivi periodi di raccolta delle quattro specie oggetto di indagine; ciò ha fornito un quadro rappresentativo delle tartufaie presenti nella regione. In questa fase si è proceduto alla georeferenziazione dei siti con strumenti GPS e sono stati rilevati i seguenti parametri: coordinate geografiche (secondo il sistema UTM) altitudine, esposizione, pendenza, densità della vegetazione arborea, piante simbionti, altre specie arboree ed arbustive presenti.
In considerazione della diffusione a livello regionale e della conseguente importanza economica lo studio di approfondimento sull'habitat delle due specie più pregiate, ha riguardato gli aspetti relativi alla pedologia, al clima e alla vegetazione.

L'indagine pedologica ha interessato 193 siti scelti tra quelli più rappresentativi per i due funghi anzidetti ed è stata eseguita attraverso lo scavo di "minipits" (pozzetti delle dimensioni di circa cm 50x50x50); gli stessi sono stati sottoposti a rilievi descrittivi in ordine a morfologia, pendenza, esposizione, quota, processi geomorfici in atto, substrato geologico, materiale di origine del suolo, vegetazione presente nella stazione. Contemporaneamente sono stati prelevati campioni di terreno sugli orizzonti individuati allo scopo di determinare, in laboratorio, i principali parametri fisici e chimici (tessitura, pH, carbonati totali, calcare attivo, sostanza organica, carbonio organico, rapporto C/N, azoto, fosforo e potassio).
L'analisi vegetazionale ha riguardato n. 27 siti rappresentativi per le due specie pregiate. Essa è stata condotta attraverso il metodo fitosociologico classico della scuola sigmatista di Zurigo-Montpellier (Braun-Blanquet, 1964) che consiste nella individuazione ed elencazione di tutte le specie vascolari presenti attribuendo a ciascuna di esse un indice di abbondanza-dominanza corrispondente al grado di ricoprimento del terreno.
Lo studio fitoclimatico è stato finalizzato ad individuare le correlazioni esistenti tra la distribuzione dei siti di *Tuber magnatum* e *T. melanosporum* e le caratteristiche bioclimatiche delle stazioni di riferimento, al fine di comprendere il ruolo del clima quale fattore ecologico responsabile di tale distribuzione.
Nell'ambito del progetto è stato altresì effettuato un monitoraggio su una serie di tartufaie coltivate realizzate nella regione nell'arco degli anni 1986-2008. Il lavoro è stato svolto attraverso interviste ai titolari delle tartufaie e con rilievi diretti in campo che hanno permesso di acquisire, oltre ai dati identificativi e la storia dell'impianto, anche le produzioni ottenute, le problematiche in essere e, in alcuni casi, i dati analitici del terreno, e quelli relativi allo stato della micorrizazione.
La mole dei dati raccolti sulle tartufaie naturali e su quelle coltivate è attualmente gestita da un database, da cui è possibile ottenere informazioni utili nell'attività di assistenza tecnica agli operatori del settore.

Risultati
I risultati del lavoro analitico costituiscono un importante punto di riferimento nella comprensione dei fattori ecologici che influiscono sulla formazione dei tartufi.
- Banca dati: gli elementi conoscitivi essenziali acquisiti durante il lavoro, che costituiscono la banca dati dei principali parametri ecologici, sono sintetizzati nelle tabelle 1 e 2.

Tab. 1 I principali elementi d'indagine delle tartufaie naturali rilevate

TARTUFAIE NATURALI			
specie indagate	**n. tartufaie censite**	**n. siti studio pedologico**	**n. siti studio vegetazionale**
Tuber magnatum	435	113	17
Tuber melanosporum	219	80	10
Tuber aestivum – uncinatum	381		
Tuber borchii	109		

Tab. 2 Principali risultati del monitoraggio degli impianti censiti

TARTUFAIE COLTIVATE
N. 83 aziende coinvolte
N. 129 tartufaie
N. 114 ha realizzati nel periodo 1986 - 2008
N. 45.000 piante a dimora di cui 32.000 micorrizate con *T. melanosporum*

Cartografia: sono state prodotte quattro carte tematiche in scala 1:250.000 relative alle specie: *Tuber magnatum*, *Tuber melanosporum*, *Tuber aestivum-Tuber uncinatum*, *Tuber borchii* e due carte di approfondimento in scala 1:100.000 per le sole specie *Tuber magnatum* (Fig. 1) e *Tuber melanosporum* (Fig. 2).

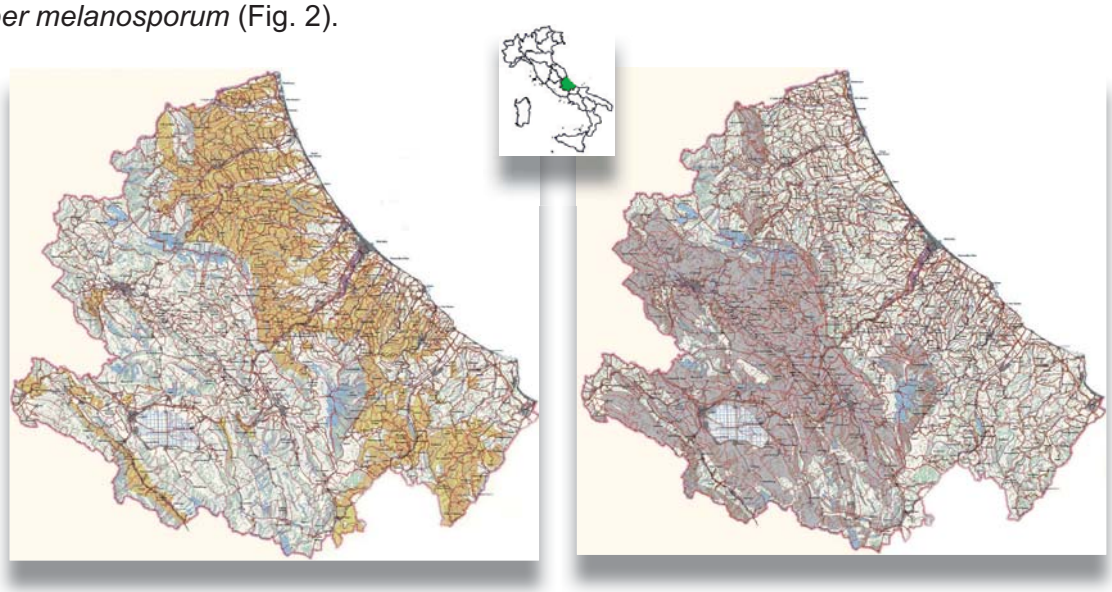

Fig. 1 Aree a vocazione per *Tuber magnatum*

La zona del *T. magnatum* interessa circa il 27% del territorio, in un range altimetrico compreso tra 109 e 1078 m s.l.m. (Fig. 3) e con esposizione prevalentemente ai quadranti Nord (Fig. 4). La zona vocata comprende una fascia di circa 50 Km che dalla costa raggiunge la zona pedemontana e si estende prevalentemente lungo il corso di fiumi e torrenti, nonché alcune valli interne quali la Valle Roveto, la media e alta Valle del Sangro.

Fig. 2 Aree a vocazione per *Tuber melanosporum*

L'area a vocazione per *T. melanosporum* interessa una superficie pari al 35% del territorio regionale, in una fascia altimetrica compresa tra 242 e 1300 m s.l.m. (Fig. 3), e con esposizione prevalentemente ai quadranti Sud (Fig. 5). La distribuzione si concentra in prevalenza nelle zone interne, nella provincia dell'Aquila dove sono stati censiti oltre l'80% dei siti naturali. Particolare interesse ecologico rivestono alcune aree del Teramano che, a differenza delle zone interne, caratterizzate da substrati prevalentemente calcarei, presentano suoli derivati da torbiditi; l'elevata componente sabbiosa e l'accentuata pendenza assicurano le condizioni pedo-climatiche di cui la specie necessita.

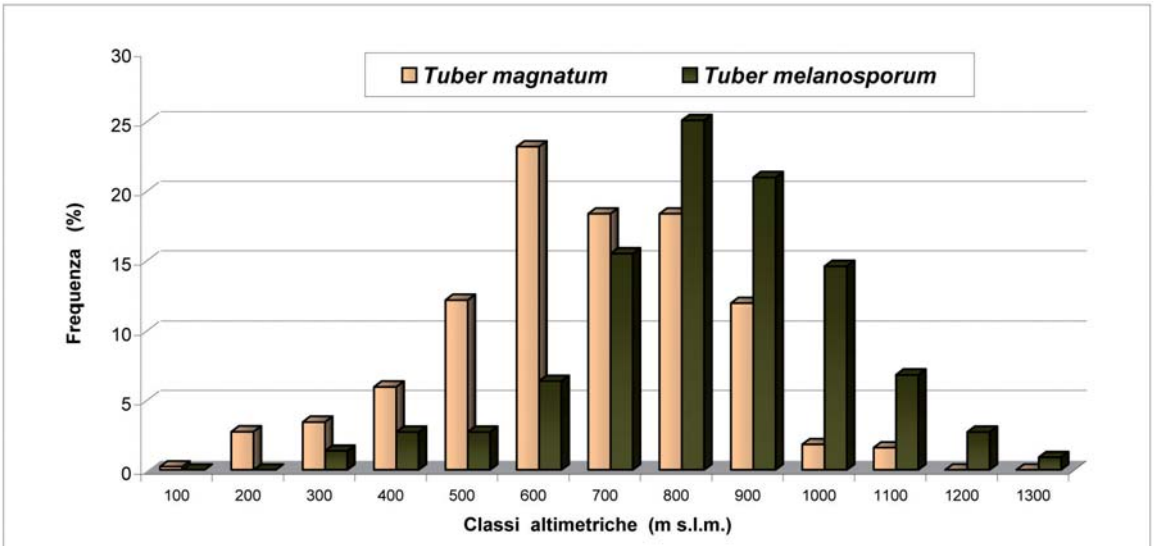

Fig. 3 Distribuzione % di frequenza per classi altimetriche di 654 siti rilevati di T. magnatum e T. melanosporum

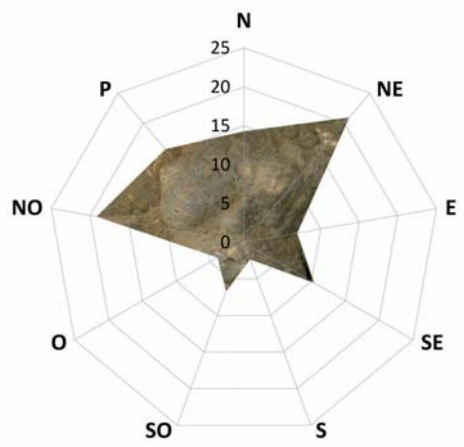

Fig. 4 - Distribuzione dei siti naturali di *Tuber magnatum* rispetto all'esposizione (%)

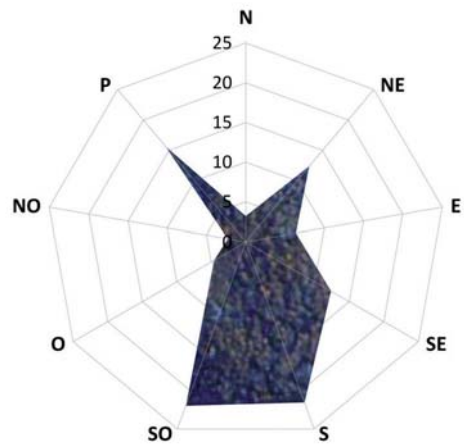

Fig. 5 - Distribuzione dei siti naturali di *Tuber melanosporum* rispetto all'esposizione (%)

Riguardo alle cartografie in scala 1:250.000 delle altre due specie indagate, risulta che:
- Il *Tuber borchii*, interessa oltre il 38% della superficie regionale. I siti naturali rilevati ricadono in una fascia altimetrica compresa tra il livello del mare (aree tipiche sono le pinete costiere) e 900 m circa s.l.m. L'area produttiva si sovrappone in buona parte a quella di *T. magnatum* con una preferenza per le aree collinari più asciutte;
- il *Tuber aestivum e/o uncinatum* è una specie piuttosto diffusa nella regione interessando circa il 70% del territorio in suoli di diversa natura e con orografia ed esposizione variabili. Dal punto di vista altimetrico lo si ritrova dal livello del mare fino a 1600 m circa di altezza.

Conclusioni

La ricerca, sulla base delle correlazioni esistenti tra la distribuzione delle tartufaie naturali delle principali specie di tartufo in Abruzzo e caratteristiche dei siti (litologia, suolo, clima, vegetazione, uso del suolo) mette in luce la notevole consistenza e la dislocazione delle aree naturalmente vocate alla produzione delle principali specie di tartufo in Abruzzo. I risultati ottenuti costituiscono un importante punto di riferimento per attività di programmazione, assistenza tecnica e valorizzazione delle risorse territoriali. Dalla elaborazione della grande mole di dati sono stati rilevati fattori ambientali discriminanti per la produzione delle principali specie di tartufi, che, qualche volta, hanno avvalorato i risultati di alcuni recenti lavori.

Nota:
Ricerca eseguita nell'ambito del progetto "Carta della vocazionalità tartuficola della Regione Abruzzo" cofinanziato dalla Regione Abruzzo, nell'ambito degli "Interventi di forestazione e valorizzazione ambientale" Programmi triennali 2001-2003 e 2004-2006 e dall'ARSSA attraverso i Programmi di Assistenza Tecnica 2001-2002-2004-2005.
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LO STUDIO DELL'UMIDITA' DEL SUOLO IN TARTUFAIE PRODUTTIVE MEDIANTE LA TECNOLOGIA FDR (FREQUENCY DOMAIN REFLECTOMETRY)

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Abstract: Frequency Domain Reflectometry technology for soil moisture detection in productive truffle orchards
The productivity of truffle cultivation of *Tuber melanosporum* Vittad. and *Tuber aestivum* Vittad. is contingent upon climate, especially rainfall and snowfall. Irrigation is an essential practice to stabilize the production to highest values (70 - 120 kg/ha). The intake of water positively affect the number and size of truffle fruit bodies, but may encourage the emergence of other mychorrhizal species more suited for moist and fresh micro-habitats.
In this research we studied changes in soil moisture in a *T. melanosporum* and in a *T. aestivum* truffle orchards located close to Spoleto.
Data were collected weekly from July 2007 to September 2008 every 0.1 m and until the depth of 0.8 m.
In the black truffle cultivation the measures were carried out from 2 productive and irrigated sites, and 2 productive non-irrigated sites.
In the summer truffle cultivation the measures were carried out in from 2 productive and hoe sites, and in 2 productive but not hoe sites.
The measurements were made using the *Diviner 2000* (Sentek Pty Ltd., South Australia) which measures the water soil content through frequency domain reflectometry (FDR) technology.
FDR systems are based on propagation speed difference of high-frequency signals (greater than 100 MHz) in the soil that consists of solid particles, air and water. These differences are due to dielectric constant (ε) fluctuations determined by changes in soil water content (for water ε = 80, for dry land ε = 4 to 8 and for air ε = 1 at 20°C).
Every frequency measure recorded by the instrument is then converted in volumetric water content by applying an appropriate calibration equation. For this work has been applied the equation proposed by the manufacturer (Default Sentek Calibration), which although lacking in respect absolute values gave very satisfactory results in terms of relatives changes of water soil content.
The results show that changes in soil moisture content are closely tied to the soil features of the site. The soils of *T. melanosporum* orchard show a steadier and more uniform moisture pattern than the *T. aestivum* one.
The summer truffle on the contrary of black truffle bears fruit even in soils where there are strong water soil content excursions, confirming its ability to adapt to different environmental conditions.
The weeding generates large moisture fluctuations in the soil surface layer. This upper layer responds with rapid increases to rain events but has also quick stages of draining; the charging effect of rainfall is exhausted more quickly than in deeper soil layers.
Key words: FDR, Diviner 2000, *Tuber*, soil moisture, dielectric constant.

Introduzione

La produttività delle tartufaie coltivate di *Tuber melanosporum* Vittad. e *Tuber aestivum* Vittad. è condizionata dall'andamento climatico e in particolare dalle precipitazioni piovose e nevose. L'irrigazione è una pratica indispensabile per incrementare e/o stabilizzare le produzioni sui valori massimi (70 – 120 kg\ha). L'apporto idrico influenza il numero e le dimensioni dei

carpofori, ma può anche favorire la comparsa di altre specie micorriziche più adatte al micro-habitat umido e fresco (Chevalier, 2008).
Con questa ricerca abbiamo studiato l'andamento dell'umidità del suolo in una tartufaia coltivata di *T. melanosporum* e in una di *T. aestivum*, situate nei pressi di Spoleto.
L'obiettivo è stato quello di sopperire alla mancanza di conoscenze scientifiche, riguardo l'incidenza del contenuto idrico del suolo nelle varie fasi del ciclo biologico dei tartufi pregiati, e di individuare i parametri per poter effettuare, in futuro, interventi idrici mirati. In particolare, per le misurazioni in campo, è stato individuato uno strumento affidabile, facile da gestire e non eccessivamente costoso, in modo che i dati possano essere raccolti anche da personale non specializzato.

Materiali e Metodi

- Le misurazioni sono state eseguite utilizzando *Diviner 2000* (Sentek Pty Ltd., South Australia) costituito da una *sonda*, formata da un sensore capacitivo posizionato all'apice di un asta graduata e da un terminale o *data unit* per il salvataggio dei dati. Esso misura il contenuto d'acqua del suolo attraverso la tecnologia *Frequency Domain Reflectometry* (FDR), basata sulla differenza di velocità di propagazione di segnali ad alta frequenza, maggiore di 100 MHz, nel terreno. Il sensore misura quindi la frequenza di oscillazione di un circuito che si crea attorno ad esso tramite un'induttanza e un sistema condensatore (Paltineanu e Starr., 1997). Dal momento che l'induttanza è fissata, la variazione della frequenza di oscillazione dipende da variazioni di capacità, determinata dall'area delle piastre del sistema condensatore dalla loro distanza e dalla costante dielettrica del materiale presente fra esse. Siccome anche le caratteristiche geometriche degli anelli conduttori sono fissate, la capacità del condensatore è a sua volta determinata dalla costante dielettrica (ε) del mezzo circostante ed in particolare dalla costante dielettrica dell'acqua, maggiore rispetto a quella del terreno asciutto e dall'aria (per acqua ε = 80 a 20°C, per terreno asciutto: ε = 4÷8; per aria ε = 1). La frequenza di risonanza varia dunque in funzione delle fluttuazioni di costante dielettrica determinate dalle variazioni del contenuto idrico del suolo. Ogni misura di frequenza registrata viene poi convertita in misura di contenuto d'acqua volumetrico (θv) applicando una opportuna equazione di calibrazione. Per questo lavoro è stata applicata *Default Sentek Calibration* proposta dal costruttore, che seppur carente per quanto riguarda valori assoluti ha fornito risultati molto soddisfacenti in termini di variazioni relative del contenuto d'acqua del suolo (Filippi, 2006; SENTEK PTY LTD, 1999).
- I dati sono stati prelevati da luglio 2007 a settembre 2008, a cadenza settimanale ogni 0.1 m fino alla profondità di 0.8 m. Nella tartufaia di *T. melanosporum* le misure sono state effettuate in 2 pianelli produttivi ed irrigati (P1B e P2B) ed in 2 pianelli produttivi non irrigati (P3B e P4B). Nella tartufaia di *T. aestivum* le misure sono state effettuate in 2 pianelli produttivi sarchiati (P3A e P4A) ed in 2 pianelli produttivi non sarchiati (P1A e P2A).
- Le misurazioni sono state effettuate facendo scorrere la sonda Diviner 2000 in pozzetti costituiti da tubi in PVC. La sonda legge i valori di frequenza in fase discendente e ascendente ogni 0.1 m fino a 0.8 m e memorizza la media fra le due letture (modalità swipe and go).
- Per avere misure dirette dei dati meteorologici è stata installata una capannina meteorologica (*Watchdog 2900 ET*) che misura i parametri relativi all'evapotraspirazione, radiazione solare, velocità e direzione del vento, temperatura e umidità relativa dell'aria, pioggia, temperatura del suolo a 5 e 15 cm di profondità, pressione barometrica.

Risultati

I suoli della tartufaia di *T. melanosporum* mostrano un andamento della frequenza più regolare ed omogeneo rispetto a quelli di *T. aestivum*. In generale, la frequenza registrata nei primi

10 cm di suolo per la tartufaia di tartufo nero (Fig. 1, 2), assume valori leggermente inferiori rispetto a quelli registrati per la tartufaia di estivo, con particolare riferimento ai siti P1A e P2A, per i quali la copertura vegetale determinata dalle piante simbionti è prossima al 100%. Come si rileva dai grafici (Fig. 3, 4), l'andamento dell'umidità del suolo registrata come frequenza nella tartufaia di *T. aestivum* mostra una sostanziale differenza tra i siti P3A e P4A rispetto ai siti P1A e P2A, in quanto situati nell'area sarchiata nella primavera del 2006.

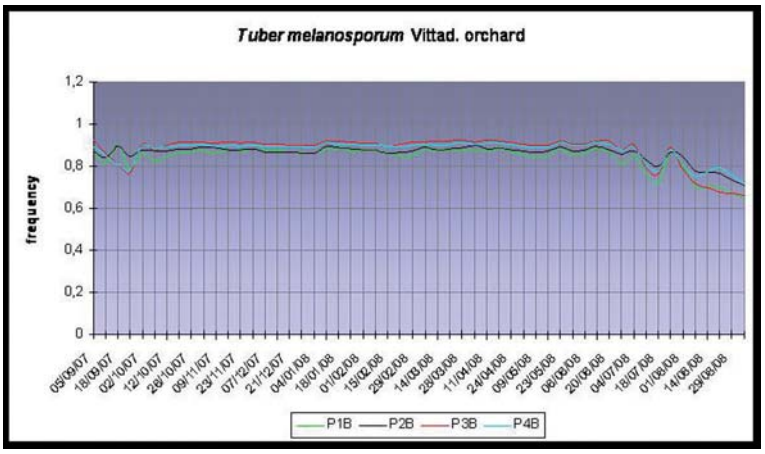

Fig. 1 andamento della frequenza misurata a 10 cm nei quattro siti della tartufaia di *Tuber melanosporum* Vittad.

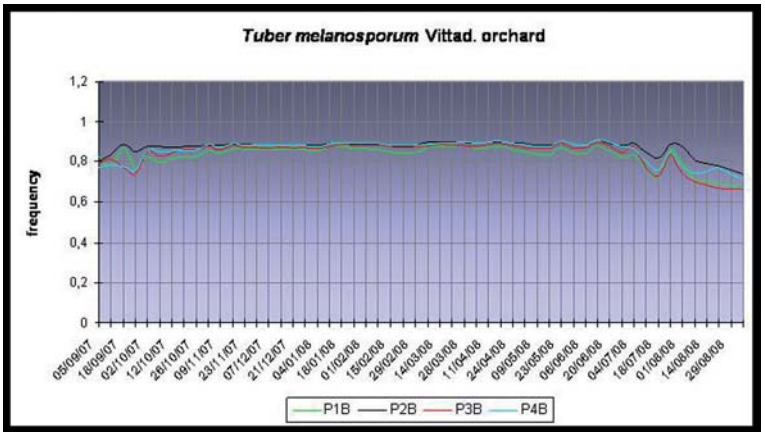

Fig. 2 andamento della frequenza misurata a 20 cm nei quattro siti della tartufaia di *Tuber melanosporum* Vittad.

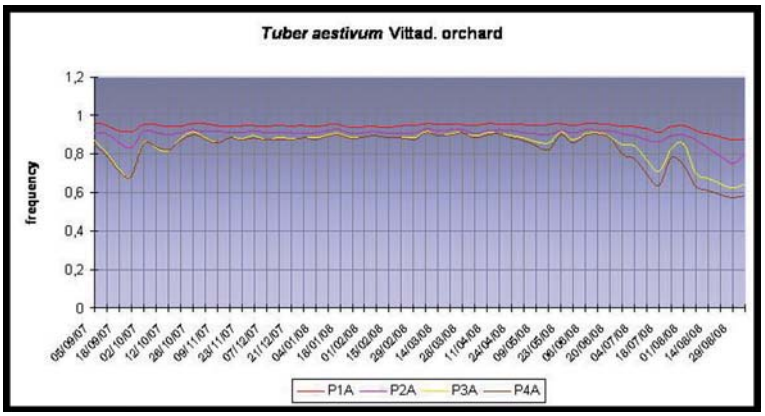

Fig. 3 andamento della frequenza misurata a 10 cm nei quattro siti della tartufaia di *Tuber aestivum* Vittad.

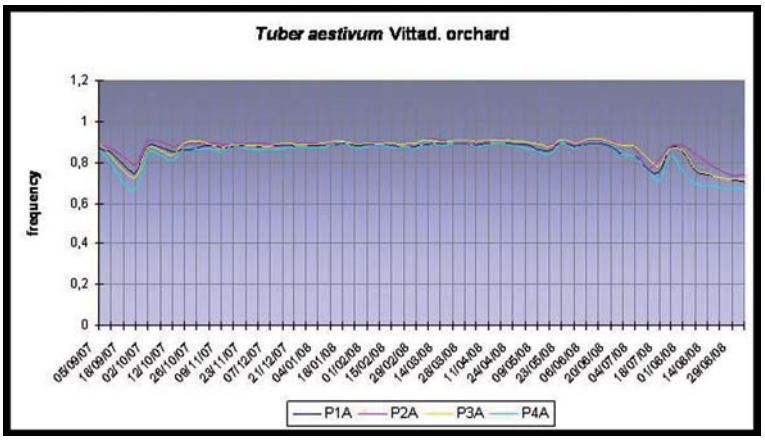

Fig. 4 andamento della frequenza misurata a 20 cm nei quattro siti della tartufaia di *Tuber aestivum* Vittad.

L'infiltrazione di acqua e l'evaporazione risultano infatti notevolmente accentuate nel periodo immediatamente successivo tale lavorazione: in particolare si hanno delle variazioni significative da luglio ad ottobre 2007, cosa che non si evidenzia per gli stessi mesi del 2008 a causa della minore quantità di pioggia, della compattazione del suolo e della ricrescita della copertura erbacea. Il contenuto idrico del suolo subisce infatti degli aumenti in corrispondenza delle precipitazioni avute per quel periodo (Fig. 5). Al contrario, nella tartufaia di *T. melanosporum*, siti irrigati (P1B e P2B) non presentano sostanziali differenze rispetto a quelli non irrigati (P3B e P4B). Come prevedibile, in entrambe le tartufaie, il periodo invernale e primaverile dell'anno sono caratterizzati da valori di frequenza tendenzialmente costanti ed omogenei, questi mesi sono inoltre meno sensibili agli eventi di pioggia. Le fluttuazioni di frequenza riscontrate sono d'entità minore in profondità rispetto a quanto rilevato in superficie (Fig. 1, 2, 3, 4): questo risulta vero soprattutto nella tartufaia di *T. aestivum*. Lo strato superiore, in cui si sviluppano la maggior parte dei corpi fruttiferi, risponde agli eventi di pioggia con incrementi molto rapidi, ma molto rapide sono anche le fasi di drenaggio; l'effetto di ricarica in seguito all'evento di precipitazione per lo strato superficiale si esaurisce quindi più velocemente rispetto a quanto accade più in profondità.

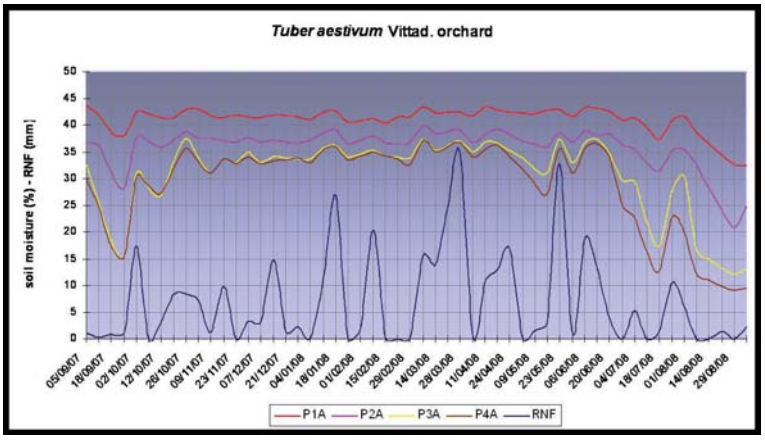

Fig. 5 andamento del contenuto volumetrico (soil moisture) nei primi 10 cm e mm di piogge cadute (RNF).

Conclusioni

Un aspetto che è emerso dall'analisi dei dati è la differenza dell'andamento dell'umidità nelle due tartufaie studiate: nei suoli dove fruttifica *T. melanosporum* l'umidità rilevata nei diversi pozzetti è molto più omogenea rispetto alla tartufaia di *T. aestivum*. Ciò conferma la maggiore capacità di adattamento ambientale del tartufo estivo rispetto al nero pregiato. Dati interessanti

sono emersi nel rilevamento dell'umidità su terreno sarchiato e non, come era prevedibile subito dopo le piogge nella parcella sarchiata si rileva una maggiore umidità nei primi 10 centimetri di suolo, data la maggiore permeabilità rispetto a quella non sarchiata.
Nei siti studiati saranno collocati dei sensori in continuo al fine di rilevare, con maggiore precisione, le variazioni del contenuto di umidità nell'arco delle ventiquattro ore.
Ciò consentirà di stabilire le relazioni che intercorrono tra il contenuto idrico del suolo, le fasi critiche del ciclo biologico e le produzioni di tartufo. Inoltre si prevede di realizzare un'equazione di calibrazione adeguata al caso studiato e che possa fornire risultati soddisfacenti per quanto riguarda i valori assoluti di umidità.

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BOTANICAL AND ENVIRONMENTAL ASPECTS OF THE NATURAL TRUFFLE BEDS

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Abstract
In research carried out between 2006 and 2008 in the province of Rieti (Central Italy), many natural truffle beds with the most important species of truffles were analysed from different points of view. The following are the preliminary results regarding the natural truffle beds of *T. magnatum* and *T. melanosporum*. The phytosociological data indicate that the sites of *T. melanosporum* are included in the phytosociological alliances of *Carpinion orientalis* Horvat 1958 and *Teucrio siculi-Quercenion cerridis* Ubaldi (1988) 1995 em. Scoppola and Filesi 1995. *T. magnatum* sites are included in the phytosociological alliances of *Salicion albae* Soó 1930 em. Moor 1958 and *Alnion incanae* Pawlowski in Pawlowski and Wallish 1928 in the riparian environment and in the alliances of *Tilio-Acerion* Klika 1955, *Carpinion orientalis* and occasionally *Geranio versicoloris-Fagion* Gentile 1970 in the hilly woods. The floristic data show several endemic taxa: this means the ecosystems are well conserved, without much human impact. The biological spectrum shows a high percentage of Hemicryptophytes, followed by Therophytes in *T. melanosporum* and by Phanerophytes in *T. magnatum*. The chorological spectrum shows a prevalence of Mediterranean s.l. chorology for *T. melanosporum* and Paleotemperate for *T. magnatum*. These data are important to support programs to conserve and manage natural truffle beds, to improve the production level and suitability of the environment for efficient truffle cultivation.
Key words: ecology, *Tuber magnatum*, *Tuber melanosporum*, flora, vegetation.

Introduction

Each species of truffles presents a specific ecology. The environments in which *Tuber magnatum* Pico and *T. melanosporum* Vittad. grow have quite different climatic and botanical characteristics (Granetti *et al*., 2005; Bencivenga *et al*., 1995; Bencivenga, 1994). It is very important to know the autoecology of the species, because this is a background for the cultivation and to preserve and improve the natural production of these precious fungi. Natural resources are essential to preserve the biodiversity of mushrooms cultivated locally. Moreover, the interruption and/or decrease of natural production is due to change in forestry management, badly managed truffle collection on too wide a scale, and climate change. For these reasons it is important, for each environment, to know the characteristics of natural productive truffle beds, and to choose the best practices to recover the non-productive sites. Thus, the aims of this research were to increase knowledge regarding the pedological, botanical and ectomycorrhizal aspects of natural truffle beds, to characterize, preserve and manage this important resource, and finally, to improve truffle cultivation.

Materials and methods

In research carried out between 2006 and 2008 in the province of Rieti (Central Italy), many natural truffle beds with the most important species of truffles were analysed from different points of view. With regard to the flora and vegetational aspects, phytosociological relevés were

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carried out during this study with Braun-Blanquet method (Braun-Blanquet, 1928, 1951, 1979; Van Der Maarel, 1976): 11 truffle beds of *T. melanosporum* and 15 truffle beds of *T. magnatum*. The syntaxonomical nomenclature has been updated up to the present day knowledge. Floristic data have been analysed using various references (Pignatti, 1982, Conti *et al*., 2005) and grouped in floristic lists, botanical families, chorological and biological *spectra*.

Results and Discussion

The following are the preliminary results regarding the natural truffle beds of *T. magnatum* and *T. melanosporum*.

The growth environment of the natural truffle beds of *T. melanosporum* is characterized by dynamic vegetation: open, dry and sunny stands, with a low vegetation coverage. The altitude is between 570 and 955 m a.s.l., the slope of the land is often elevated, the most frequent exposures are E-S-E and S-W (Tab. 1).

Tab. 1 Topographical data of natural truffle beds of *T. melanosporum* Vittad.

Relevé n°	Date	Altitude m a.s.l.	Exposure	Inclination in degrees	Surface m^2	Total coverage %
5	14/6/06	955	SW	14	12	60
4	14/6/06	950	SW	17	15	60
3	14/6/06	940	SSW	11	15	25
1	14/6/06	860	SE	16	15	5
1a	14/6/06	860	SE	11	8	10
2	14/6/06	800	NNE	20	6	40
19	10/7/07	735	SSE	18	20	70
8	16/6/06	675	ESE	18	8	60
7	16/6/06	670	ESE	18	10	90
20	10/7/07	650	SW	30	50	95
6	16/6/06	620	ESE	5	10	45
9	16/6/06	570	SSW	17	10	90

The natural truffle beds are located in former farmland and open woods, with *Quercus* spp. as symbiotic plants. The total coverage is very low. In these 11 truffle beds of *T. melanosporum*, the symbiotic plants are: *Quercus pubescens* Willd., *Q. dalechampii* Ten., *Q. cerris* L., *Ostrya carpinifolia* Scop. and *Corylus avellana* L..

The natural truffle beds of *T. magnatum* are mainly located in two situations: climatophylous woods and riparian vegetation. In any case these truffle beds require an undisturbed environment where the structure of vegetation is complex, with all layers present and total coverage (Fig. 1, Tab. 2).

Fig. 1 Natural truffle bed of *Tuber magnatum* Pico

Tab. 2 Topographical data of the *T. magnatum* Pico natural truffle beds.

Relevé n°	Date	Altitude m a.s.l.	Exposure	Inclination in degrees	Surface m^2	Total coverage %
26	11/7/07	933	WSW	10	30	90
23	11/7/07	805	SW	7	20	100
22	11/7/07	804	WSW	15	30	100
25	11/7/07	762	-	-	20	100
24	11/7/07	627	WSW	10	30	100
14	16/6/06	610	NNW	20	15	100
15	16/6/06	610	-	-	20	100
18	16/6/06	610	ENE	15	20	100
17	16/6/06	595	W	18	20	95
16	16/6/06	585	NNW	35	20	100
21	10/7/07	550	NNW	7	15	100
13	15/6/06	160	-	-	20	100
12	15/6/06	150	WNW	8	15	100
10	15/6/06	95	SW	27	20	100
11	15/6/06	90	-	-	15	100

These conditions are very hard to maintain, because the riparian environment is over exploited and threatened by agriculture, urban and industrial settlements. In climatophylous woods the moisture level has to be quite high and constant. In the 15 truffle beds detected the altitudinal range is from 90 to 933 m a.s.l. with a very variable slope and exposure from WSW to NNW (Tab. 2). The most frequent symbiotic species are: *Populus alba* L., *P. canescens* (Aiton) Sm., *P. nigra* L., *Salix alba* L., *Corylus avellana* L. and *Quercus cerris* L..

The flora of *T. melanosporum* shows 141 species distributed over 37 botanical families (Tab. 3).

Tab. 3 Number and percentage of species in the families of *Tuber melanosporum* Vittad.

Family	**n° of species**	**% of species**
Gramineae	21	14,9
Leguminosae	20	14,2
Compositae	12	8,5
Caryophyllaceae	9	6,4
Labiatae	9	6,4
Rosaceae	7	5,0
Cruciferae	6	4,3
Umbelliferae	6	4,3
Rubiaceae	6	4,3
Crassulaceae	4	2,8
Fagaceae	3	2,1
Cistaceae	3	2,1
Scrophulariaceae	3	2,1
Orchidaceae	3	2,1
Cupressaceae	2	1,4
Corylaceae	2	1,4
Ranunculaceae	2	1,4
Papaveraceae	2	1,4
Euphorbiaceae	2	1,4
Primulaceae	2	1,4
Others	17	12,0
Total	**141**	**100,0**

The flora of *T. magnatum* shows 141 species distributed over 44 botanical families (Tab. 4).

Tab. 4 Number and percentage of species in the families of *Tuber magnatum* Pico

Family	**n° of species**	**% of species**
Rosaceae	14	9,9
Gramineae	12	8,5
Compositae	10	7,1
Leguminosae	10	7,1
Labiatae	8	5,7
Orchidaceae	7	5,0
Rubiaceae	7	5,0
Salicaceae	6	4,3
Umbelliferae	6	4,3
Aceraceae	4	2,8
Corylaceae	4	2,8
Fagaceae	4	2,8
Liliaceae	4	2,8
Ranunculaceae	4	2,8
Caprifoliaceae	3	2,1
Cyperaceae	3	2,1
Violaceae	3	2,1
Boraginaceae	2	1,4
Cornaceae	2	1,4
Equisetaceae	2	1,4
Oleaceae	2	1,4
Ulmaceae	2	1,4
Others	22	15,6
total	**141**	**100**

The floristic data show several endemic taxa: in *T. melanosporum* they are *Erysimum pseudorhaeticum* Polatschek, *Polygala flavescens* DC, *Digitalis micrantha* Roth, *Festuca centro-appenninica* (Mgf.-Dbg.) Mgf.-Dbg., *Phleum ambiguum* Ten., in *T. magnatum* are *Equisetum telmateja* Ehrh, *Populus nigra* L., *Salvia glutinosa* L., *Polygala flavescens* DC.,. This means the ecosystems are well conserved, without much human impact.

The chorological spectrum shows a prevalence of Mediterranean s.l. chorology for *T. melanosporum* (Fig. 2) and Paleotemperate for *T. magnatum* (Fig. 3).

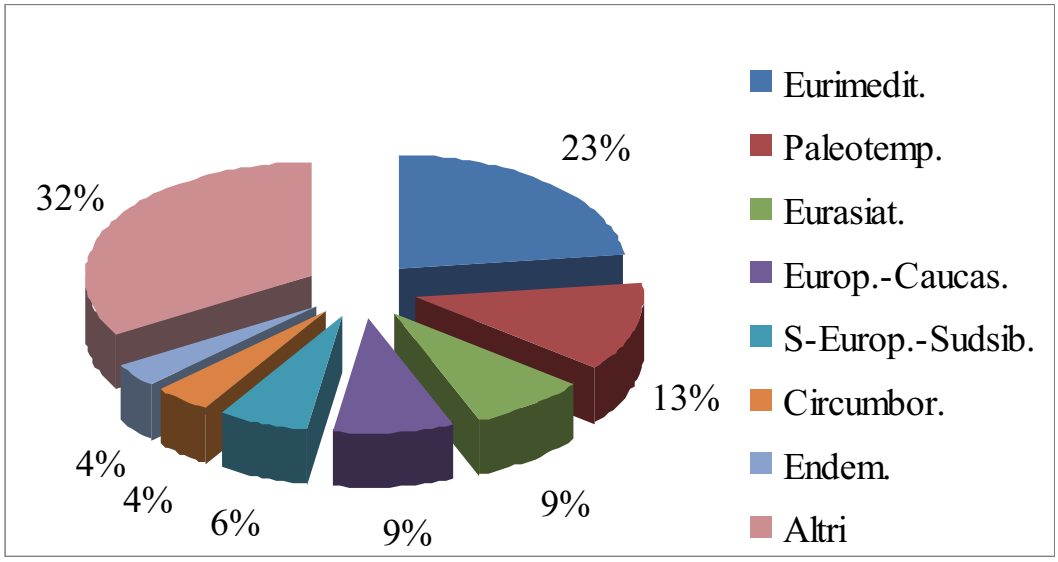


Fig. 2 The chorological spectrum of *Tuber melanosporum* Vittad.

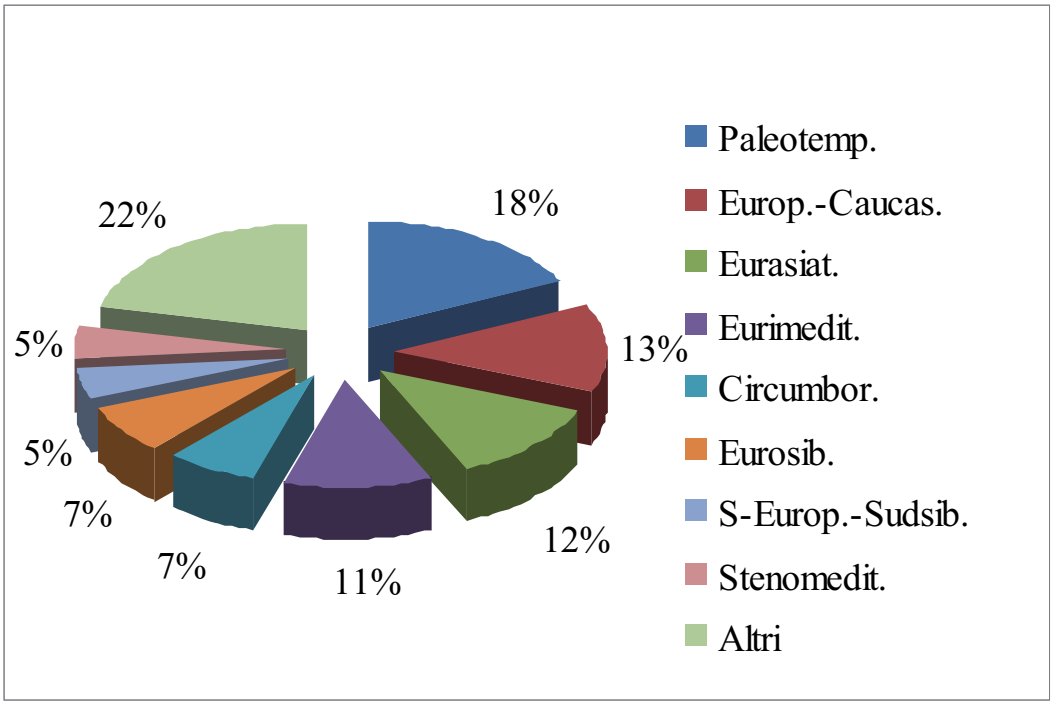
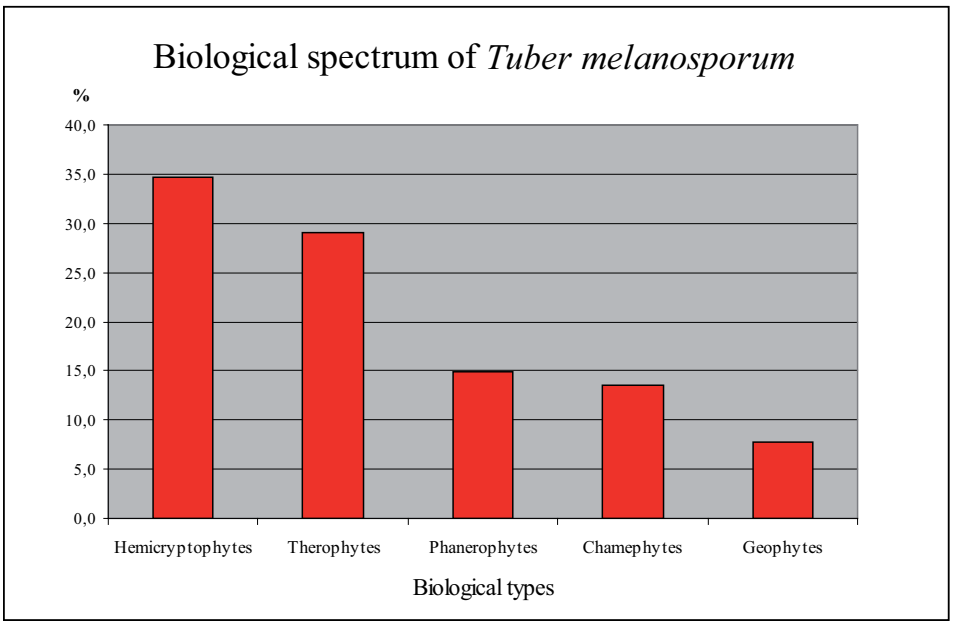
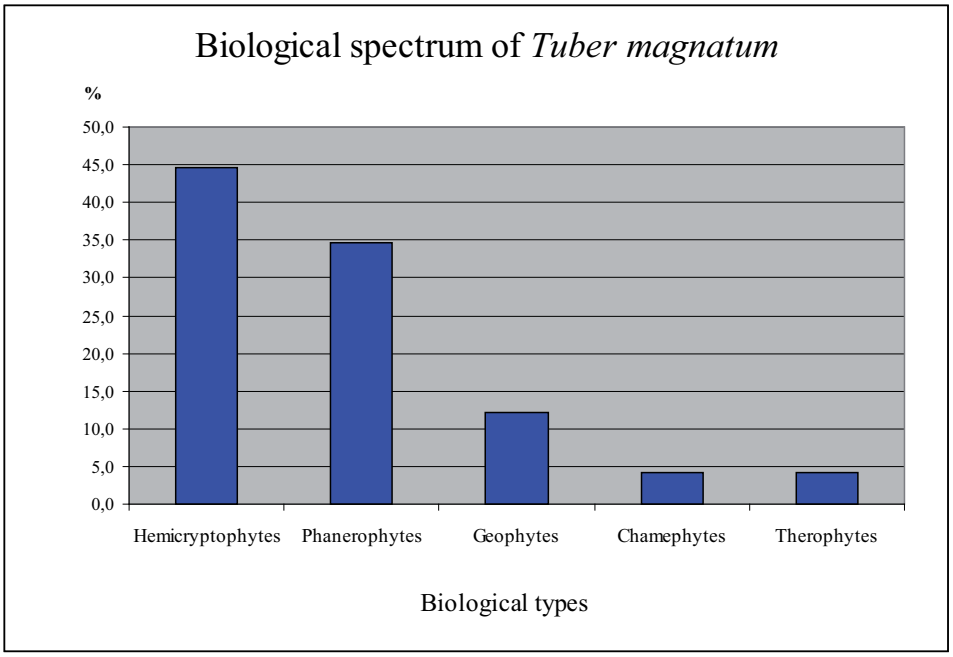


Fig. 3 The chorological spectrum of *Tuber magnatum* Pico

The biological spectrum shows a high percentage of Hemicryptophytes, followed by Therophytes in *T. melanosporum*: this confirms arid and thermophilous environments.



The biological spectrum shows a high percentage of Hemicryptophytes and Phanerophytes in *T. magnatum*: here we have woods with a very rich herbaceous stratum.



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Conclusions
The natural environments where *T. melanosporum* and *T. magnatum* grow show quite different

botanical characteristics: from open and dry woods to riparian vegetation, without much human impact, although there is the threat of excessive collection of truffles.
The research indicated certain specific vegetational types which need further study to better define their relationship to the truffles.
This knowledge must be supplemented with pedological information and is important to support programs to conserve and manage natural truffle beds, to improve the production level and suitability of the environment for efficient truffle cultivation.

Acknowledgements
We would like to thank Dr. Daniela Gigante for checking the phytosociological classification.

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INDAGINE PRELIMINARE SULLA MICOFLORA RIZOSFERICA IN QUERCETO E PIOPPETO CON DIFFERENTI LIVELLI DI PRODUTTIVITÀ DI *TUBER MAGNATUM*

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Abstract: Preliminary survey on micoflora rhizosphere under poplar and oak plantations with different productivity of *Tuber magnatum.*
Proves of specific relationships between soil micro-organisms and plant roots are so evident that a new word has been coined, 'rhizosphere', which indicates soil microbic flora (fungi) placed near the root apexes. *Tuber magnatum* fructification is still a mysterious phenomenon, maybe influenced by micro-environmental factors among which the 'rhizosphere'. This research has the aim to prove, by a quali-quantitative comparison, hypothesised relationships between the 'mycorhizospheres' of a poplar plantation and those of an oak plantation. In the Cuneo province two natural truffle-grounds were chosen as study-sites so far enough to be not reciprocally influenced (Priero oak plantation and Monchiero poplar plantation). Interesting, even if still preliminary, results were obtained by isolation fungal, carried out using the 'subsequent dilutions method'. No relations were detected between the fungal intensity and *T.magnatum* productivity, even if at the oak site mean values were slightly higher. Qualitative differences of fungal flora were demonstrated to be dependent on study-area, host plant species and *T. magnatum* productivity.
At Priero site thirty-five fungal forms were isolated with prevalence from the unproductive area. Six of these forms seem to be dependent on productivity (*Gliocladium viride*, *Penicillium nigricans*, *Acremonium* sp., *Penicillium terreus* etc), vice versa others were isolated only on the rhizospheric soil of the unproductive oak site (*Aspergillus terreus*, *Monilia* sp., *Dictyuchus* Leitg., *Gliocladium roseum*, ecc.). At Monchiero site thirty-one fungal forms were identified, among which eleven seem to be dependent on the poplar site productivity (*Aspergillus terreus*, *Gliocladium roseum*, *Penicillium nigricans*, *Aspergillus niger*, ecc), vice versa other thirteen were isolated from the 'rhizosphere' of the unproductive species (*Mucor* sp., *Penicillium albicans*, *Aspergillus ochraceus*, *Rhizopus stolonifer*, *Trichurus spiralis*, *ecc.)*. Comparison of qualitative data from all the samples is a difficult operation as some 'mycetes' (i.e. *Penicillium albicans)*, are ubiquitous and not correlable to host plant, productivity and site location. Few fungal forms seem to be dependent on productivity as they were found out only under productive oak and poplar (rhizoctonic mycelium, *Penicillium nigricans*). Finally the presented preliminary results about 'rhizosphere' micoflora telluric are encouraging research continuation.
Key words: ecology, micoflora telluric, white truffle.

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Introduzione

Tra i microrganismi tellurici e le radici delle piante esistono stretti rapporti influenzati dall'ospite arboreo, dai funghi simbionti presenti e da una ampia gamma di fattori abiotici. La fruttificazione del *Tuber magnatum*, ad esempio, rappresenta tuttora un enigma; sicuramente fattori ecologici che possono condizionarne o favorirne lo sviluppo, importanti ma poco conosciuti, sono quelli microambientali ed in particolare quelli relativi alla flora microbica del suolo prossimo alle radici recanti l'infezione micorrizica o ai carpofori stessi. Ancora scarse sono le conoscenze sulla rizosfera delle tartufaie; nel 1940 Sappa formulava già l'ipotesi dell'effetto stimolante la germinazione sporale del *Tuber* esercitato dagli enzimi prodotti dai microganismi del suolo. Ciò premesso, è evidente l'interesse che può derivare da una indagine quali-quantitativa sui microrganismi rizosferici e della loro possibile influenza sulla fruttificazione del tartufo bianco

pregiato, tanto più che le attività antropiche possono modificarne i reciproci rapporti (Luppi *et al*., 1970; Garbaye, Bowen, 1989).
Col presente lavoro, di tipo preliminare, sono riportati alcuni dati relativi alla composizione ifomicetica presente nella rizosfera di un querceto e di un pioppeto di aree piemontesi con differenti livelli di produttività di *T. magnatum*.

Materiali e metodi

Aree di saggio e campionamenti

Le aree di studio sono state individuate in 2 tartufaie naturali ubicate nei pressi di Cuneo (Piemonte), sufficientemente distanti e tali da non influenzarsi a vicenda: un querceto a farnia in Cascina Canonicato nel Comune di Priero e un pioppeto monoclonale (*Populus deltoides* cv "Carolin") in Valle S. Stefano nel Comune di Monchiero. In ciascuna tartufaia si sono individuate due sub-aree caratterizzate da differente produttività di *Tuber magnatum*. In ognuna di queste, su indicazione dei "trifulau" e nel mese di novembre, si è effettuato da piante produttive e non produttive il prelievo di campioni di suolo.
Per ogni sub-area produttiva e non produttiva, distanti l'una dall'altra nella prima località circa 50m e nella seconda circa 25m, si sono prelevati campioni in 5 punti distinti, nell'arco di circa 10m e a una profondità di circa 20cm, in prossimità delle terminazioni radicali.
Tutte le operazioni di prelievo e di stoccaggio del suolo sono state eseguite con la massima accortezza per evitare l'apporto di eventuali contaminazioni. Prima di attuare le analisi di laboratorio i campioni sono stati unificati tenendo conto della zona di origine (Priero/ Monchiero), della specie arborea (quercia/pioppo) e della segnalata capacità tartufigena. La loro identificazione è riassunta in tabella 1.

Tab. 1 campioni di suolo rizosferico

denominazione del campione	Località	Specie arborea ospite	Produttività di *T. magnatum*
A	Priero	Querceto	sì
B	Priero	Querceto	no
C	Monchiero	Pioppeto	sì
D	Monchiero	Pioppeto	no

Isolamento microbiologico

Si è adottata la tecnica di laboratorio delle "diluizioni successive"; nello specifico si sono utilizzati quantitativi di 5 g di suolo umido rizosferico, omogeneizzati in mortaio, con una procedura comprensiva di diverse fasi.
In avvio, viene preparata, in soluzione acquosa di pirofosfato di sodio (0,1%), una prima diluizione del campione alla 10^{-1}; in seguito si eseguono, con successiva progressione geometrica (da 10^{-1} a 10^{-6} …), le diluizioni successive in soluzione acquosa di NaCl allo 0,9% (soluzione fisiologica). Si procede quindi all'inoculazione di piastre Petri in ragione di 1 ml per ciascuna diluizione, allestendo tre ripetizioni per ognuna. In particolare le diverse diluizioni di suolo rizosferico sono amalgamate con 20ml di terreno nutritivo agarizzato (agar malto saccarosato all'1%). Il materiale viene poi collocato in termostato alla temperatura di 25°C sino a fine prova, per un totale di 9 giorni di coltura.

Valutazioni quali-quantitative della carica fungina

L'analisi quantitativa è stata effettuata su base visiva, a intervalli di 3 giorni e per una durata di 9; in particolare, per ciascuna ripetizione, si è espresso un giudizio d'intensità relativo alla presenza totale delle colonie fungine. Per l'espressione dei giudizi si fa riferimento ai valori indice espressi in tabella 2.

Tab. 2 Valori indice adottati nelle valutazioni quantitative delle colonie fungine

Valore indice (% visiva di presenza colonie)	Intensità di presenza (n° colonie)
0	Assenza (0)
1	Presenza scarsa (1-6)
2	Presenza mediocre (7-12)
3	Presenza elevata (13-19)
4	Presenza assai elevata (≥ 20)

I risultati sono inoltre suddivisi, per epoca di lettura dopo 3, 6 e 9 giorni e per crescente diluizione (da 10^{-1} a 10^{-6}).
I valori di intensità media di carica fungina vengono ricavati dalla media dei valori degli indici ottenuti nel corso delle diverse letture per singola ripetizione.
L'analisi qualitativa ha richiesto il trapianto delle singole colonie miceliari, per ottenere, "in vitro", miceli puri e idonei alla determinazione tassonomica; l'identificazione delle forme fungine è stata condotta su base morfologica, mediante osservazioni allo stereomicroscopio e al microscopio ottico.
Allo stereomicroscopio si sono analizzati l'aspetto, il colore, la capacità di accrescimento e di fruttificazione delle colonie. Per il controllo microscopico, si sono allestiti preparati trattati con acido acetico per il fissaggio dei conidi ai conidiofori e acido lattico e Tripan blu, per colorare le diverse strutture. Le analisi qualitative si sono condotte mediante consultazioni della bibliografia specializzata (Gilman, 1957; Arx, 1970; Ellis, 1971; Hanlin, 1997; Barnett, Hunter, 1998; Watanabe, 2002; Dugan, 2005).

Risultati
Per quanto concerne la quantificazione della carica fungina la figura 1 riassume gli esiti conseguiti a livello di querceto e di pioppeto con differente capacità produttiva.

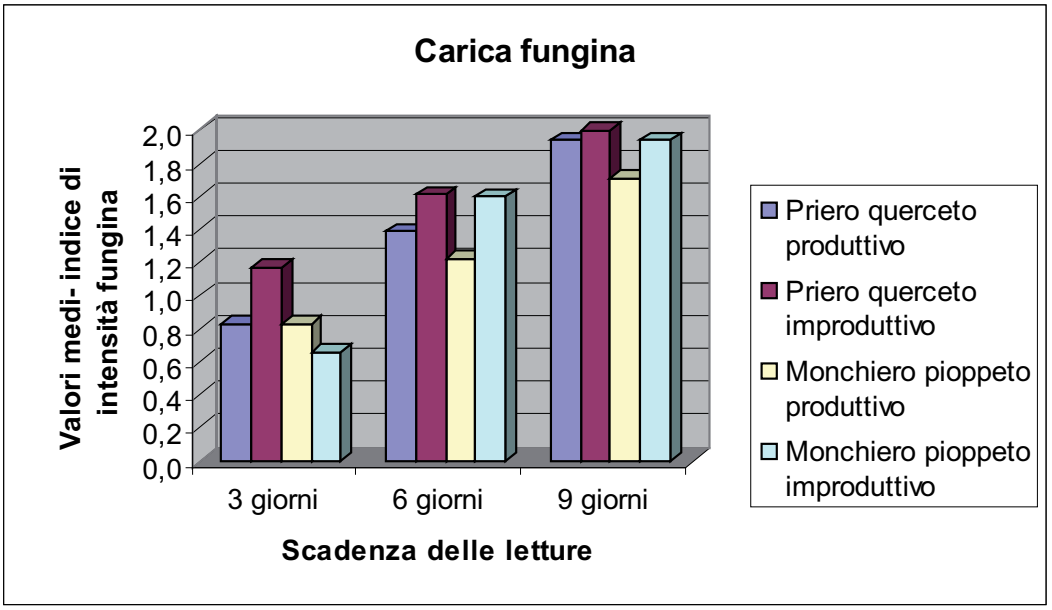

Fig. 1 Valori medi – indice di intensità fungina per periodo di lettura e per pianta ospite a diversa produttività di *T. magnatum*

Gli istogrammi della figura 1 offrono spunto alle considerazioni che seguono:

- a fine prova non appaiono sostanziali differenze di intensità fungina tra il suolo rizosferico di Priero e Monchiero nei diversi livelli di produttività del *T. magnatum*;
- nel querceto produttivo di Priero si riscontra un regolare incremento di intensità fungina e, per l'area improduttiva, una carica fungina da subito più intensamente presente (dopo 3 giorni), perchè forse caratterizzata da specie a più pronta fruttificazione;
- nel pioppeto di Monchiero la carica fungina è apparentemente più intensa a livello del suolo rizosferico improduttivo.

Di un certo interesse sono gli esiti relativi alle analisi qualitative sulla micoflora. Nello specifico dalla figura 2 si evince che:

- il numero delle specie e forme fungine isolate varia, sia in relazione all'ospite arboreo, sia in funzione della produttività;
- a Priero il suolo rizosferico del querceto improduttivo manifesta, rispetto a quello produttivo, una microflora fungina di maggiore difformità;
- nel pioppeto di Monchiero si riscontra un andamento opposto.

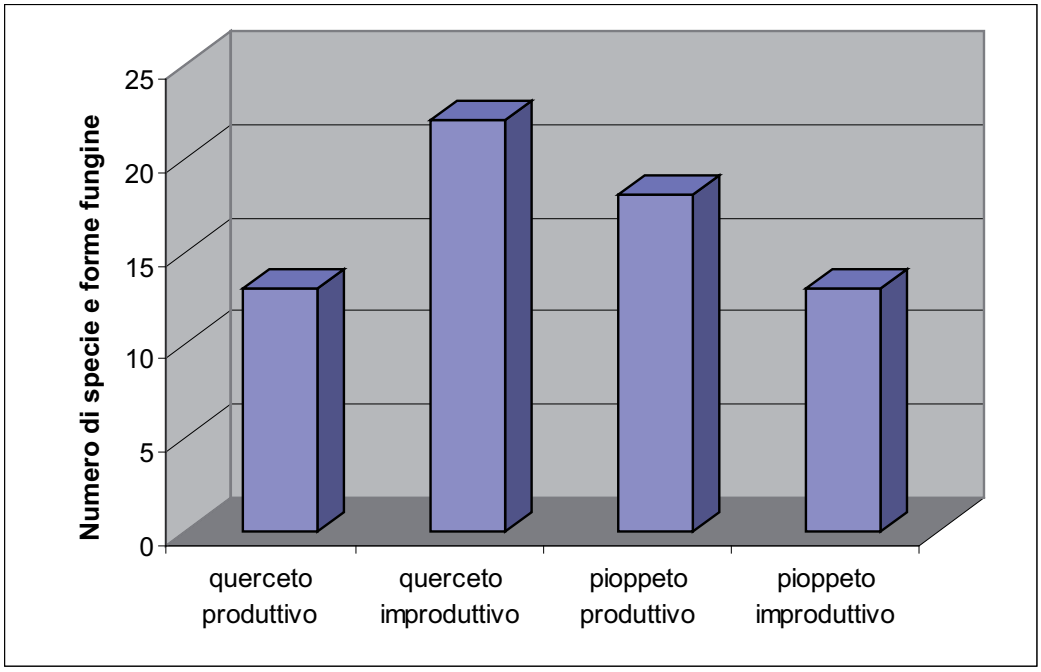

Fig. 2 Numero di specie e forme fungine isolate in funzione delle aree di studio a diversa produttività

In tabella 3 sono riportati in dettaglio i risultati delle analisi qualitative, diversificati per area, ospite arboreo e capacità produttiva di *T. magnatum*.

Tab. 3 Specie e forme fungine isolate nei diversi campioni di suolo rizosferico

AREA DI SAGGIO	FORME FUNGINE IDENTIFICATE
PRIERO QUERCETO IMPRODUTTIVO	*Aspergillus terreus*, *Penicillium resticulosum*, *Mucor* **sp.**, *Trichoderma* **sp.**, *Penicillium albicans*, **un micelio sterile di colore rosa**, *Eupenicillium* **sp. (colonia azzurra), micelio sterile di colore arancione**, *Pythium* **sp.**, *Eupenicillium* **sp. (colonia verde),** *Monilia* **sp.**, *Dictyuchus* **Leitg.**, *Gliocladium roseum*, *Verticillium* **sp.**, *Bispora* **sp.**, *Aspergillus candidus*, *Diplodia* **sp.**, *Chrysosporium* **sp.**, *Rhinocladiella anceps*, *Gonatobotrys* **sp.**, *Penicillium* **sp. (con aspetto bianco e ramificazione biverticellata simmetrica),** *Eupenicillium* **sp. (colonia rosa)**
PRIERO QUERCETO PRODUTTIVO	*Mucor* **sp**, *Trichoderma* **sp.**, *Penicillium albicans*, *Eupenicillium* **sp. (colonia azzurra)** *Pythium* **sp.**, *Verticillium* **sp.**, *Aspergillus candidus*, *Gliocladium viride*, **un micelio tipo** *Rhizoctonia* **sp.**, *Penicillium nigricans*, *Acremonium* **sp.**, *Penicillium terreum* **e un micelio sterile color salmone**
MONCHIERO PIOPPETO IMPRODUTTIVO	*Mucor* sp., *Penicillium albicans, Pythium* sp., *Verticillium* sp.,*Tilachlidium* sp., *Aspergillus ochraceus, Rhizopus stolonifer, Trichurus spiralis, Trichoderma pseudokoningi, Acremonium roseum, Sporotrichum* sp., *Fusarium* sp., un micelio sterile di colore marrone.
MONCHIERO PIOPPETO PRODUTTIVO	*Aspergillus terreus*, *Penicillium albicans*, *Pythium*, *Monilia* **sp.**, *Gliocladium roseum*, *Verticillium* **sp., un micelio tipo** *Rhizoctonia* **sp.**, *Penicillium nigricans*, *Aspergillus ochraceus*, *Rhizopus stolonifer*, *Trichoderma pseudokoningi*, **un micelio sterile di colore marrone**, *Penicillium* **sp (colonia verde),** *Aspergillus niger*, *Talaromyces* **sp. (colonia rosa), un micelio bianco che forma abozzi**, *Ophistoma* **sp.**, *Phialocephala* **sp;**

Emergono differenze di popolazione fungina in funzione della specie arborea simbionte, dell'area in esame e della produttività al *T. magnatum*.
Nel querceto di Priero l'area improduttiva presenta un maggior numero di forme fungine isolate: in particolare sono 22 contro 13 dell'area produttiva. Risulta inoltre che:

- 7 forme fungine ossia, *Mucor* sp., *Trichoderma* sp., *Penicillium albicans* (figura 3), *Eupenicillium* sp.(colonia azzurra), *Pythium* sp., *Verticillium* sp., *Aspergillus candidus*, sono presenti in entrambi i campioni di suolo rizosferico (produttivo/improduttivo), manifestando così una particolare ubiquità;
- 6 forme fungine sono state isolate solo nel suolo rizosferico del querceto produttivo: *Gliocladium viride*, un micelio tipo *Rhizoctonia* sp., *Penicillium nigricans*, *Acremonium* sp., *Penicillium terreu*s e un micelio sterile color salmone;
- alcuni miceti paiono, nella quercia, correlabili con l'assenza di produttività perché isolati unicamente dal suolo rizosferico di sub-area non produttiva: *Aspergillus terreus*, *Penicillium resticulosum*, alcuni miceli sterili di colore rosa ed arancione, E*upenicillium* sp.(colonia verde), *Monilia* sp., *Dictyuchus* Leitg., *Gliocladium roseum*, *Bispora* sp., *Diplodia* sp., *Chrysosporium* sp., *Rhinocladiella anceps*, *Gonatobotrys* sp., *Penicillium* sp. (con aspetto bianco e ramificazione biverticellata simmetrica), *Eupenicillium* sp. (colonia di colore rosa).

Nel pioppeto di Monchiero, a differenza del querceto di Priero, un numero maggiore di forme fungine è stato isolato dal suolo produttivo: nello specifico 18 forme contro 13.
Altri confronti a livello qualitativo evidenziano che:

- 8 forme fungine sono state isolate indipendentemente dalla produttività, in quanto rinvenute in entrambi i campioni: *Penicillium albicans*, *Pythium* sp., *Verticillium* sp., *Aspergillus ochraceus* (figura 4), *Rhizopus stolonifer*, *Trichoderma pseudokoningi* e un micelio sterile di colore marrone;

- 6 sono state rinvenute esclusivamente nel suolo rizosferico improduttivo: *Mucor* sp., *Tilachlidium* sp., *Trichurus spiralis*, *Acremonium roseum*, *Sporotrichum* sp., *Fusarium* sp;
- 11 da quello produttivo per *T. magnatum*: *Aspergillus terreus*, *Monilia* sp., *Gliocladium roseum*, un micelio tipo *Rhizoctonia* sp., *Penicillium nigricans*, *Penicillium* sp. (colonia di colore verde), *Aspergillus niger*, *Talaromyces* sp. (colonia di colore rosa), un micelio che forma abbozzi di fruttificazioni, *Ophistoma* sp. e *Phialocephala* sp..

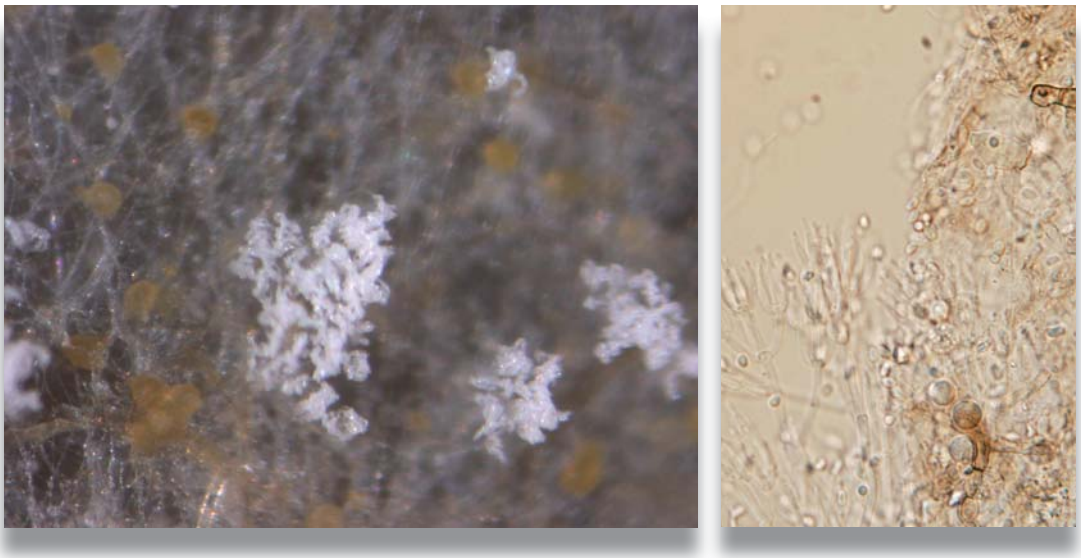

Fig. 3 *Penicillium albicans*: allo stereomicroscopio sono visibili le fruttificazioni conidiche caratterizzate da una colorazione bianca. A destra è visibile la fruttificazione conidica osservata al microscopio ottico

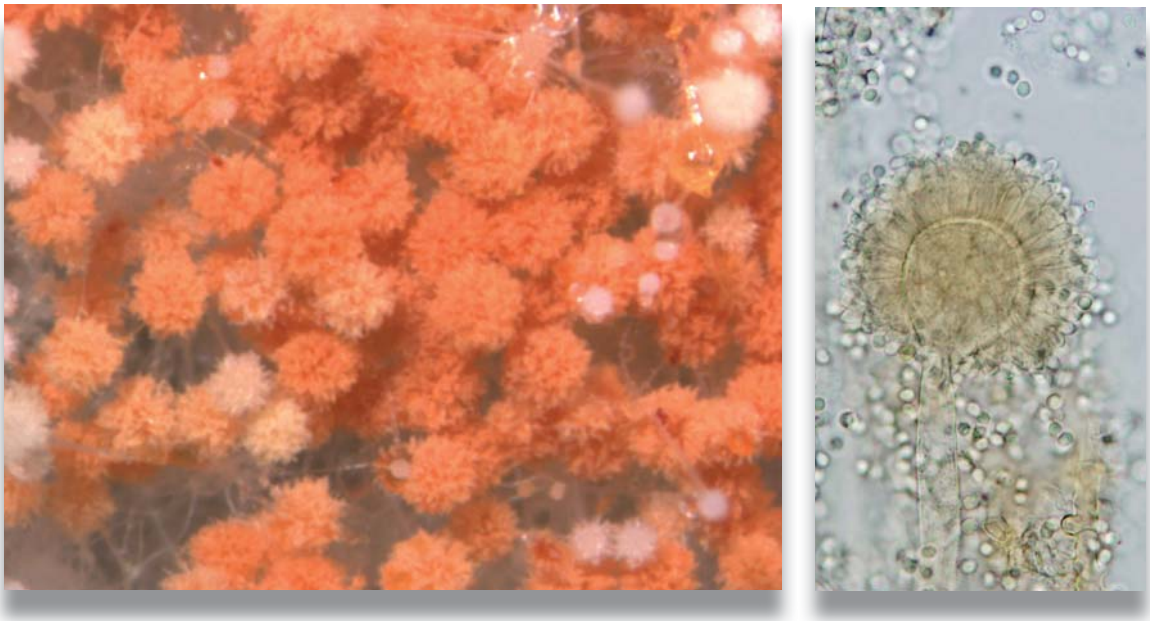

Fig. 4 *Aspergillus ochraceus*. I conidiofori portano conidi colorati. Al microscopio ottico i fialidi sono disposti in due file e i conidi con l'età ingialliscono

Conclusioni

I risultati conseguiti forniscono alcuni preliminari orientamenti sul possibile effetto selettivo esercitato da esemplari di farnia e pioppo monoclonale, localmente segnalati produttivi e non di *T. magnatum*, sulla micoflora della loro rizosfera; allo stesso tempo evidenziano le grandi difficoltà che subentrano quando si intenda approfondire le conoscenze su tali problematiche.

In sintesi i primi esiti offrono spunto alle seguenti considerazioni:

- il numero di forme della micoflora rizosferica associabili alla presenza del tartufo bianco, indipendentemente dalla pianta ospite e dal sito di campionamento, è risultato scarso;
- non si sono individuate forme fungine che, indipendentemente dalla pianta ospite, paiono legate alle condizioni di improduttività;
- per contro si sono rinvenute forme fungine che nel querceto paiono in sintonia con l'improduttività, mentre nel pioppeto sembrano connesse alla condizione di produttività.

Sulla base di questi primi risultati risulta indispensabile procedere all'analisi di un più cospicuo numero di campioni, prelevati da diverse piante simbionti, in differenti località, produttive ed improduttive e, eventualmente, in diversi periodi dell'anno. Solo così operando sarà possibile mettere in luce, con maggior sicurezza, gli effetti esercitati dalla pianta ospite su alcuni fattori macro e micro ambientali della propria rizosfera, influenti, sulla selezione della micoflora tellurica convivente in tali ambiti, da individuare, come organismi spia, della ottimale condizione ecologica di nicchia per il *Tuber magnatum*.

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INDICATOR-BASED APPROACH FOR MAPPING AND ASSESSING *TUBER MAGNATUM* PICO PRESENCE IN WESTERN LIGURIA (ITALY)

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Abstract

The availability of a potential distribution map of truffles represents a powerful tool for an effective preservation of the areas where these precious species grow or might grow.

The paper presents an original approach to prepare this kind of maps exploiting data gathered both in the field and from several indexes, commonly used in GIS analysis. Specifically, the proposed method consists of two steps: in the first one, a set of data concerning the pedological and botanical characterization of investigated localities is collected by a number of on field-surveys. In the second step a potential map is built on the basis of information obtained by combining collected data with an index derived from a 3D-elevation model of soil. A map has been created and the potential productiveness of some sample sites was been checked. The results achieved encourage further studies on this issue and extensive mapping of further areas.

Key words: ecology, multilayer binary mapping, white truffle.

Introduction

The white truffle presence in Liguria (Fig. 1A) is known to a few people so far. The most important area for truffle production in Liguria is Val Bormida, located in the north – midwestern zone, bordering with Piedmont "Langhe". Since the Ligurian white truffle is traditionally sold in Piedmont markets and it is not adequately valorised as typical Ligurian product, Val Bormida (Fig. 1B) truffle hunters are trying to advertise the local production in order to enhance the Ligurian truffle market. "Comunità Montana Alta Val Bormida" funded a research aimed at exploring the Ligurian zone of the Val Bormida to map the potential distribution of the truffles.

The first step of the project consisted of compiling a detailed check-list of productive places where truffles are found, with the collaboration of "Associazione Ligure tartufai e Tartuficoltori". The aim was to realize a potential distribution map of white truffle, on the basis of the presence *Tuber magnatum* Pico distribution in Liguria, and to provide a tool for an ecological territorial assesment planning.

Nowadays, there are different truffle maps of some Italian regions (Baglioni & Gardin, 1998; Tibiletti & Zambonelli, 1999; Biagioni *et al*., 2005; web site). These maps were exclusively based on overlay queries of data layers in GIS environment.

A modern approach includes mapping by means of a complex model based on some kind of mathematical analysis performed on each data layer employed. This model was already applied to different subjects (Guisan *et al.,* 2000; Fleishman *et al.,* 2001; Augustin *et al*., 2001; Ottaviani *et al*., 2004; Leopold & Völkel, 2007). The final resulting map is a qualitative complex layer combination, with exhaustive and reliable information. The methodological approach above described is at the basis of this study. The map was realized by using predictive quantified variables, among which the topographic wetness was applied so far in morphometric studies (Gessler *et al.,* 1995; Moore *et al*., 1991, 1993). This potential map plays an important role for the local territory valorisation and management.

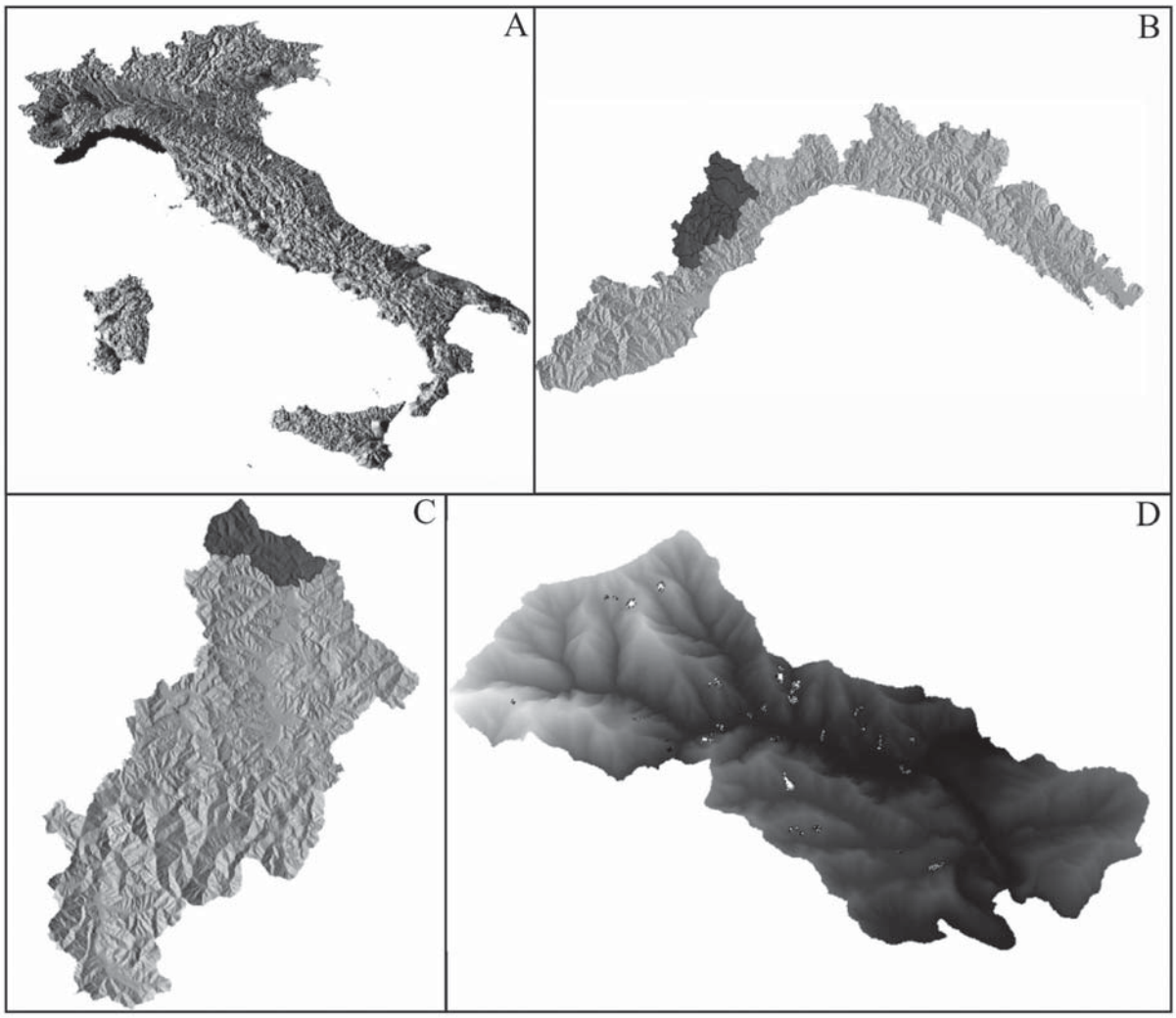

Fig. 1 A) Ligurian Region (dark - grey); B) Alta Val Bormida (dark - grey); C) study area (dark - grey); D) truffle ground sites (white spots).

Study Area

Liguria (Fig. 1A and 1B) is a NW Italy region to the border with Piedmont.

The main production zone of *Tuber magnatum* in Liguria is "Alta Val Bormida" (Fig.1B).

Study area (30.000 Ha Fig. 1C, detail in Fig. 1D) is located in the municipality of Piana Crixia (geographic coordinates in WGS 84 system: 8.16° longitude, 44.49° latitude). Study area altitude ranges from 290 to 600 m a.s.l. Lithology is dominated by sedimentary rocks, which are known, in the regional geological literature, as sediments of Tertiary Piedmont Basin (TPB). Basement sedimentary rocks are constituted by Voltri Group metaophyolite complex (Vanossi *et al*., 1984; Chiesa *et al.,* 1975). The latter is composed of serpentinites, metabasites and metasediments. However, the ophyolitic rocks crop out only in a restricted spots of the south sector of the study area. The sedimentary rocks along the eastern side of "Bormida di Spigno" river valley mainly consist of conglomerate sandstones (Molare Formation, Gelati *et al.,*1993), while the western sector is characterized by outcrops of marls and silty sandstones (Rocchetta and Monesiglio Formations, Gelati & Gnaccolini, 1998 and 1996).

The intense erosion processes on the large marls outcrops produced in the past an extensive badlands landscape and a complex geomorphology with several steep, scarp slope and knife-edge ridges. These characteristic badland landforms are known in Italy as "calanchi" (Castiglioni, 1933; Alexander, 1982; Pinna and Vittorini, 1989).

The local climate type is "Mediterranean oceanic" (Rivas-Martinez, 2004), the average annual

temperature is 12.9°C. The soil moisture regime is xeric, with a mesic soil temperature regime, according to Soil Taxonomy (USDA, 2006). Vegetation area is characterized by a mixed oak woods and chestnut woods. The dominant trees in mixed oak woods are *Quercus pubescens* Willd. and *Q. petraea* (Matt.) Liebl.; *Populus alba* L. and *Tilia cordata* Mill. are present along the river valleys. Agricultural activity is limited by the stony soil and the steep slope; moreover, only fluvial terraces and plains are cultivated.

Materials and Methods

Over the past two years, a lot of surveys were carried out in the study area and for each survey, geographic coordinates of the related sample areas were acquired by a GPS receiver. Specifically, 40 surveys were performed in natural well-known white truffle-grounds. Field investigations were carried out by a research team accompanied by trained dogs. The method employed is based on the investigation of ecological characteristics of the study area and on production of different thematic maps. All the maps were processed and developed in a GIS environment and the geographic database was produced. Fig. 2 shows the working scheme developed.

Mycology map and fungi analysis

The hypogeous and epigeous fungi of natural truffle-ground were collected and identified. A truffle-ground geodatabase was created; then a vector layer of survey areas was produced.

Vegetation map and plant analysis

Trees and shrub species were identified and plant communities were analysed in order to produce a vegetation map of the study area. Each plant community was classified into two categories: suitable or unsuitable for *Tuber magnatum* growth. This map was created using the G.R.A.S.S. – (Geographic Resources Analysis System) package and converted from a vector to raster layer with a grid cell dimension of 5m.

The classification for each vegetation class is reported in Table 1.

Thereby, a new raster layer (VL) was created whose binary pels represent the class (suitable or unsuitable) wich the corresponding pel in the vegetation layer belongs to.

Table 1 - Plant communities and their suitability for *T. magnatum* growth.

Plant communities	**Dominant species**	**Suitability for *T. magnatum***
Chestnut woods	*Castanea sativa*	0
Mixed chestnut woods with scots-pine woods	*Castanea sativa*, *Pinus sylvestris*	0
Scots-pine woods	*Pinus sylvestris*	0
Mixed *Tilia* spp. and *Acer* spp. woods	*Tilia cordata, Acer* spp.	1
Mixed oaks woods	*Quercus pubescens*, *Quercus cerris*	1
Poplar and willow woods	*Polpulus alba, Salix alba*	1
Quercus pubescent - woods	*Quercus pubescens*	1
Shrubs	*Juniperus communis*	0
Grassland	*Brachypodium* spp., *Bromus* spp.	0
Sowable lands		0

Pedological map and soil analysis

The studied soil profiles were characterized by field description, bulk sampling, routine physical and chemical analyses. Field descriptions were performed according to the methods and terminology of I.S.S.D.S (2006). Laboratory routine analyses were performed in compliance with proposed Italian official methods (MiPAF 1999). The soil profiles were described up to the bedrock (if present) or to a depth of 50 cm because *Tuber magnatum* grows in a short depth range (0 – 30 cm, Verlhac *et al*., 1990). Soils were explained according to their properties defined in terms of diagnostic horizons, properties and materials, following the criteria of the World Reference Base (Soil Groups, FAO, 2006). Several pedological units were mapped over a specific lithostratigraphic unit and correlated with different slope gradient and land use. Finally, the wet soils (groundwater influence) were derived using the topographic wetness index calculation.

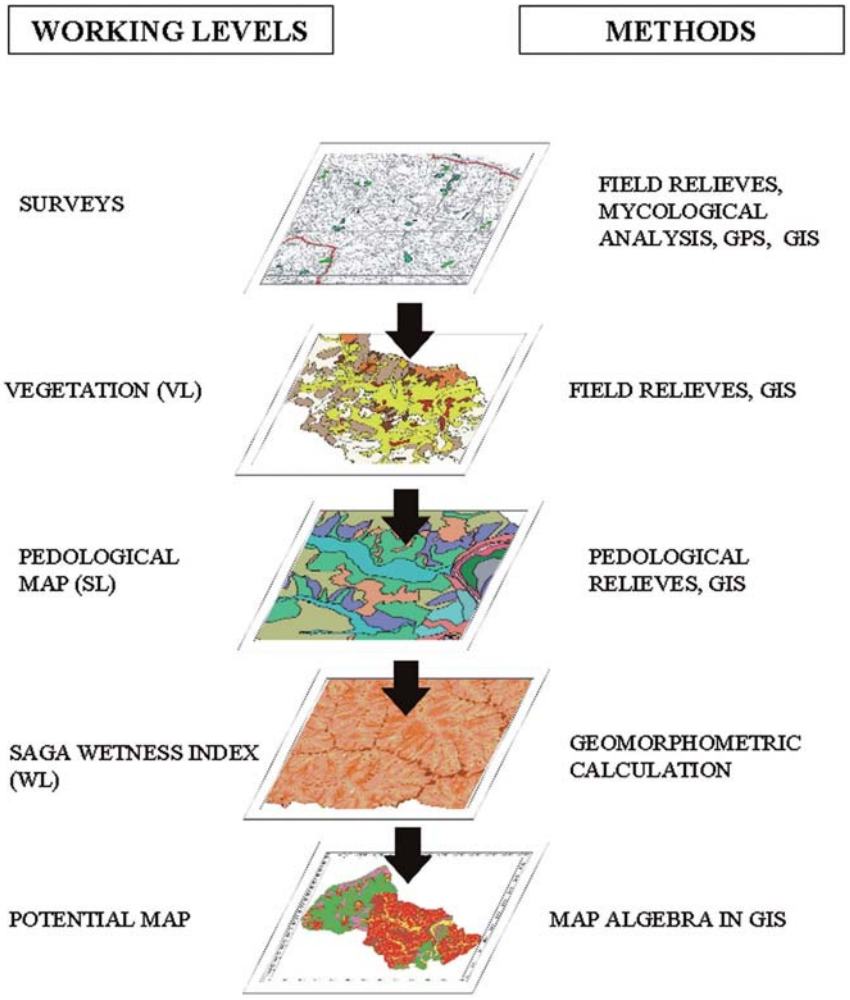

Fig. 2 The developed working schemes and methods.

Each pedological unit was classified into suitable or unsuitable for *Tuber magnatum* growth through the comparison with the physical and chemical average values of the studied truffle-ground soils. The classification for each soil composition is shown in Table 2.

As similarly done for vegetation a new raster layer (SL) was created whose binary pels represent the class (suitable or unsuitable), which the corresponding pel in the soil layer belongs to.

Wetness index map

A Digital Elevation Model (DEM) with 5 m cell size resolution was created using a grid elaboration

in the GRASS GIS. Wetness index was calculated from the previous DEM by means of a specific GIS system for automated geoscientific analysis. The wetness index characterizing the hydrological nature of each grid, is defined as:

$w_i = \ln(As / \tan\beta)$

where w_i = wetness index, A*s* = specific catchment area, β = slope angle of soil in degrees.

The SAGA WETNESS index (Boehner *et al*., 2002) used in this study is based on a modified catchment area calculation, which does not think of the flow as very thin film. As a result it predicts for cells situated in valley floors with a small vertical distance to a more realistic channel, higher potential soil moisture compared to the standard "topographic wetness index calculation ".

The wetness index values range from 1.45 to 14 while the truffle-grounds (40 sites) are characterised by a wetness index value always higher than 8.

A threshold wetness index (value >8) was used to separate the typical truffle-grounds soils from the other typologies. Hence, if wetness index is greater than 8, we set the wetness suitability metric to 1, otherwise 0.

As similarly done for previous layers, a new raster layer (WL) was created whose binary pels represent the class (suitable or unsuitable) which the corresponding pel in the wetness index layer belongs to.

Potential map

The potential distribution map was obtained by combining the values of the pels in the three layers (VL, SL and WL) above described.

Thus each pel in the potential map can assume three values, namely 0, 1, 2 that represent the labels of suitability for white truffle growth.

In the potential map, each region which presents all the three suitability metrics set to 1 is classified as an area were the white truffle can potentially grow. Seven of the potential spotted area resulted have been tested by means of field relieves with the help of trained truffle dogs.

Results and Discussions

At first, we prepare the map of truffle grounds. The studied truffle grounds are mostly found in wild oak and poplar woods; they are found or at the edge of cultivated ground under *Quercus* spp. and *Tilia* spp.; or in moll valleys, or ditches under the shade of *Populus* spp. and *Salix* spp. As far as the presence of macrofungi is concerned, there are genera common to all studied white truffle-grounds, such as *Inocybe* (Fr.) Fr*., Cortinarius* (Pers.) Gray and *Genea* Vittad. This type of research was carried out only for two years and so the findings should not be completely reliable. Surely more reliable data call for a longer study and further surveys.

The second map regards vegetation.

There are different dominant plant communities in the study area: chestnut woods sometimes mixed with scots – pine, *Quercus pubescens* woods and *Q. cerris* woods; and along the rivers *Populus alba*, *Salix alba*. Obviously, for the potential map we have more weighted the communities dominated by plants potentially able to form ectomycorrhyzal association with white truffle.

The investigated truffle-grounds show the presence of the following soil groups: Calcaric Gleyic Fluvisol, Hypereutric Stagnic Regosol or Epistagnic Fluvic Cambisol. The truffle-grounds developed on a wide range of unconsolidated materials, mainly fluvial, in depression areas (also on slope) and low landscape positions with shallow groundwater. These soils with clear signs of groundwater influence are not constantly saturated; the redoximorphic features are common, especially in the lower part of the profile.

In the superficial horizons the soil reaction varies from weakly to moderately alkaline, the total $CaCO_3$ has high values, ranging from 7% to 25% and the exchangeable bases show very high amounts of Ca, which ranges from 2300 mg/kg to 4400 mg/kg so. These soils are also characterised by high saturation rate (90-100%). Organic matter varies from about 1.1% to 3.8%, CEC from 11 to 23 cmol(+)/kg and it is well correlated with the organic matter content.

The spatial distribution and variability of the most frequent soil groups are reproduced in the GIS. Eighteen pedological units were identified and characterized in detail (Table 2). These units were the basis of the pedological map.

Tab. 2 Pedological units and their chemical and physical proprieties.

Lithostratigraphic Unit	Landscape Unit	Pedological Unit	Suitability for *T.magnatum*	WRB Soil Group	Texture	pH	Organic Matter	% tot. $CaCO_3$	C.E.C	K	Ca	Mg	B.S.R %
Alluvial and detrital deposits													
	River sediment with bank vegetation	UP1, UC13	1	Endoskeletic Calcaric Fluvisol	FS	8	2	6,6	12,7	45	1995	102	100
	Alluvial plain, sowable land	UP2, UC14	1	Hypereutric Fluvic Cambisol	FL	6,1	0,2	6,6	13,7	44	1329	214	98
	Recent fluvial terraces, sowable land	UP3, UC15	0	Hypereutric Fluvisol	FS	6,7	1,3	1,8	16,1	101	1846	361	99
	Fluvial terraces with colluvial cover, sowable land	UP4, UC18	0	Chromic Fluvic Cambisol	FS-FL	6,5	0,6	0,5	12,1	25	1030	237	99
	Thick colluvial and slope deposits, sowable land	UP5, UC17	0	Chromic Fluvic Cambisol	FL	5,8	1,7	0	12,3	58	985	212	98
Rocchetta F., marls													
	Incised and moderatly steep slopes, forest	UP6, UC05_06	1	Hypereutric-Calcaric Cambisol	FS-F	6,8	0,8	4,5	23	60	1100	1800	99
	slightly steep slope, discontinuous forest	UP7, UC07_08	1	Calcaric Cambisol	FS	7,9	2,2	5,2	24,5	84	4150	310	100
	Flat summitslope, sowable land	UP8, UC09	0	Cutanic Luvisol	F	6,4	2,2	1,3	20,5	115	2000	730	99
	Very steep slope with rock outcrops, shrubbery	UP9, UC10	1	Calcaric Epi-Endoleptic Regosol	SF	7,1	1,7	3,2	13,3	48	1400	280	99
	badlands with low vegetation cover	UP10, UC11	1	Calcaric Leptosol	FL	8,3	0,8	25,4	11,1	121	2457	114	100
	Fluvial terraces, sowable land	UP11, UC12	1	Calcaric Fluvisol	FS	8,2	1,5	13,6	17,8	150	2841	230	100
Monesiglio F., conglomerate and marls													
	Flat summitslope, sowable land	UP12, UC22	0	Calcaric Cambisol	FL	8,2	3,1	22,5	14,5	150	3501	179	100
	moderatly steep slopes with terrace-cultivation	UP13, UC23	1	Calcaric Regosol	FL	8,4	2,7	19,5	19,1	164	3236	425	100
	Very steep slope, forest	UP14, UC24	0	Calcaric Cambisol	F	7,1	2,9	2,2	16,3	156	2805	401	100

Lithostratigraphic Unit	Landscape Unit	Pedological Unit	Suitability for *T.magnatum*	WRB Soil Group	Texture	pH	Organic Matter	% tot. $CaCO_3$	C.E.C	K	Ca	Mg	B.S.R %
Molare f., conglomerate and sandstones													
	Very steep slope with rock outcrops, forest	UP15, UC01_02	0	Calcaric Endo-Epileptic Regosol	FS	6,4	1,9	1,8	20	70	800	1700	99
	moderatly steep slope, discontinuous forest	UP16, UC03_04	0	Endoskeletic-Hypereutic Cambisol	FS	6,9	2,1	2,5	20	80	1100	1200	99
Voltri Gruop U., Ophyolite													
	Serpentinite, Steep slopes, forest	UP17, UC19	0	Episkeletic Epileptic Regosol	FS	6,7	1,3	5,5	19,6	143	700	1300	99
	Calcschist, Steep slopes, forest,	UP18, UC20_21	0	Episkeletic Endoleptic Cambisol	FS	7,5	0,7	1,8	26	190	3700	220	100

The final map of *T. magnatum* distribution (Fig. 3) is a layer raster grid with 5 m of resolution; this is the same resolution used for the DEM and the other layers employed. The map shows 3 different classes: from class 3 (the best situation) to class 1 representing areas unsuitable for *Tuber magnatum* growth. Class 2 represents areas only partially suitable for *T. magnatm* growth (vegetation and soil suitable).

For visualization purpose, the following lookup table was adopted:
class1 RGB combination 254, 251, 141 -
class 2 RGB combination 166, 86, 40 -
class 3 RGB combination 255, 255, 51 -

Fig. 3 Potential distribution map of *Tuber magnatum* realized.

Seven areas among those classified as suitable (class 3), were random chosen in order to validate the final map. In these areas, the presence of *Tuber magnatum* was confirmed. Further field relieves are planning to be performed, but the positive results obtained suggest that the proposed method can also be successfully applied in other areas.

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VEGETATION ANALYSIS: A TOOL TO IDENTIFY TRUFFLE VOCATED AREAS IN MOLISE REGION

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Abstract
Owing to the climatic and soil characteristics in Molise region, the truffles are there widely diffused. Since this Region is one of the most important white truffles (*Tuber magnatum* Pico) area in Italy, the aim of the present study is to provide some preliminary information about the ecology of *T. magnatum* plantations in Molise region. The study, carried out during three years (2005-2007) in Busso (Molise region), investigated the relationships between the plant community and the truffle presence as well as its frequency in order to plan a flexible approach in preserving natural regional resources (Paura *et al*., 2008). Truffle presence, in fact, is closely related not only to symbiotic plants and pedosphaere environment but also to phytocoenosis characteristics (i.e. plant association, forest structure). In this connection, several data have been recorded in order to identify floristic, vegetation and structural features, in a natural white truffle plantation. The truffle plantation experimental area (100x100 m) was divided in 16 plots (25x25 m), to improve the accuracy of the data detected. In each plot were adopted the following methods:

a) phytosociological method to analyse the truffle plantation by means of the floristic and ecological characterization of plant communities;
b) analysis of the relationships between forest structure (i.e. diametric classes and canopy) and presence/frequency of white truffle;
c) correlation between the presence of floristic species and the white truffle presence/ frequency.

A lot of white truffle occurrence in the plots were linked with the *Daphno laureolae-Quercetum cerridis* Taffetani & Biondi, 1995 association. Moreover the occurrence of truffle seems to be related to the landscape use history. For instance, the lack of *T. magnatum* seems to be associated to ancient pasture re-colonized by wood, the latter was attested by the presence of *Brachypodium rupestre*. In the some way a "buffer effect" was also recognized owing to the absence of the shrub in the truffle plantation peripheral areas that caused an higher surface invested by radiation energy and consequently allowing dryness environment. All the studied parameters let us to edit thematic maps, in according to the model elsewhere reported (Marchetti *et al.,* 2006). A deep in knowledge on the truffle plantations ecology will be useful to identify the most suitable areas for truffle cultivation and to improve the correct management of truffle production.
Key words: *Tuber magnatum* Pico, Plant ecology, Phytosociology

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PEDOLOGICAL ASPECTS OF NATURAL TRUFFLE BEDS OF *TUBER MAGNATUM* PICO IN THE RIETI PROVINCE (CENTRAL APENNINES, ITALY)

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Abstract
A study on natural truffle beds of *Tuber magnatum* Pico in the Rieti Province was carried out. Two zones, which belong to different elevation belts, were considered: the Lower Sabina and the Salto river valley. The analysis of climatic data, according to the phytoclimatic map of the Latium (Central Italy), indicates that the truffles sites are all located inside the Temperate Region. The truffle beds were discovered on terraced hilly slopes, often of anthropic origin, or along the banks of little rivers, both affected by superficial landslide and solifluction, or above alluvial plains. The parent material of the soils is represented by sandy and marly sediments in Lower Sabina, while the silty arenaceous rocks and marls are dominant in the Salto river valley. The results of chemical analysis show that the average values of pH measured in water and KCl, are 8.0-8.1 and 7.3-7.4 respectively and that the minimum values of pH in KCl do not fall below 7.09. The content of total $CaCO_3$ is low in both the environments if compared to the mean value of other zones, suitable for *Tuber magnatum* growth outside the Rieti Province. The particle size composition indicates that the soils in Lower Sabina differ from those of the Salto river valley for a lower presence of sand and a higher content of silt. In this initial phase of the study the limited number of examined cases would seem to indicate that the truffle sites are associated neither with a particular type of parent material nor with a well defined "external" climate. It could be hypothesized that the factor which unites the different studied cases (Lower Sabina and Salto river valley) is due to particular situations of geomorphological dynamics that activate non-catastrophic processes of slope, associated to particular local microclimatic conditions in Lower Sabina.
Key words: soil, *Tuber magnatum* Pico, truffle ecology

Introduction
At present the studies on white truffle are not sufficient for a complete understanding of this hypogeous fungus ecology; they are unsatisfactory for a modern truffle cultivation. This work aims at widening the knowledge in this economically important sector. For this purpose, a comparison among the pedological and macroclimatic growth conditions of *Tuber magnatum* Pico, was carried out in two different elevation belts in the Rieti Province.

Materials and methods
The study area is located in the Rieti Province (Latium, Central Italy). In particular two zones were considered: the Lower Sabina and the Salto river valley, which are characterized by different elevation belts from a macroclimatic point of view: from 100 m a.s.l. to 250 m a.s.l. in Lower Sabina and from 550 m a.s.l. to 900 m a.s.l. in mountainous inland Sabina (Salto river valley). The main parent materials from which the soils take origin are represented by pebbles, sands and marly sediments of plio-pleistocenic age in Lower Sabina. On the other hand silty arenaceous rocks and marls of miocenic age are prevalent in the Salto river valley. Field observations were carried out on natural truffle beds of *Tuber magnatum* Pico by digging shallow pits in the productive areas. For each soil profile every pedological horizon was described and sampled in accordance with the F.A.O. Manual (1990). Soil profile description was associated with site characteristics (elevation, exposure, type of vegetation, parent

material). Special attention was given to the understanding of the type and intensity of erosive phenomena and the morphologic dynamics acting in the site. Soil samples were air dried and sieved through a 2 mm mesh. Laboratory analyses were performed according to the "Official Methods of Chemical Analyses of the Soil" (MiPAF, 2000). In particular the soil texture was determined after dispersion in sodium hexametaphosphate, using the pipette method for clay and silt fractions, and wet sieving to separate the different sand size fractions. The measured values of sand, silt and clay were introduced in the calculation of the Texture Index (TI), as referred by Sillanpää (Sillanpää, 1990), on the basis of the formula: TI = 1.0 x (% clay) + 0.3 x (% silt) + 0.1 x (% sand). Soil pH was measured by potentiometric method in both water and a solution of potassium chloride 1 N, using a 1:2.5 (weight/vol.) solid/liquid ratio. Total carbonates and active $CaCO_3$ contents were analysed by Dietrich-Fruehling calcimeter and Drouineau method respectively. Total organic carbon was determined by the Walkley-Black method. Metal concentration of Fe and Mn microelements was determined by flame atomic absorption spectrometry (FAAS) after extraction with a solution of ammonium acetate 0.5 M + EDTA buffered at pH 4.65.

Results and discussion

The analysis of the climatic data, according to the phytoclimatic map of the Latium (Blasi, 1993), indicates that the truffle sites are all located inside the Temperate Region. In particular the sites of the Lower Sabina belong to lower hilly thermotype, lower moist ombrotype of the mesaxeric Region. On the other hand the sites of the Salto river valley are included in upper hilly thermotype, upper moist ombrotype of the same mesaxeric Region. The ombrotermic diagrams of two sites (*Tarano* and *Balze Santa Lucia*), representative of the studied zones, are displayed in Figure 1. They are in agreement with the phytoclimatic map and differ from each other mainly by showing a slight summer dryness in the Lower Sabina but not in the Salto river valley.

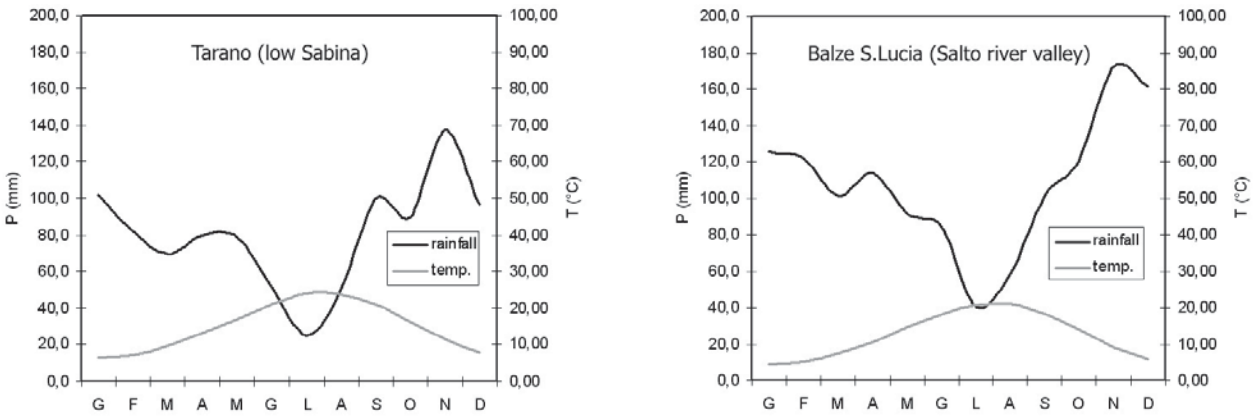

Fig. 1 Ombrotermic diagrams of two sites representative of the studied zones.

The geomorphological observations stress that the natural truffle beds of *Tuber magnatum* Pico in Rieti province are located on terraced hilly slopes, often of anthropic origin, along the banks of little rivers, both affected by superficial landslide and solifluction, or above alluvial plains. The main descriptive statistics of the results of chemical analysis on truffle soils are displayed in Table 1.

Tab. 1 Main descriptive statistics of chemical analysis results.

Environments / Parameters	Lower Sabina					Valley of the Salto river				
	Mean	St. Dev.	Min.	Max.	CV (%)	Mean	St. Dev.	Min.	Max.	CV (%)
sand (%)	30,0	8,85	23,1	42,2	29,6	34,9	11,72	7,6	57,6	33,6
silt (%)	52,9	11,18	39,0	69,8	21,1	50,4	6,70	38,8	64,5	13,3
clay (%)	17,1	6,25	5,5	23,5	36,5	14,7	8,26	2,2	38,4	56,2
texture index	36	4,60	29	42	12,8	33	7,84	21	54	23,5
pH (H_2O)	8,02	0,20	7,76	8,21	2,4	8,10	0,21	7,69	8,41	2,6
pH (KCl)	7,31	0,11	7,16	7,43	1,5	7,36	0,14	7,09	7,59	1,9
total $CaCO_3$ (%)	11,8	7,41	5,6	24,0	62,6	5,7	3,28	0,0	12,7	57,4
active $CaCO_3$ ($g\,kg^{-1}$)	39	44,59	0	99	114,7	10	24,01	0	70	232,7
organic carbon (%)	3,14	2,76	0,88	7,71	87,9	1,74	1,72	0,19	7,14	98,8
Fe-EDTA ($mg\,kg^{-1}$)	151,2	48,44	89,9	198,9	32,0	160,9	67,53	72,3	293,8	42,0
Mn-EDTA ($mg\,kg^{-1}$)	98,5	16,07	77,2	118,5	16,3	125,0	54,46	49,4	278,3	43,6

They show that the average values of pH measured in water are comparable in the two environments (8.0-8.1) as well as those measured in KCl (7.3-7.4) and that the minimum values of pH in KCl do not fall below 7.16-7.09. The content of total $CaCO_3$ in both the studied areas is low if compared to the average value found in other environments outside the Rieti Province. The values of the Texture Index, which reflects the whole soil particle size composition and rises with the increase of the fine fraction, indicate that the coarse component is higher in the sites of the Salto river valley with respect to those of the Lower Sabina. The mean values of Fe and Mn microelements indicate a good condition of oxygenation of the carpophore growth environment (De Simone *et al*., 1993).

Conclusions

The examined cases, in this preliminary phase of study of the pedological aspects of natural truffle beds of *Tuber magnatum* Pico in Rieti Province, would seem to indicate that the truffle sites are associated neither with a particular type of parent material nor with a well defined “external” climate. In fact the rocks that constitute the parent material of the soils are quite different from each other and they give rise to soils different in particle size composition, but the particular geomorphological dynamics, that characterized the productive sites, produce soils similar in evolutionary characters. The same soil similarity is present in the chemical parameters. The minimum value of pH measured in KCl remains an important factor of threshold below which the soil suitability for the carpophore growth is not assured. The macroclimatic conditions, as indicated by ombrothermic diagrams, differ from each other by showing the occurrence of a slight summer dryness in Lower Sabina but not in the Salto river valley. It could be hypothesized that the factor which unites the two studied areas is due to particular local morphologic conditions in Lower Sabina (narrow and very shaded valleys) which create in the truffle sites microclimatic conditions independent of the local macroclimate and similar to those of the Salto river valley.

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TRUFFLE POPULATIONS IN SALENTO (SOUTHERN ITALY): ASSOCIATION WITH *QUERCUS ILEX* AND *PINUS HALEPENSIS*

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Abstract
Morphological and molecular characters of *Tuber borchii* Vittad. and *Tuber aestivum* Vittad. populations have been investigated in the eastern and central Salento (Puglia, southern Italy) area throughout one year of sampling. From this preliminary study, they represent the most frequently encountered *Tuber* species in Salento and, on the basis of where they were collected, *Quercus ilex* and *Pinus halepensis* Miller have been identified as the symbiotic plants.
Key words: Ectomycorrhiza, Salento, *Tuber borchii* Vittad., *Tuber aestivum* Vittad.

Introduction
The role of symbiotic mycorrhizal fungi in soil–plant systems is fundamental; they influence the soil environment in different ways, either through direct interactions with the mineral or organic substrates that they colonise, or indirectly, via interactions with their host plants, increasing plant growth and nutrient uptake (Smith and Read, 1997). Mycorrhizal fungi inoculation of plants improve physical, chemical and biological soil properties (Requena *et al*., 2001) which render their presence of high importance especially in the restoration of ecosystems.
Fungi of the genus *Tuber* are able to form ectomycorrhizal symbiosis with roots of both trees and shrub species. They are of great interest for forestry as well as for the organoleptic properties of some species, such as *T. aestivum* Vittad. and *T. borchii* Vittad. largely found in Salento area (Puglia, southern Italy).

Matherials and Methods
Morphological identification of fruit bodies (Montecchi and Sarasini, 2000; Agerer, 2002) collected with the help of dogs, was confirmed through Polymerase Chain Reaction (PCR) analysis (White *et al*., 1990; Amicucci *et al.,* 2008).
Samples were employed for the mycorrhizal inoculation of local selected plants as described by Bencivenga (1982).

Results
Morphological and molecular characters of *T. aestivum* (Fig. 1A) and *T. borchii* (Fig. 1B) populations have been investigated in the eastern and central Salento area throughout one year of sampling. In Figure 2 are reported the sites were samples were collected. From this preliminary study, they represent the most frequently encountered *Tuber* species in Salento and, on the basis of where they were collected, *Quercus ilex* L. (Fig. 3) and *Pinus halepensis* Miller (Fig. 4) have been identified as the symbiotic plants (Accogli *et al,* 2008). In particular, *T. borchii* has been found in association with both kind of plants, while *T. aestivum* mainly with *P. halepensis* (Tab. 1). Moreover, *T. borchii* and *T. aestivum* were found in about 76% and 59% of sampled sites, respectively.
Greenhouse tests to assess the capacity of both *Tuber* species investigated to perform an association with local flora (especially *Q. ilex*) are in progress, taking into account the main objectives of this research: (1) vegetal reconstruction processes and (2) increment of local truffle production.

Fig. 1 Samples of *T. aestivum* (A) and *T. borchii* (B) collected in Salento.

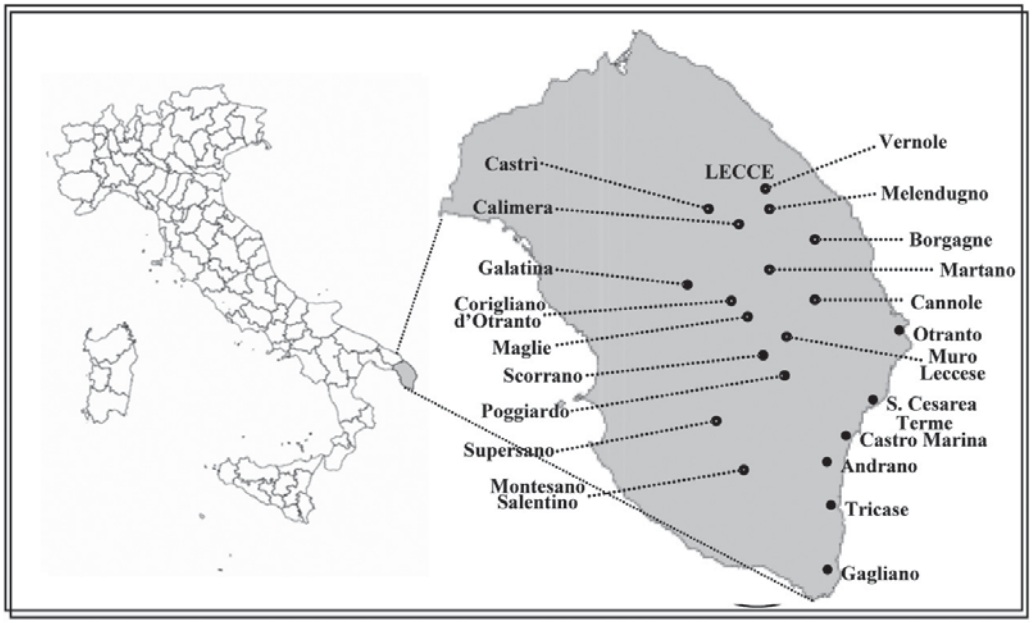

Fig. 2 Sites in Salento (Puglia, Italy) where samples were collected.

Fig. 3 *Quercus* wood of (A) and Pines wood (B) of Salento.

Tab. 1 Plant species encountered in the sampling sites where *T. aestivum* and *T. borchii* fruit bodies were collected

Sampling site	Plant species	*T. aestivum*	*T. borchii*
Andrano	*Quercus ilex*	+	+
Borgagne	*Quercus ilex*	+	+
Calimera	*Quercus ilex*	+	+
Cannole	*Pinus halepensis*	-	+
Castrì di Lecce	*Q. ilex + P. halepensis*	+	+
Castro Marina	*Pinus halepensis*	+	-
Corigliano d'Otranto - site 1	*Pinus halepensis*	+	+
Corigliano d'Otranto - site 2	*Quercus ilex*	+	-
Gagliano del Capo	*Quercus ilex*	+	-
Galatina	*Pinus halepensis*	-	+
Lecce - site 1	*Pinus halepensis*	-	+
Lecce - site 2	*Quercus ilex*	+	+
Lecce - site 3	*Pinus halepensis*	-	+
Maglie	*Quercus ilex.*	+	+
Martano	*Pinus halepensis*	-	+
Melendugno	*Quercus ilex*	+	+
Montesano Salentino	*Pinus halepensis*	-	+
Muro Leccese	*Quercus ilex*	+	-
Otranto - site 1	*Quercus ilex*	+	+
Otranto - site 2	*Quercus ilex*	+	+
Otranto - site 3	*Pinus halepensis*	-	+
Poggiardo - site 1	*Quercus ilex*	-	+
Poggiardo - site 2	*Pinus halepensis*	-	+
Poggiardo - site 3	*Quercus ilex*	+	-
Santa Cesarea Terme	*Pinus halepensis*	-	+
Scorrano	*Quercus ilex*	+	-
Supersano	*Pinus halepensis*	-	+
Tricase - Depressa	*Quercus ilex*	+	-
Vernole	*Q. ilex + P. halepensis*	-	+

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CLIMATE CHANGE THREATENS THE TRUFFLE HARVEST

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Abstract
History is full of poor or disastrous crops, as well as of environmental disaster.
Such arguments in themselves would not help to explain either the range or the quality of modern climate change. Rather, one might be led to consider it almost as a matter of routine.
However, worries and warnings are coming from the scientists about what is in store for us in a not so distant future. And it is just in this light that we should start considering poor crops, as they turn out to be directly connected with the radical changes which are taking place.
In the field of growing and harvesting all sorts of truffles, a quite remarkable decline in production has been recorded since last year.
It has all started from the drought in the second half of the year 2007, whose effects are still noticeable in spite of the late, heavy springs rains.
Having through the years gained a good deal of experience in this field, as far back as my memory goes I can say that an event of such prominence has never been recorded before.
Truffles are known to be reliable environmental indicators. Their presence is guarantee of the woods' healthy condition, and a drastic reduction in truffles must be seriously considered as the ringing of an alarm bell, as it goes beyond the specific field to show what is likely to happen in a more general context.
Anybody can perceive these worryng signs on going to the market or to the grengrocer's. There is less fruit on sale, and it is more expensive.
Being confronted with such an alarming prospect, we'd rather make a virtue of necessity- as the sayng goes.
This is why we now welcome the Truffle International Conference, which will be held in Italy in the next autumn.
It will be a good opportunity to investigate the changes, reflect upon the prospects in the field, and adopt the necessary measures which will protect the truffle's life and biodiversity, so that such a niche economy- which is so important not just in agriculture, but also in the marketing of quality products-may continue to exist.
Key words: Climate change, truffle.

Taxonomy Biology and Ectomycorrhizae Session

TAXONOMIC AND QUALITY ISSUES ASSOCIATED WITH THE COMMERCIALIZATION OF *TUBER BORCHII*

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Abstract
Tuber borchii Vittad., commonly called bianchetto (whitish truffle), is an edible truffle with excellent culinary qualities. However, bianchetto is often regarded as one of the lesser truffles because it is often sold mixed with morphologically similar species in the Puberulum clade (Jeandroz *et al.,* 2008) such as *Tuber maculatum* Vittad.*, Tuber dryophilum* Tul. & C. Tul. and *Tuber puberulum* Berk. & Broome which have very poor culinary characteristics. In this paper we define the characteristics of the commercial species *T. borchii* describing its morphological and genetic characteristics.
Key words: *Tuber borchii*, bianchetto, taxonomy, commerce, genetic variability.

Introduction

Tuber borchii Vittad. is an edible truffle with excellent culinary qualities. To distinguish it from the more expensive Italian white truffle (*Tuber magnatum* Pico) it is commonly called “bianchetto” (whitish truffle) in Italy. Other common names are “marzuolo” (the truffle of March) and “tartufo di pineta” (pine forest truffle) which refer to the maturation time and its natural habitat. This truffle has a culinary tradition only in Italy in the area between Ferrara and Ravenna of Emilia Romagna region (Zambonelli *et al.,* 2002). In the famous Italian cookery book “La scienza in cucina e l’arte di mangiar bene” (Artusi, 1891) the culinary qualities of this truffle are recorded “*I bianchi di Piemonte, sono da tutti riconosciuti pregevoli, e i bianchi di Romagna, che nascono in terreno sabbioso, benché sappiano d’aglio, hanno molto profumo.....”* (the white truffle of Piedmont are appreciated by everybody and the white truffle of Romagna, which grows in sandy soils, although tasting of garlic, has an excellent perfume….).

T. borchii is adapted to a wide range of soils and climates and is found throughout Europe (Hall *et al.,* 2007). In Italy it is commonly found in sandy calcareous soils typical of the littoral areas but is also common in the Apennines in the same areas where *T. magnatum* and *T. melanosporum* grow naturally where the pH is 7 to 8. It is also common in moderately acidic soils with pH 5 to 7 which is unusual for commercial truffles (Zambonelli *et al.,* 2002; Gardin, 2005).

T. borchii cultivation is not as complex as for other commercial truffles**,** in particular *T. magnatum* (Zambonelli *et al.,* 2002)**,** because infected plants can be easily produced in greenhouses by inoculating either with spores or mycelial cultures (Zambonelli & Iotti*.,* 2006). Infected plants can also be produced *in vitro* using sterile seedlings of *Alnus cordata* (Loisel.) Desf.*, Castanea sativa* Mill. or micropropagated plants of poplar, *Cistus* sp. or *Tilia platyphyllos* Scop. (Zambonelli *et al.*, 2002)*. T. platyphyllos* infected with *T. borchii in vitro* have been particularly useful by providing a system for studying fungus-plant molecular interactions under controlled conditions in the laboratory (Giomaro *et al.,* 2005).

In the field this truffle competes strongly with other ectomycorrhizal fungi and produces truffles earlier than other species making it more attractive to investors. It was successfully

cultivated first in Italy late in the 1990s (Zambonelli *et al.,* 2000a; Vinay & Pirazzi, 2001) and was successfully cultivated in commercial quantities in New Zealand in 2008 on *Quercus robur* and *Corylus avellana* only four years after planting (Hall, 2008).

Morphological and molecular methods for the identification of *T. borchii*

T. borchii was firstly described by the Italian mycologist Vittadini (1831) with numerous other Italian mycologists (Montecchi & Sarasini, 2000; Zambonelli *et al.,* 2000b; Ceruti *et al.,* 2003) providing more microscopic details of the Vittadini type specimens deposited in the Mattirolo herbarium, or fresh ascomata collected from the same areas where Vittadini collected his types, or collected from other parts of Italy.

In many parts of Italy and throughout Europe *T. borchii* is very often confused with other *Tuber* species in the Puberulum clade (Jeandroz *et al.,* 2008) such as *Tuber maculatum* Vittad., *Tuber dryophilum* Tul. & C. Tul. and *Tuber puberulum* Berk. & Broome which have poor culinary characteristics (Montecchi & Sarasini, 2000). Mixtures of these truffles often smell like *T. borchii* but the flavour of food containing them is often a disappointment (Hall *et al.,* 2007). *T. dryophilum* is a common contaminant because it often fruits in the same areas as *T. borchii*, at the same time of year (Iotti *et al.,* 2009 submitted), and has a similar appearance (Figure 1), particularly when covered with soil, even though its aroma is somewhat different (Gioacchini *et al.,* 2005).

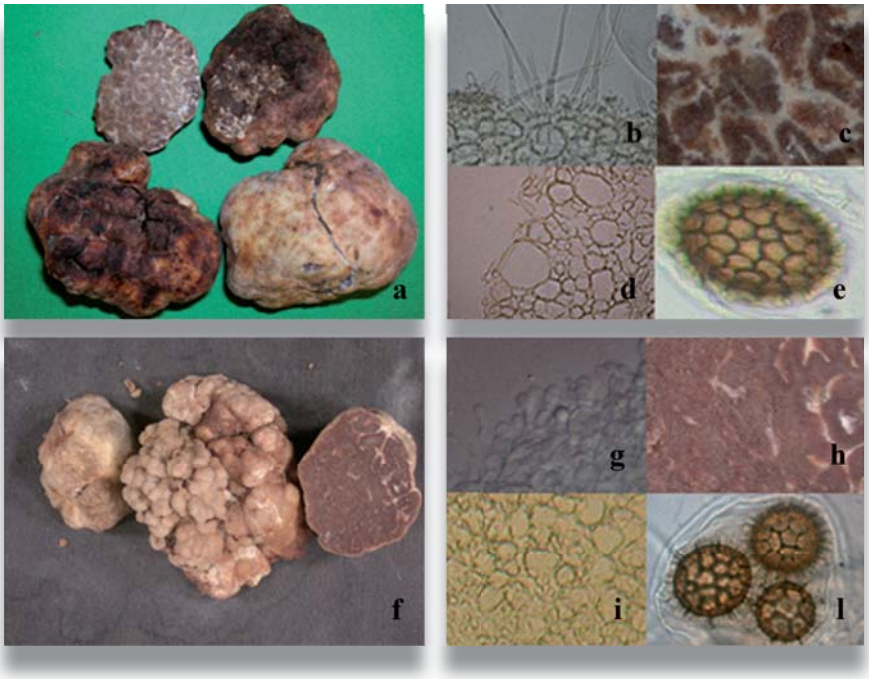

Fig. 1 Comparison between *T. borchii* ascomata (a), peridium cystidia (b), particular of a gleba section (c), peridium external anatomical structure (d), spores (e) and *T. dryophilum* ascomata (f), peridium cystidia (g), particular of a gleba section (h), peridium external anatomical structure (i), spores (l).

Other species of whitish truffles with morphological features similar to *T. borchii* are *Tuber oligospermum* (Tul. & C. Tul.) Trappe, which is common in southern Europe and in northern Africa, the northern European *Tuber rapaeodorum* Tul. & C. Tul and *Tuber scruposum* R. Hesse (Riousset *el al.,* 2001; Ceruti *et al.,* 2003; Badalyan *et al.,* 2005), the American *T. whetstonense* Frank, Southworth & Trappe, *T. californicum* Harkn., *T. gibbosum* Harkn., *T. oregonense* Trappe & Bonito, and the Chinese *Tuber latisporum* Juan Chen & P.G. Liu and *Tuber liui* A S. Xu (Chen & Liui, 2007; Trappe *et al.,* 2007, Jeandroz *et al.,* 2008).

Recently Italian researchers have developed reliable morphological and molecular tools for

the identification of *T. borchii*. For example, *T. borchii* specific primers have been used in simple or multiplex PCR reactions to show the presence of several species of *Tuber* in a single step reaction (Amicucci *et al.*, 1998; Bertini *et al.*, 1998; Mello *et al.*, 1998; Paolocci *et al.*, 1999). *T. borchii* specific primers were also able to successfully amplify *T. borchii* Vittadini's herbarium specimens (Mello *et al.*, 2000). However, despite this work some of the sequences for *T. borchii* reported in the Genbank database are incorrect because incorrect morphological identifications were made prior to sequencing (Mello *et al.,* 2006, El Karkouri *et al.,* 2007). In addition, some internal transcribed spacers (ITS) sequences for *T. borchii* are labelled as *T. puberulum* and some sequences of *T. dryophylum* are labelled as *T. borchii.* This has created considerable confusion between mycologists who have assumed that sequences deposited in Genbank are correct, when they are clearly not, which in turn has led to spurious conclusions being reached in ecological and phylogenetic studies (Hall *et al.,* 2007). This situation must be rectified.

T. borchii intraspecific variability

Many studies have been focused on the analysis of the intraspecific variability of truffles with the aim to define the relationship between their genotype, their oganoleptic proprieties, and/or their geographical origins (Mello *et al*., 2006). However, few studies have been conducted on *T. borchii* even though if was considered to have a high degree of genetic diversity (Gandeboeuf *et al.*, 1997; Mello *et al.,* 2006).

Our research group in collaboration with the Middle Tennessee State University and University of California has recently studied the genetic structure of *T. borchii* using 61 representative specimens with a broad distribution throughout Italy (Bonuso *et al.,* 2009 submitted). The analyses were conducted using a combined data set of four single locus marker analyses (Internal Transcribed Spacer region, Intergenic spacer region, β-tubulin, and Protein Kinase C genes). Within the specimens we found two distinct haplotypes but without any differences in their morphological or organoleptic qualities (cryptic species), or geographical distribution (Figure 2). The variability inside the two haplotypes was very low. The presence of a cryptic species within a commercially valuable species of truffle, should be taken into consideration when developing marketing and cultivation strategies.

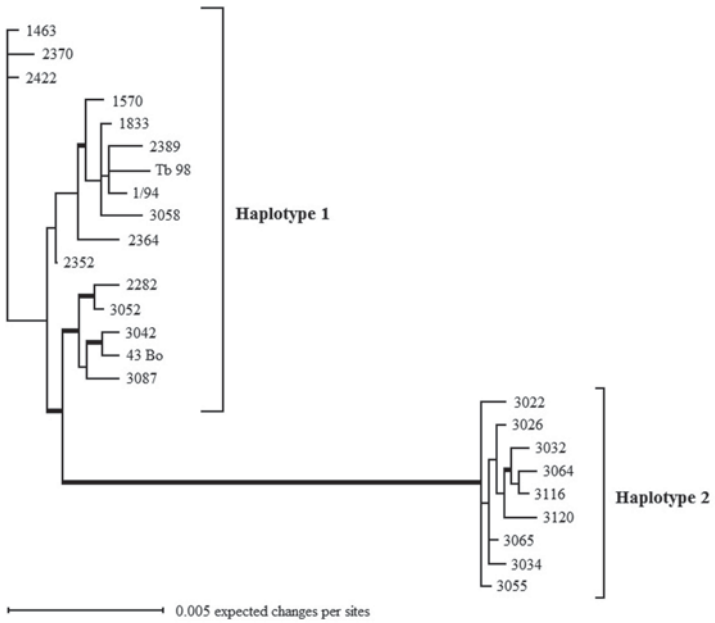

Fig. 2 *T. borchii* phylogenetic tree for the combined dataset of four nuclear loci (ITS, IGS, β-tubulin and PKC) performed using both maximum likelihood for each single locus and Bayesian estimation. Thickened branches represent posterior probability percentage of 100.

Conclusion

T. borchii is a very good truffle which can be commercialized only if first correctly identified. To this end we suggest only using the descriptions of Italian researchers as references (Montecchi & Sarasini, 2000; Zambonelli *et al*., 2000b; Ceruti *et al.,* 2003) and Italian sequences. Also both cryptic species should be included in the species perhaps by using a sensu lato definition in a manner similar to those used for *Tricholoma matsutake* and *Boletus edulis* (Chapela & Garbelotto 2004; Hall *et al.,* 2003). We also suggest using the common name "bianchetto" only for *T. borchii* and not other whitish species (bianchetti in Italian) to avoid confusion in the marketplace. However, because of the morphological similarity of *T. borchii* and *T. dryophilum* and are harvested from the same habitats and fruiting seasons we suggest that in the marketplace a small amount of contamination by *T. dryophilum* could be tolerated. In contrast, when *T. borchii* is used for producing infected plants for truffle cultivation molecular tools should be used to check for contaminants and in particular *T. dryophilum.*

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IS TYROSINASE EXPRESSION DURING *TUBER MELANOSPORUM* DEVELOPMENT DUE TO ENZYME INHIBITION OR GENE SWITCHING OFF?

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Abstract

During *Tuber melanosporum* development tyrosinase activity decreases reaching a minimum at the sporal stages (5 and 6) as biochemically and histochemically detected, moreover tyrosinase activity was found to be inhibited *in vitro* by truffle flavours such as dimethyl sulfide and bis (methyl thio) methane. To make clear if the decreasing tyrosinase activity during truffle development is due to enzyme inhibition or gene expression repression we investigate here by real-time RT-PCR the levels of tyrosinase mRNA during *Tuber melanosporum* development. The histochemical control of tyrosinase activity has been also done. The real-time RT-PCR shows that the *Tuber melanosporum* tyrosinase mRNA levels decrease as the ascocarp ripens and also the histochemical control supports a decrease of tyrosinase activity (DOPA oxidase) with ascocarp ripening. The findings at this stage suggest that tyrosinase gene expression or pre-mRNA processing are involved in the control of tyrosinase levels during *Tuber melanosporum* development.

Key words: Truffle, tyrosinase, development; cell biology.

La melanina (allomelanina) ed i suoi precursori giocano un ruolo fondamentale nel differenziamento sessuale di Ascomiceti quali, ad esempio, *Neurospora crassa* (Hirsch, 1954) e *Tuber melanosporum* (Miranda *et al.* 1997). Le allomelanine vengono prodotte per ossidazione dell'1,8-diidrossinaftalene o derivati cumarici, mentre le eumelanine e feomelanine di origine animale derivano dall'ossidazione della tirosina ed altri monofenoli o difenoli, in assenza o presenza di cisteina o glutatione. Comunque, sia nella produzione di allomelanine (batteriche, fungine o vegetali) che in quella di eumelanine e feomelanine l'enzima chiave è la tirosinasi (EC 1.14.18.1; monofenolo-o-difenolo ossigeno, ossidoreduttasi), ubiquitario nella scala filogenetica.

In precedenti ricerche avevamo dimostrato la presenza di tirosinasi nei tartufi neri e bianchi (Miranda *et al.,* 1997) ed evidenze biochimiche ed istochimiche avevano rivelato una correlazione tra l'attività enzimatica, la sua distribuzione nell'ascocarpo ed il differenziamento di quest'ultimo (Miranda *et al*., 1997). Si era anche osservato che l'attività tirosinasica, valutata utilizzando come substrato sia tirosina (attività creolasica) che L-3,4-diidrossifenilalanina (attività catecolasica), diminuisce di pari passo con la maturazione dell'ascocarpo (Miranda *et al*., 1992), parallelamente con un aumento di concentrazione degli aromi volatili solforati, quali il dimetilsolfuro ed il bis-metiltiometano (per i tartufi neri e bianchi, rispettivamente). Altri studi riportavano che gli aromi solforati dei tartufi neri e bianchi inibiscono reversibilmente la tirosinasi di *Agaricus bisporus* e lo stesso fu osservato per la tirosinasi purificata da *Tuber melanosporum* (Zarivi *et al*., 2003). Era quindi lecito chiedersi se la diminuzione dell'attività tirosinasica durante lo sviluppo di *Tuber melanosporum* fosse da attribuire ad inibizione da parte degli aromi solforati e/o a repressione dell'espressione del gene della tirosinasi.

Per fare chiarezza su questo punto si è deciso di andare a determinare i livelli di mRNA per la tirosinasi di *Tuber melanosporum* nel corso dello sviluppo dell'ascocarpo mediante real-time RT-PCR. A tale scopo si sono messi a punto dei protocolli sperimentali per l'analisi dell'espressione trascrizionale del gene della tirosinasi. Nel contempo si è controllata l'espressione dell'attività tirosinasica a vari stadi di sviluppo del tartufo per via istochimica.

In fig.1 A e B, è presentata la colorazione Giemsa di sezioni di ascocarpi di *T. melanosporum* agli stadi 3 ("vein stage") e 5 ("sporal stage"). Per gli stessi stadi di sviluppo la figura.1 mostra in C e D la distribuzione della attività cresolasica (L-tirosinasi idrossilasica) e in E ed F la distribuzione delle attività catecolasica (L-3,4 diidrossifenilalanina ossidasica) della tirosinasi di tartufo nero.

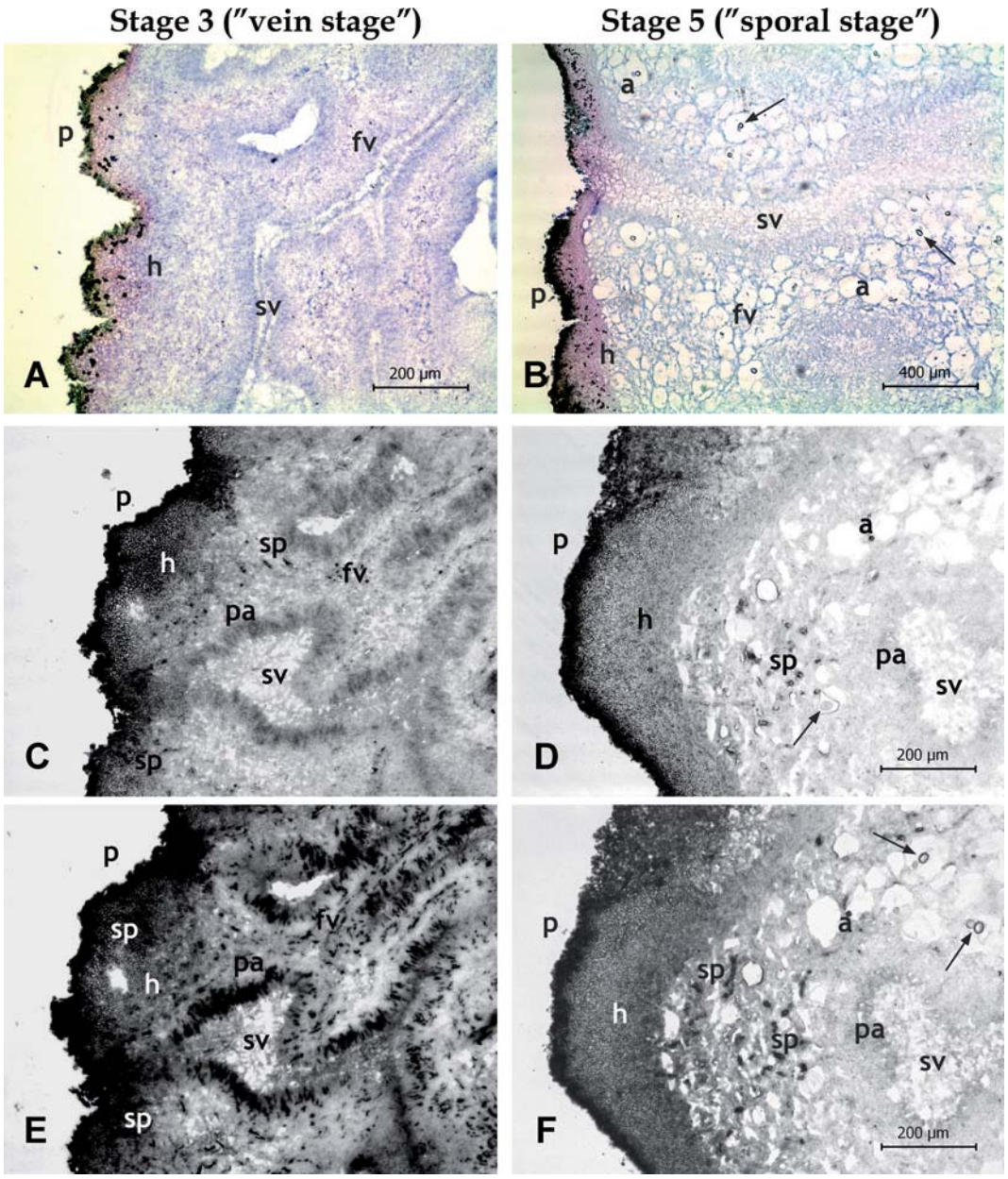

Fig. 1 *Tuber melanosporum* ascocarp section: A–B, Giemsa staining; C–D, cresolase activity of tyrosinase; E–F, catecholase activity of tyrosinase. Stage 3 and and 5, developmental stages of *Tuber melanosporum.* Arrows, ascospores; a, asci; fv fertile ven; h, hypothecium; p, peridium; pa, paraphyses; sp, sporogenic hyphae; sv, sterile vein.

Come si evince dalla fig.1, C e D allo stadio 3 si ha reazione positiva. L'attività è più intensamente rivelata con L-DOPA che con L-tirosina quale substrato; ma è noto che l'attività catecolasica della tirosinasi è circa 10 volte maggiore di quella cresolasica. L'attività enzimatica, in tale stadio, è principalmente localizzata a livello delle ife sporogeniche e delle parafisi. D'altro canto, pochissima attività è presente allo stadio 5, localizzata nelle parafisi, negli aschi e nelle spore. La fig. 2 mostra l'intensa attività tirosinasica (catecolasica), nelle ife sporogeniche (A,

stadio 4) e nelle spore immature (B, stadio 5). Tale attività è, del resto, quasi assente nelle spore mature (B, stadio 5). Così dallo stadio 3 allo stadio 5 si ha una diminuzione di attività tirosinasica nell'ascocarpo.

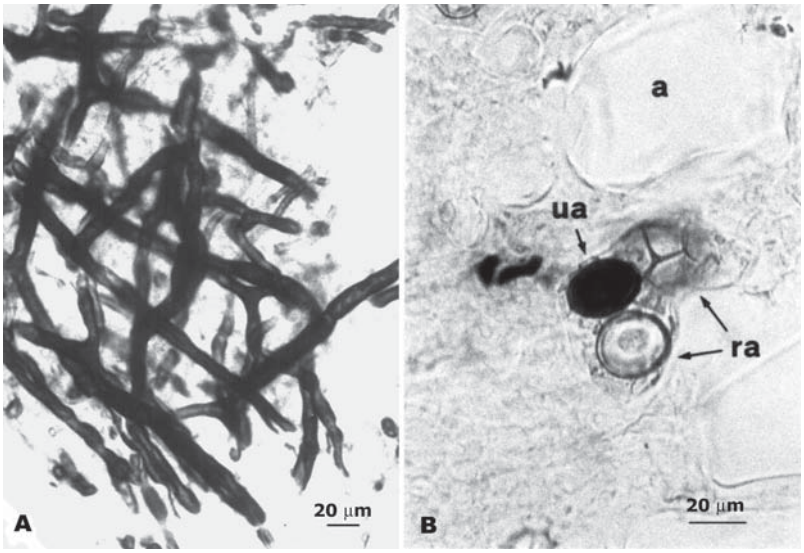

Fig. 2 Dopa oxidase reaction in *Tuber* sporogenic hyphae (A, developmental stage 4) and ascospores (B, developmental stage 5); a, ascus; ra, ripe spores; ua, unripe spores.

Le indagini preliminari di real-time RT-PCR indicherebbero, invece, che l'mRNA per la tirosinasi vada aumentando dallo stadio 3 allo stadio 5, in apparente contraddizione con la diminuzione dell'attività. Una possibile spiegazione potrebbe essere la risposta adattativa all'aumento di aromi solforati, che inibiscono la tirosinasi, volta a garantire il mantenimento di un'attività sufficiente a sostenere lo sviluppo delle pareti cellulari.

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QUERCIRHIZA QUADRATUM: A REVISION OF THE CHARACTERS AND IDENTITY OF THE AD TYPE ECTOMYCORRHIZA

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Abstract
The well-known AD type, described first by Giraud in 1988, is considered as a competitor in black truffle (*Tuber melanosporum* Vittad.) plantations. It has been mainly observed in *T. melanosporum* and *T. magnatum* Pico plantations in France and Italy. This ectomycorrhiza has always been observed on roots of oak (*Quercus ilex* L. and *Q. faginea* Lam.) and hazelnut (*Corylus avellana* L.) plantations with "burnt" areas around the trees, even in those that do not produce black truffle sporocarps, so it can create false expectations in young plantations. The AD type has also been described in nurseries, as a competitive ectomycorrhiza on seedlings inoculated with black truffle. In Spain, AD type has been detected in black truffle plantations and natural holm oak stands in Navarra, Soria, Huesca, Zaragoza, Teruel, Castellón and Valencia. In 2005, De Román & De Miguel, suggested that AD type could be a telephoroid type due to its anatomical and morphological characters. In 2006, Baciarelli-Falini *et al.* using molecular techniques identified this type as an Ascomycotina belonging to Pezizales. The detailed anatomical, morphological and molecular study of the AD type led to a description as *Quercirhiza quadratum* (Águeda *et al*. 2008). Based on the anatomical and morphological characters, the AD type belongs to the Ascomycotina. The presence of Woronin bodies on hyphal septa, and the sometimes slightly dissolved septa, are two typical characters of this group. The DNA sequences obtained from the AD types studied showed close similarities with members of Pyronemataceae and Sarcosomataceae (Pezizales). Both taxonomic groups correspond to the same AD type as found by Baciarelli Falini *et al*., (2006). One of the studied sequences showed a close identity (100% maximum identity, 84% coverage) with *Trichophaea woolhopeia* (Cooke & W. Phillips) Arnould, although records of this fungal species are scarce in the Iberian Peninsula.
Key words: ectomycorrhizae, ecology, population biology, taxonomy.

Introduction

The well-known AD type, described first by Giraud in 1988, is considered as a competitor in black truffle (*Tuber melanosporum* Vittad.) plantations. It has been mainly observed in *T. melanosporum* and *T. magnatum* Pico plantations in France and Italy. This ectomycorrhiza has always been observed on roots of oak (*Quercus ilex* L. and *Q. faginea* Lam.) and hazelnut (*Corylus avellana* L.) plantations with "burnt" areas around the trees, even in those that do not produce black truffle sporocarps, so it can create false expectations in young plantations. The AD type has also been described in nurseries, as a competitive ectomycorrhiza on seedlings inoculated with black truffle. In Spain, AD type has been detected in black truffle plantations and natural holm oak stands in Navarra, Soria, Huesca, Zaragoza, Teruel, Castellón and Valencia.

The aim of this work is to give the complete description of this ectomycorrhiza in order to put some light in its identity.

Material and methods

Following Agerer & Rambold (2004-2008) methods, ectomycorrhizae from five different locations in Spain were characterized and described. Reference specimen for *Quercus* ectomycorrhizae: Spain, Castilla y León, Prov. Soria, La Quiñonería, coord.: 41° 34' 36.11" N, 2° 3' 13.68" W; 06.10.2000; ecology: *Quercus ilex* stands with *Tuber melanosporum* production; soil core and myc. isol.: B. Águeda, herb. Departamento de Investigación y Experiencias Forestales de Valonsadero (JCyL), VALONSADERO – MYCORRHIZA 050 (in Soria, Spain). - *Further material studied:* Spain, Navarra, Oloriz, coord.: 30TXN12 (special coordinates of University); 02.04.2008; ecology: *Q. faginea* and *Q. ilex* subsp. *ballota* stands; soil core and myc. isol.: A.M. de Miguel, herb. Universidad de Navarra, PAMP-Mycorrhiza 200 (in Pamplona, Spain). - Spain, Aragón, Prov. Teruel, UTM coord.: 30T 0691507/4444998; 18.03.08; *Q. ilex* seedling from a nursery, inoculated with *Tuber melanosporum*; myc. isol.: A.M. de Miguel, herb. Universidad de Navarra, PAMP-Mycorrhiza 203 (in Pamplona, Spain). Ectomycorrhizae used for molecular analyses: Spain, Navarra, Oloriz, coord: 30TXN12; 15.06.2006; ecology: *Q. faginea* stand; soil core and myc. isol.: A.M. de Miguel, herb. Universidad de Navarra PAMP-Mycorrhiza 150 (in Pamplona, Spain), GenBank accession number: EU822505. - Spain, Navarra, Ollogoyen, coord: 30TWN72; 6.11.2007; ecology: *Q. ilex* subsp. *ballota* stands; soil core and myc. isol.: A.M. de Miguel, herb. Universidad de Navarra PAMP-Mycorrhiza 201 and PAMP-Mycorrhiza 202 (in Pamplona, Spain), GenBank accession numbers: EU822506 and EU822507, respectively.

Molecular characterization of different mycorrhiza samples was carried out by sequencing fragments of the nuclear ribosomal DNA region. DNA extraction was performed with the PowerSoil™ DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) according to the manufacturer's instructions. Amplifications of ITS rDNA sequences were carried out with an Applied Biosystems 9700 PCR thermocycler using the primers ITS1F (5'-TCCGTAGGTGAACCTGCGG-3') (Gardes & Bruns, 1993) and ITS4 (5'-TCCTCCGCTTATTGATATGC- 3') (White *et al.,* 1990). Nucleotide sequences of the amplified region were obtained with a Applied Biosystems 3730 DNA analyzer and registered in the NCBI GenBank database with the following accession numbers: EU822505, EU822506 and EU822507. Fungal identification was carried out by searching highly similar sequences in the GenBank and Unite databases.

Results

Short description of the ectomycorrhizae

This brown ectomycorrhiza is well characterized by its rectangularly ramified emanating hyphae and by a two-layered pseudoparenchymatous mantle, the outer one is covered by mounds of mostly globose cells, which are lacking on the very tip. Emanating hyphae straight close to the mantle, continuing in rather irregularly shaped branches with thick (often unevenly) cell walls, smooth or patchily covered by distinct warts, without clamps. Ends of emanating hyphae simple, ramified or tortuous, even screw-like, sometimes forming almost ball-like structures, always thin walled. Septa accompanied by Woronin bodies and, sometimes, slightly dissolved. Rhizomorphs and cystidia absent.

A complete description of *Quercirhiza quadratum* can be found in Águeda *et al*., (2008).

Molecular characterization

The sequence EU822505 showed 100% identity with DQ402507 (84% query coverage) identified as a member of Pyronemataceae (Baciarelli-Falini *et al*., 2006). The sequences EU822506 and EU822507 showed 87 and 89% maximum identity with DQ402506 (97 and 87% query coverage, respectively) identified as a member of Sarcosomataceae (Baciarelli Falini *et al.,* 2006).

Discussion

"*Quercirhiza quadratum*" can easily be distinguished from similar ectomycorrhizae. Most distinctive are the predominantly rectangularly branched, clampless emanating hyphae,

Woronin bodies associated septa, and a pseudoparenchymatous mantle with angular cells that is covered by mounds of globose cells. From the similar *Genea* ectomycorrhizae they differ in the presence of heaps of cells, whereas in *Genea* only solitary cells occur on the mantle surface, and *Genea* forms in addition only a few emanating scarcely ramified hyphae (Agerer, 2006; Brand, 1991a, b; Jakucs & Bratek, 1998; Jakucs *et al*., 1998). *Tomentella* ectomycorrhizae with similar mantle types have either cystidia, and/or clamps, lack Woronin bodies and show differently ramified emanating hyphae (Agerer, 2006; Agerer & Rambold, 2004-2008). Some *Tuber* ectomycorrhizae also present mantles with angular cells, but typical awl-shaped cystidia are usually and emanating hyphae, when present, are smooth (Agerer, 2006; Agerer & Rambold, 2004-2008).

"Quercirhiza quadratum" belongs to the well-known AD type, described first by Giraud (1988), who chose the name, "angle droit" in French language, referring to the abundant rectangular ramification of its emanating hyphae. This ectomycorrhiza is considered as competitor in black truffle (*Tuber melanosporum* Vittad.) plantations and it had been observed mainly in France (Giraud, 1988; Sourzat *et al*., 1993; Sourzat, 1994; Verlhac *et al*., 1990), and also in Italy in *T. melanosporum* and in *T. magnatum* Pico plantations (Bencivenga *et al*. 1992, Bencivenga *et al.,* 1995; Granetti & Baciarelli Falini, 1997; Baciarelli Falini & Granetti, 1998). This ectomycorrhiza has always been observed on oak roots (*Q. ilex*, *Q. faginea* Lam.) and hazelnuts (*Corylus avellana* L.) that always showed "burnt-areas" around trees. The AD type has also been described in nurseries, as a fearsome competitive ectomycorrhiza on seedlings inoculated with black truffle (Bencivenga *et al.,* 1995; Di Massimo *et al.,* 1996).

In Spain, there are proofs of its presence in black truffle plantations in Navarra, Soria, Huesca, Zaragoza, Teruel, Castellón and Valencia. In fact, "*Q. quadratum"* was one of the first types identified as ectomycorrhizal competitor in black truffle plantations established in Navarra (De Miguel & Sáez, 1997, 2005; Etayo & De Miguel, 1998), and also in Soria (Águeda *et al*., 2001) and in Castellón (Domínguez-Núñez *et al*., 2005). De Román & De Miguel (2005), reported this type for the first time in natural holmoak (*Quercus ilex*) stands, suggesting that it was a telephoroid type due to its anatomical and morphological characters.

In 2006, Baciarelli Falini *et al*., using molecular techniques to characterize the ectomycorrhizae of a black truffle plantation, identified their samples Ecm7 and Ecm8 (both showing AD morphotype) both as Ascomycotina belonging to Sarcosomataceae and Pyronemataceae (Pezizales), respectively. Similarly, the molecular analyses performed with "*Q. quadratum"* in the present study showed a maximum identity with both fungal families. However, our sequence EU822505 showed also a close identity (100% maximum identity, 84% coverage) with *Trichophaea woolhopeia* (Cooke & W. Phillips) Arnould, Pezizales, Pyronemataceae (GenBank sequence DQ200835). As there are only five records of *T. woolhopeia* in Iberian Peninsula, very scattered and no one in productive black truffles areas (Pando, 2000), it could not be concluded that "*Quercirhiza quadratum*" belongs to this species. Moreover, three additional species of *Trichophaea* are reported for Spain (s. below).

The members of Pezizales are a considerable proportion of the ectomycorrhizal symbionts in mature boreal deciduous and coniferous forests (Tedersoo *et al.,* 2006), with the potential to rapidly recolonize the site after a disturbance. This could be transferable to Mediterranean stands but there are few records of pezizalean taxa due to the fact that this fungi produce inconspicuous or hypogeous sporocarps that are easily overlooked unless specifically searched for. Molecular techniques could help to increase the knowledge of the role of these group of fungi under different environmental situations.

Ectomycorrhizae of the Pyronemataceae are, in general hydrophylic, of the contact or short-distance exploration type with few rough and clampless emanating hyphae (Agerer, 2006). There are only five ectomycorrhizal genera of the Pyronemataceae known until now: *Genea*, *Humaria*, *Sphaerosporella, Tricharina* and *Trichophaea*. The hypogeous genus *Genea* is the only member of this family known today to form pseudoparenchymatous mantles with globular cells on the surface (Agerer, 2006). *Humaria* ectomycorrhizae have also angular outer mantle layers, but epidermoid inner mantle layers and warted, thick-walled emanating hyphae (Erős-

Honti *et al*., 2008), although all mantle layers of *H. hemisphaerica* are plectenchymatous according to Ingleby *et al*., (1990). *Sphaerosporella brunnea* ectomycorrhizae have densely packed plectenchymatous mantles with infrequent emanating hyphae (Danielson, 1984; Meotto & Carraturo, 1988). *Tricharina* ectomycorrhizae have plectenchymatous mantles and also infrequent emanating hyphae (Ingleby *et al*., 1990). *Trichophaea* ectomycorrhizae form pseudoparenchymatous outer mantle layers, but plectenchymatous to pseudoparenchymatous inner mantle layers, with infrequent emanating hyphae (Tedersoo *et al*., 2006). According to the molecular analysis, the sequence EU822505 would be closely related to the genus *Trichophaea*. On the other hand, no significant coincidences in the ITS sequences were found with the *Humaria* and *Genea* ectomycorrhiza characterized by Erős-Honti *et al.* (2008).
In the Iberian Peninsula there are nine records for *Genea*: *G. fragans* (Wallr.) Paoletti, *G. hispidula* Berk., *G. klotzschii* Berk. & Broome, *G. pulchra* Corda, *G. sphaerica* Tul. & C. Tul., *G. subbaetica* Moreno-Arroyo, Gómez & Calonge, *G. thaxterii* Gilkey, *G. vagans* Mattir. and *G. verrucosa* Vittad., five records for *Humaria*: *H. coccinea* (Fr.) Quélet, *H. hemisphaerica* (F.H. Wigg.: Fr.) Fuckel, *H. melaloma* Karsten, *H. sabranskyana* Bäuml., *H. superans* Bond., one for *Sphaerosporella*: *S. brunnea* (Alb. & Schwein.) Svrček & Kubička, three for *Tricharina*: *T. fibrillosa* (Currey) Yang & Korf, *T. gilva* (Bond. ex Cooke) Eckblad, and *T. praecox* (Karst.) Dennis, and four for *Trichophaea*: *T. gregaria* (Rhem.) Bond., *T. hemisphaerioides* (Mouton) Graddon, *T. paraphysinscrustata* Donadini, Torre & Calonge and *T. woolhopeia* (Pando, 2000).

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ATTIVITÀ ALLELOPATICA, ANTIBATTERICA ED ANTIOSSIDANTE DI ESTRATTI METANOLICI DI *TUBER MAGNATUM* E *T. MELANOSPORUM*

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Abstract: Biological activities of methanolic extract from *Tuber magnatum* and *T. melanosporum*

In this study the potential allelochemical, antimicrobial and antioxidant activities of methanolic extracts of *Tuber magnatum* Pico and *T. melanosporum* Vittad. were investigated.

The allelopathic effect of the truffle extracts was evaluated using a Petri dish assay. Twenty seeds of each plant species (*Lotus corniculatus* L., *Melica ciliata* L., *Silene vulgaris* (Moench) Garcke) were sown on filter paper in a Petri dish with 7 ml of water plus various doses of the MeOH extracts. The Petri dishes were incubated at 25°C in a greenhouse with 10 h of artificial light (250 µmol photons m^{-2} s^{-1}) daily. The root length and seedling height of the tested plant species were measured after 28 d. The antibacterial activity of the truffle methanolic extract against *Streptomyces* species was determined by broth microdilution method as recommended by CLSI (NCCLS, document M24-A) guidelines. The antioxidant activity of the methanolic extracts was tested with the DPPH test. The methanolic extracts of truffle had a dose-dependent inibitory effect on seedling height and root length and showed significant antibacterial activity against *Streptomyces* species.. The results of the antioxidant activity showed that between 24.9 and 22.6 mg were necessary to reduce 50% of the DPPH coloration. There was no linear correlation between antioxidant activities and the polyphenol content.

Key words: Allelopathic activity, antibacterial activity, antioxidant activity, methanolic extracts, total phenolic compounds, *Tuber magnatum, Tuber melanosporum*.

Introduzione

I Macromiceti sono un'importante fonte di prodotti naturali, in grado di fornire una ricca varietà di metaboliti secondari biologicamente attivi con enorme importanza nell'industria farmaceutica e nella moderna agricoltura eco-compatibile (Liu, 2004). A tale riguardo, i metaboliti secondari coinvolti in interazioni biochimiche inibitorie o stimolanti varie specie di piante e di microrganismi (allelochimici), rappresentano una potenziale sorgente di prodotti agrochimici e farmaceutici che potrebbero essere usati a risolvere molti problemi connessi ad inadeguate pratiche di coltivazione o ad un abuso di erbicidi sintetici. Poco è conosciuto sulle attività biologiche dei metaboliti secondari prodotti da *Tuber* spp. In uno studio di Montacchini *et al.* (1977) è emersa un'azione fitotossica di estratti acquosi di ascocarpi e di filtrati colturali di micelio di *T. melanosporum* coltivato *in vitro*, sulla germinazione dei semi di piante erbacee coltivate e spontanee e sulle giovani piante. Pacioni (1991) ha rilevato l'azione fitotossica dei composti volatili prodotti dai tartufi sulle specie vegetali che circondano la pianta ospite. Splivallo *et al*. (2007) hanno evidenziato che le sostanze volatili di *Tuber melanosporum* Vittad., *Tuber borchii* Vittad. e *Tuber indicum* Cooke & Massee inibiscono lo sviluppo di *Arabidopsis thaliana* Heinh e ne modificano il suo metabolismo ossidativo.

Altre ricerche realizzate da Tirillini e Granetti (1995) e Tirillini e Stoppini (1996), hanno rilevato la presenza di composti fenolici derivati in estratti metanolici di ascocarpi di *T. aestivum* Vittad., *T. borchii* Vittad., *T. magnatum* Pico e *T. melanosporum* Vittad. e di ectomicorrize di *Tuber magnatum* Pico e *Tuber borchii* Vittad.

Lo scopo del presente lavoro è stato quello di studiare le possibili attività allelopatica, antimicrobica ed antiossidante di estratti metanolici di *T. magnatum* e *T. melanosporum* che potrebbero essere correlate alla presenza dei derivati fenolici.

Materiali e metodi

Materiale vegetale - Semi di *Lotus corniculatus* L. e *Silene vulgaris* (Moench) Garcke e cariossidi di *Melica ciliata* L. (specie vegetali caratteristiche degli ambienti in cui vive *T. melanosporum*) sono stati raccolti in alcune tartufaie dell'Umbria. Un campione di ogni specie è stato depositato presso l'erbario della sezione di Biologia vegetale e Geobotanica dell'Università degli Studi di Perugia.

Tartufi - *Tuber magnatum* Pico e *T. melanosporum* Vittad. sono stati acquistati presso l'azienda "Paolo Tartufi" di Spello (PG) ed identificati morfologicamente (Granetti *et al*., 2005).

Batteri - I ceppi batterici utilizzati nella sperimentazione, provenienti dalla collezione DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) e DBVPG (Dip.to Biologia Applicata, Università degli Studi di Perugia), sono i seguenti: *Streptomyces griseus* subsp. *griseus* (DSMZ 40236), *S. anulatus* (DSMZ 40361), *S. albus* subsp. *albus* (DSMZ 41209), *S. parvus* (DBVPG 8605), *S. prasineus* (DBVPG 8609), *S. virginiae* (DBVPG 8615). Il controllo di qualità del test dell'attività antibatterica degli estratti metanolici di *Tuber* spp., è stato realizzato utilizzando *Staphylococcus aureus* ATCC 29213, come raccomandato dalle linee guida CLSI (Clinical and Laboratory Standard Institute, documento M24-A).

Preparazione estratti metanolici di *Tuber* spp.
Per l'ottenimento degli estratti metanolici di *Tuber* spp., 100 g di ascocarpo di ciascuna specie di tartufo sono stati trattati con 200 ml di metanolo (96% v/v) per 7 giorni. Successivamente l'estratto è stato filtrato con carta da filtro (No. 2) ed evaporato a secco.

Attività allelopatica
Prima di procedere all'esperimento, i semi di *Lotus corniculatus* e *Silene vulgaris* e le cariossidi di *Melica ciliata* sono stati superficialmente sterilizzati con l'1% di NaClO per 30 minuti, risciacquati con acqua distillata e vernalizzati "overnight" per 1 settimana.
Per ogni singola specie di Tartufo sono state sperimentate tre differenti dosi pari a 100 mg, 50 mg e 10 mg di estratto secco. Per la valutazione dell'attività allelopatica ciascuna dose di estratto secco di *Tuber* spp. è stata disciolta in 0,5 ml di metanolo e versata su 5 dischi sovrapposti di carta da filtro Whatman collocati in una piastra Petri di 9 cm. Il metanolo è stato poi evaporato in stufa ventilata a 37°C. Successivamente la carta da filtro nella piastra Petri è stata bagnata con 7 ml di soluzione acquosa contenente Tween 20 (0,05%) e 20 semi o cariossidi di ciascuna specie vegetale sono stati posti sopra. Nelle piastre di controllo la carta da filtro è stata bagnata con la soluzione acquosa contenente Tween 20 senza estratto. Ogni prova è stata realizzata in triplo e per ciascuna specie di tartufo sono state allestite 27 piastre (9 per ciascuna specie vegetale). Le piastre, quindi, sono state poste ad incubare in una camera di crescita alla temperatura di 24°C e con un fotoperiodo di 10 ore (250 µmol photons m^{-2} s^{-1}) per 4-6 settimane, a seconda della specie vegetale. Dopo la germinazione è stata misurata la lunghezza dei giovani germogli e delle radici.

Attività antibatterica
L'attività antimicrobica degli estratti metanolici di *Tuber* spp. nei confronti di *Streptomyces* spp. è stata valutata con il metodo della microdiluizione seriale utilizzando il substrato colturale liquido di Mueller-Hinton, in accordo alle linee guida CLSI, documento M24-A. Per ciascuna specie di *Tuber* sono state sperimentate le seguenti dosi di estratto secco: 10, 5, 2,5, 1,25 e 0,625 mg/ml. Le sospensioni microbiche utilizzate per l'inoculo sono state preparate a partire

da colture di 3 giorni ed aggiustate alla torbidità di 0,5 McFarland mediante spettrofotometro. Le sospensioni sono state ben miscelate su un vortex mixer e distribuite usando una apposita pipetta in ciascuno dei 24 pozzetti di una piastra contenenti sia il substrato colturale liquido (0,2 ml) che l'estratto secco a concentrazioni seriali. Le piastre inoculate sono state poste ad incubare a 35°C. La concentrazione minima inibitoria (CMI) è stata definita come la più bassa concentrazione degli estratti in grado di inibire una crescita visibile. La lettura delle CMI è stata effettuata dopo 72 ore per tutti i ceppi. Le prove sono state realizzate in triplo. Il ceppo di controllo di qualità (*Staphylococcus aureus* ATCC 29213) è stato testato nello stesso modo.

Polifenoli totali

Il contenuto dei polifenoli totali negli estratti metanolici di *T. magnatum* e *T. melanosporum* è stato determinato mediante il saggio di Folin-Ciocalteau (Singleton *et al*., 1971). Ad 1 ml di estratto metanolico sono stati aggiunti 0,5 ml di reattivo di Folin-Ciocalteau (Merck), diluito due volte con acqua deionizzata, e 5 ml di sodio carbonato (Na_2CO_3) al 10%. Dopo due ore i campioni sono stati analizzati allo spettrofotometro a 765 nm. Il bianco è stato preparato con 1 ml di metanolo, 0,5 ml di reattivo di Folin-Ciocalteau e 5 ml di sodio carbonato (Na_2CO_3) al 10%. Conoscendo l'equazione della retta di regressione lineare standard, è stata determinata la concentrazione fenolica totale (espressa come equivalenti di acido gallico) degli estratti metanolici di *Tuber* spp. Dalla quantità esatta di campione secco sono stati calcolati i mg di polifenoli totali su 100 mg di sostanza secca.

Attività antiossidante

L'attività antiossidante degli estratti di *Tuber magnatum* e *T. melanosporum* è stata valutata mediante una soluzione metanolica di DPPH (2,2-diphenyl-1-picrylhydrazyl), secondo il metodo di Hatano *et al*. (1988). 100 µl di estratto metanolico sono stati uniti a 0,9 ml di metanolo puro e 4 ml di soluzione DPPH (concentrazione finale di DPPH: 2×10^{-4} M). Trascorsi trenta minuti è stata misurata l'assorbanza a 517 nm. Il grado di decolorazione è stato indice della capacità del campione aggiunto di ridurre il radicale libero DPPH. Una soluzione bianca è stata preparata con 100 µl di metanolo. L'attività *radical scavenging* degli estratti metanolici nei confronti del radicale stabile DPPH è stata espressa come EC50 che è la concentrazione dell'estratto testato necessaria per indurre un decremento del 50% di assorbanza rispetto a quella della soluzione del bianco.

Analisi statistica

I dati sono stati elaborati statisticamente mediante l'analisi della varianza e il test di Fisher PLSD ($p \leq 0,05$), utilizzando il programma SuperAnova (Abacus Concept).

Risultati e discussioni

Per quanto riguarda l'attività allelopatica è risultato che, a seconda delle dosi, gli estratti metanolici possono inibire la germinazione dei semi, influenzare la crescita dei giovani germogli e ridurre la lunghezza delle radici (Tabb. 1-2).

Tab.1 Effetti degli estratti metanolici di *Tuber* spp. impiegati a differenti dosi, sulla percentuale di germinazione dei semi di alcune specie vegetali.

	Lotus corniculatus				*Melica ciliata*				*Silene vulgaris*			
Estratti metanolici												
Tuber spp.	100 mg	50 mg	10 mg	controllo	100 mg	50 mg	10 mg	controllo	100 mg	50 mg	10 mg	controllo
T. magnatum	0 a	11,6±1,6 b	18,3±1,6 c	76,6±1,6 d	0 a	0 a	25,0±2,8 b	86,6±1,6 c	5±0,0 a	18,3±4,4 b	25,0±2,8 b	76,6±3,6 c
T. melanosporum	0 a	0 a	23,3±3,3 b	78,3±1,6 c	0 a	0 a	18,3±4,4 b	90,0±2,8 c	0 a	10,0±2,8 ab	16,6±1,6 b	81,6±6,0 c

I valori sono la media di tre ripetizioni ± errore standard. I dati lungo la stessa riga seguiti da lettere differenti tra parentesi, indicano differenze significative secondo il test di Fisher PLSD ($p \leq 0,05$).

La Tab. 1 evidenzia che la germinazione dei semi delle specie vegetali esaminate è risultata significativamente inibita dai due estratti, in modo particolare da quello di *T. melanosporum*. Per quanto riguarda gli effetti degli estratti sull'accrescimento delle plantule, le tre specie vegetali hanno evidenziato un diverso comportamento (Tab. 2).

Tab. 2 Effetti degli estratti metanolici di *Tuber* spp. impiegati a differenti dosi, sulla crescita del giovane germoglio di alcune specie vegetali.

	lunghezza radice				altezza germoglio				lunghezza radice				altezza germoglio			
	Estratto metanolico di *T. melanosporum*				Estratto metanolico di *T. melanosporum*				Estratto metanolico di *T. magnatum*				Estratto metanolico di *T. magnatum*			
Specie vegetali	100 mg	50 mg	10 mg	controllo	100 mg	50 mg	10 mg	controllo	100 mg	50 mg	10 mg	controllo	100 mg	50 mg	10 mg	controllo
Lotus corniculatus	0 a	0 a	0,6±0,2 b	0,6±0,2 b	0 a	0 a	1,5±0,4 b	5,1±0,3 c	0 a	0,4±0,1 b	0,3±0,1 b	0,5±0,1 b	0 a	1,5±0,4 b	1,8±0,8 b	5,5±0,3 c
Melica ciliata	0 a	0 a	1,3±0,2 b	1,2±0,1 b	0 a	0 a	3,3±0,6 b	3,7±0,3 c	0 a	0 a	0,4±0,8 b	1,4±0,2 c	0 a	0 a	3,9±0,6 b	3,5±0,3 c
Silene vulgaris	0 a	0 a	0 a	0,5±0,1 b	0 a	0,6±0,1 a	0,7±0,1 a	3,4±0,3 b	0,3±0,1 a	0,4±0,1 a	0,4±0,1 a	0,5±0,3 a	0,8±0,1 a	2,8±0,3 b	3,2±0,3 b	3,3±0,2 b

I valori sono la media di tre ripetizioni ± errore standard. I dati lungo la stessa riga seguiti da lettere differenti tra parentesi, indicano differenze significative secondo il test di Fisher PLSD ($p \leq 0,05$).

S. vulgaris, alquanto resistente all'estratto di *T. magnatum*, è stata totalmente inibita dalla dose più alta dell' estratto di *T. melanosporum* (100 mg). *L. corniculatus* e *M. ciliata*, sono state completamente inibite alle dosi maggiori degli estratti di *T. magnatum* e *T. melanosporum*, mentre *M. ciliata* ha manifestato un effetto stimolante nell'accrescimento dei germogli alla dose minima (10 mg) di *T. magnatum*. Molto evidente è risultato l'effetto brachizzante, a volte molto marcato a livello dell'apparato radicale, soprattutto nel caso dell'estratto di *T. melanosporum* nei confronti di *S. vulgaris*.
Questi risultati evidenziano negli estratti metanolici dei tartufi la presenza di allelochimici che potrebbero essere potenzialmente usati per il controllo delle infestanti erbacee delle tartufaie.

Per quanto riguarda l'attività antibatterica è risultato che gli Streptomiceti, batteri largamente diffusi nei terreni tartuficoli, hanno presentato un'elevata sensibilità all'azione tossica degli estratti metanolici dei tartufi, sia pure in modo diversificato
I risultati dell'attività antibatterica dei 2 estratti di *Tuber* spp. nei confronti di alcuni ceppi di *Streptomyces* spp. hanno mostrato valori delle CMI (Concentrazione Minima Inibitoria) compresi tra 1,12-6,29 mg/ml (Tab. 3).

Tab. 3 Concentrazione minima inibitoria (CMI) degli estratti metanolici di *Tuber* spp. nei confronti di *Streptomyces* spp.

	CMI (mg/ml)	
Streptomyces spp.	*T. magnatum*	*T. melanosporum*
S. griseus subs. *griseus* DSMZ 40236	3,96	1,25
S. anulatus DSMZ 40361	6,29	1,57
S. albus subsp. *albus* DSMZ 41209	5,00	1,12
S. parvus DBVPG 8605	3,14	1,25
S. prasineus DBVPG 8609	2,50	2,50
S. virginiae DBVPG 8615	3,96	1,32
I valori sono la media geometrica di tre ripetizioni.		

L'estratto metanolico di *T. melanosporum* ha registrato i valori delle CMI più bassi, in modo particolare nei confronti di *S. albus* subsp. *albus* DSMZ 41209.
A conferma di quanto sopra, nelle aree bruciate (o cave) determinate da *T. melanosporum* e *T. aestivum*, la popolazione streptomicetica è sempre inferiore alle zone di controllo (Mamoun e Olivier, 1990).
Sebbene gli esperimenti realizzati in laboratorio abbiano mostrato che entrambi gli estratti metanolici di *T. magnatum* e di T*. melanosporum* possiedano attività allelopatica ed antibatterica, in campo, tali proprietà potrebbero essere influenzate da molti fattori capaci di mascherarne gli effetti (caratteristiche del suolo, luce, temperatura). Le caratteristiche fisiche, chimiche e biologiche del terreno, ad esempio, influenzano la disponibilità quantitativa e qualitativa degli allelochimici e di conseguenza i loro effetti. Inoltre, i suoli con livelli elevati di sostanze organiche o di argilla, generalmente ritengono i composti fenolici molto più dei suoli sabbiosi (Bezuidenhout e Laing, 2006). Probabilmente, è da ricercare in una di queste cause la motivazione del fatto che, in natura, l'azione tossica sulle piante e sui microrganismi degli allelochimici solubili nei suoli di *T. magnatum*, più profondi e umidi rispetto a quelli dei tartufi neri, sia poco o affatto rilevante.
Per quanto riguarda l'attività antiossidante degli estratti di *T. magnatum* e *T. melanosporum* è stato rilevato che, valori medi corrispondenti a 22,6 e 24,9 mg (differenze medie non statisticamente significative) di estratto, riducono del 50% la colorazione del DPPH. Tale attività è risultata alquanto elevata, soprattutto in considerazione del basso contenuto di polifenoli (Tab. 4).

Tab. 4 Dosaggio dei polifenoli totali ed attività antiossidante degli estratti metanolici di *Tuber* spp.

		DPPH
Estratto metanolico	% polifenoli	EC50 (mg)
T. magnatum	15,1±2,2 a	22,6±0,6 a
T. melanosporum	19,4± 2,6 b	24,9±1,3 a
I valori sono la media di tre ripetizioni ± errore standard. I dati lungo la stessa colonna seguiti da lettere differenti tra parentesi, indicano differenze significative secondo il test di Fisher PLSD ($p \leq 0,05$).		

Sulla base di questi risultati, si ipotizza che altre classi di composti possano giocare un importante ruolo nella capacità antiossidante dei due estratti studiati.
Ulteriori studi sono necessari per stabilire la natura dei componenti attivi presenti negli estratti

metanolici di *Tuber* spp. e per valutare la loro attività sia in condizioni controllate di laboratorio che in condizioni di campo, al fine di meglio comprendere il loro meccanismo di azione.

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DISTRIBUTION OF *TUBER MELANOSPORUM* MYCORRHIZAS ON ROOTSTOCKS OF HOLM-OAKS (*QUERCUS ILEX*) IN PRODUCTION

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Abstract

Alcina is currently developing molecular diagnosis based on PCR technology to determine the potential of a tree to produce truffles, using either roots (mycorrhiza DNA) or soil (spore or mycelia DNA) samples. Concerning the production potential of a tree, an optimal mycorrhiza sampling protocol should be established to ensure efficient and reproducible data. The aim of the present study was to localize an area around the oak tree in which fine roots bearing mainly *Tuber* mycorrhizas would be particularly abundant. The study involves three 13 years-olds-holm-oak trees (*Q. ilex*) that are producers of black truffles (*T. melanosporum*). For each tree, 3 soil profiles were carried out on opposite side of the trunk at the following distances: 20cm, 50cm (corresponding to the burned area) and 120cm. The profiles were 100-120cm deep and 100cm wide. The trees were localized in the middle of the profile width. All cardinal orientations were studied. For each profile the number of fine (diameter<2mm) and medium (diameter>2mm) roots carrying mycorrhizas was counted at 20 cm intervals in the vertical axis. The presence from 3 *Tuber* species (*T. melanosporum*, *T. aestivum* and *T. brumale*) was analyzed using PCR multiplex. The results showed that root density does not depend on the cardinal orientation. The 3 trees revealed similar rooting profiles for both fine and medium roots. There were more fine roots between 20 and 40cm depth. Fine root density profile below 40cm depth was the same for all trees and root density decreased with the depth. Two trees produced fine roots below 100cm depth. *Tuber* mycorrhizas were scarce within the first 20 cm whatever the distance from the trunk. At 120cm from the trunk, almost all mycorrhizas were found below 40cm depth. At the burned limit, *T. melanosporum* was detected in 70% of the analyzed samples that were performed between 20 and 40 cm depth. Near the trunk, *T. brumale* mycorrhizas were detected near the surface (0-40cm). No *T. aestivum* mycorrhiza was found. Most of the analyzed roots found below 60cm depth were mycorrhized with *T. melanosporum*. which it could even be found below 120cm in depth. *T. melanosporum* mycorrhizas were found throughout the depth of the studied profiles, for all distances from the trunk and all orientations. However, the present results proved that to ensure the most efficient *T. melanosporum* production diagnosis by PCR technology, sampling must be done at the burn area limit and between 20-40 cm depth which corresponds to the richest area for both fine roots and mycorrhizas belonging to this *Tuber* species.

Key words: molecular diagnosis, mycorrhiza, Polymerase Chain Reaction, root density, *Tuber.*

Introduction

La truffe noire (*Tuber melanosporum*) est un champignon dont la fructification est très renommée pour ses arômes. La récolte de ce champignon se fait soit en milieu naturel soit dans des plantations d'arbres à vocation truffière. Or, depuis une quinzaine d'années une méthode de gestion des espaces naturels en vue de produire des truffes a été remise au goût du jour: la sylviculture truffière (Diette and Lauriac, 2004). Cette méthode a pour objectif de revaloriser les espaces naturels où la truffe était présente. Le postulat est que les zones

où la truffe a déjà été ramassée peuvent à nouveau produire si les conditions écologiques nécessaires à la fructification du champignon sont rétablies. Toutefois la sylviculture truffière n'est possible que si le champignon est encore présent dans la parcelle choisie. En effet, après l'arrêt de production de la forme commercialisable du champignon (la truffe), ce dernier reste présent dans le sol sous des formes biologiques diverses difficilement détectables comme les mycorhizes ou sa forme de conservation qui est la spore.
Aujourd'hui, le diagnostic du potentiel truffier est basé sur les connaissances acquises sur l'écologie du champignon (caractéristiques du sous-sol et du sol, topographie, végétation, ensoleillement, précipitations…). Ce diagnostic permet de déterminer les zones qui peuvent potentiellement produire ce champignon, mais il ne donne aucune indication quant à la présence du champignon dans le sol.
Depuis une quinzaine d'année, grâce à la biologie moléculaire et la technique de PCR, il est possible d'identifier les différentes espèces de *Tuber* sous forme d'ascocarpes, de mycorhizes ou de mycélium grâce à l'identification de l'ADN (Henrion *et al.,* 1994; Paolocci *et al.,* 1999).
A partir de ces techniques, nous avons développé au laboratoire des méthodes de diagnostics moléculaires applicables à un grand nombre d'échantillons et qui s'adressent aux trufficulteurs et aux professionnels de la filière. Au cours de la validation du diagnostic mycorhize sur des échantillons issus du terrain, il est apparu indispensable de mettre au point des méthodes d'échantillonnage qui soient simples et rapides à mettre en oeuvre et dont le résultat après analyse soit fiable et reproductible.
Dans cette optique, nous avons étudié le système racinaire d'arbres producteurs de truffes avec l'objectif de mettre en évidence la présence d'une zone autour de l'arbre qui soit la plus favorable à l'échantillonnage en vue du diagnostic de potentiel truffier d'une arbre.

Materiel et methodes
Matériel: Trois chênes verts (*Quercus ilex*) producteur de truffe noire (*Tuber melanosporum*) situés sur une truffière à Saint Mathieu de Tréviers (France) ont été choisis pour cette étude. Ces arbres ont été plantés en 1995 et produisent des truffes depuis 2002. L'étude a été faite en mai 2008.

Méthodes:
Pour chaque arbre, 6 profils de sol de 100cm de large à 100-120cm de profondeur sont creusés à l'aide d'une pelle mécanique pour le comptage des racines et la collecte de racines fines mycorhizées. Le profil est creusé de manière à ce que l'arbre soit au milieu de la largeur du profil. Pour chaque arbre, deux profils de part et d'autre du tronc sont effectués à 20cm, 50cm (limite du brûlé) et 120cm du tronc. Pour l'arbre A, les profils sont placés de part et d'autre du tronc selon l'axe Nord-Sud. Pour l'arbre B, un profil est placé selon l'axe Nord-Sud et l'autre perpendiculairement selon l'axe Est-Ouest. Pour l'arbre C, les profils sont placés de part et d'autre du tronc selon l'axe Est-Ouest. Ainsi, toutes les orientations ont été étudiées.
Pour chaque profil, une grille (100cm de large/100cm de hauteur) dont le maillage est de 20cm/20cm est placée sur le profil de sol de façon à ce que le tronc soit au centre de la largeur de la grille. Dans chaque maille des comptages de racines fines de diamètre inférieur à 2mm et de racines de diamètre supérieur à 2mm sont référencés. Les racines fines sont prélevées sur toute la largeur du profil dans chaque bande de 20 cm d'épaisseur pour l'analyse de mycorhizes.
Les résultats de l'analyse des densités de racines (inférieures et supérieures à 2mm) et de fréquence de mycorhizes est une moyenne des trois arbres étudiés.

Caractérisation des mycorhizes de *Tuber* par PCR multiplex.
Pour chaque horizon de 20 cm d'épaisseur, 4 prélèvements de mycorhizes sont effectués et placés dans des tubes pour analyse. L'ADN est extrait des mycorhizes selon le protocole utilisé par Paolocci *et al*., 1999.
Pour l'amplification par PCR multiplex, des couples d'amorce spécifique à 3 espèces de truffes

couramment rencontrées dans le sud de la France sont utilisés. Pour *T. melanosporum* MELF et MELR et *T. brumale* SYLV1 et SYLV2 (Douet *et al.*, 2004) et pour *T. aestivum* BTMEL-F et BTAEMB-Rev (Schiaffino, 2006). L'amplification avec ces primers est faite sur un PX2 THERMO, Electron Corporation, en utilisant 0,3-5 ng de DNA cible isolé à partir des mycorhizes prélevées sur les différents horizons: 0-20cm, 20-40cm, 40-60cm, 60-80cm, 80-100cm et 100-120cm uniquement pour les profils à 50 et 120cm du tronc. Les conditions d'amplification sont les suivantes: dénaturation 5' à 95°C; 35 cycles de 30s à 95°C, 1min à 59°C, 1min30s à 72°C et une étape d'élongation de 10min à 72°C.
L'amplification se fait dans un volume final de 20µl contenant 0,5 unité de Taq polymérase et 1,6 µL de son tampon de réaction (EurobioTaqpolymérase, EUROBIO), 0,075µM de chaque primer, 800µM de chaque dNTP et 7,5 mM de MgCl2.
Le produit de la PCR (15µl) après addition de 2µl de tampon 6X est analysé sur gel d'agarose à 1,8% dans un tampon de TAE et coloré avec du BET et visualisé sous lampe UV.

Analyses statistiques
Des comparaisons de moyennes de fréquence de mycorhizes par horizons ont été réalisées:

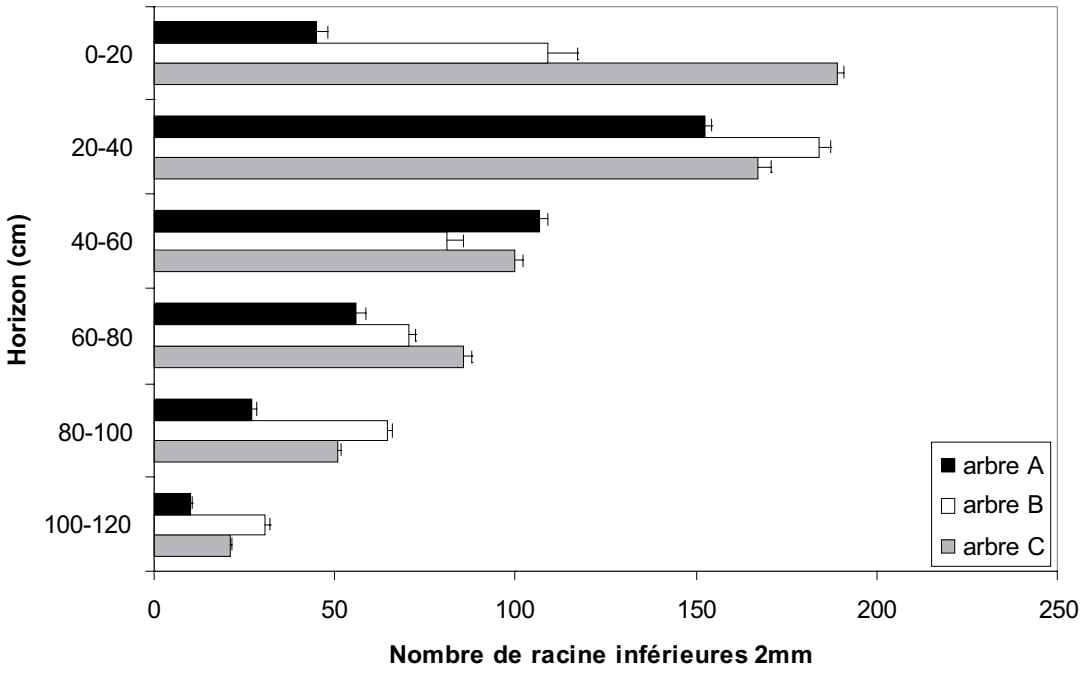
- soit en comparant les moyennes de fréquence deux à deux par profondeur d'horizon à distance fixe du tronc
- soit par distance au tronc à profondeur fixe.

Après vérification de l'égalité des variances par le test de Snedecor, les moyennes de fréquence ont été comparées par le test de Student.

Resultats

Densité racinaire

La densité racinaire n'est pas affectée par l'orientation. Ceci permet dans cette étude de regrouper les données analysées des trois arbres par distance au tronc.
Le profil d'enracinement des racines fines (diamètre < 2mm) n'est pas significativement différent entre les 3 arbres A, B et C (Fig. 1A). Seul dans l'horizon situé entre 0-20cm de profondeur la colonisation des racines fines est variable selon les arbres. De même, le profil d'enracinement des racines supérieures à 2 mm n'est pas significativement différent entre les 3 arbres sauf pour l'horizon 20-40cm de profondeur (Fig.1B).



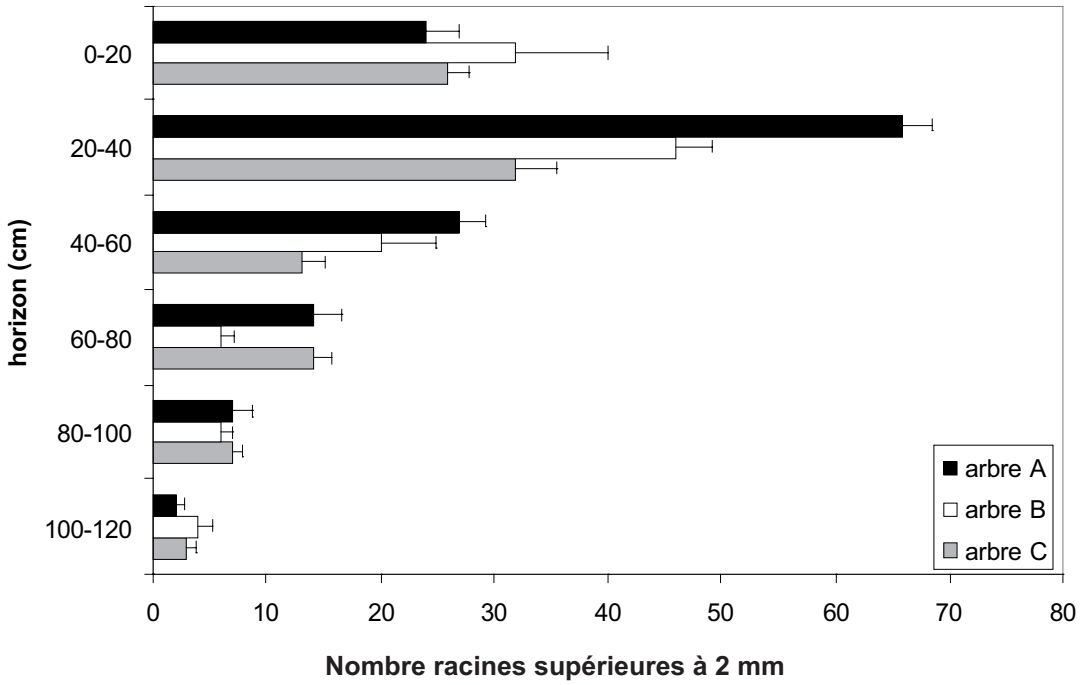


Fig. 1: A - Nombre de racines inférieures à 2mm et B supérieures à 2mm dans des horizons de 20cm d'épaisseur sur 120cm de profondeur. Pour chaque horizon, le nombre de racines est obtenu à partir de la moyenne des 6 profils étudiés.

Face à ce constat, les profils d'enracinements des racines fines et moyennes présentés sont calculés sur la base d'une moyenne des trois arbres étudiés (A, B et C).
Pour les trois profils étudiés (20cm, 50cm et 120cm du tronc), le nombre de racines inférieures à 2mm diminue avec la profondeur (Fig. 2 A et B).

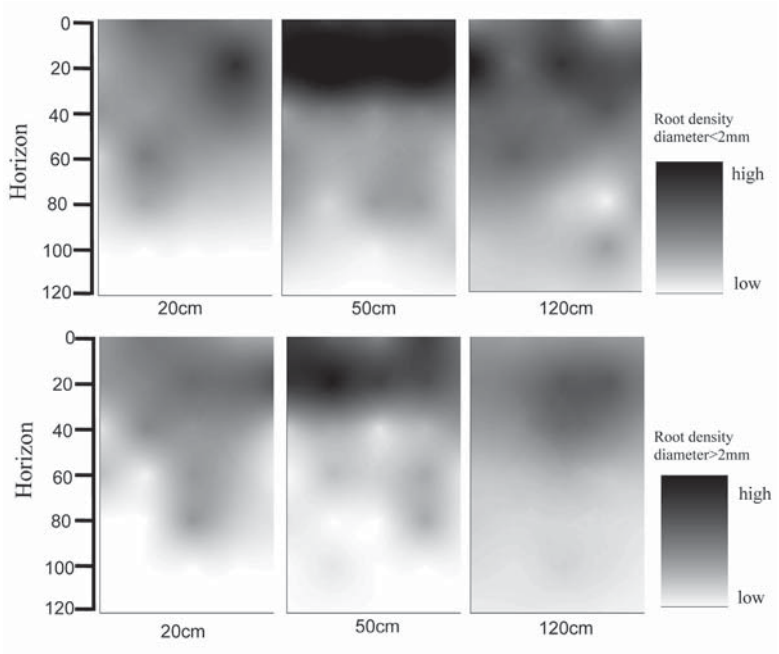


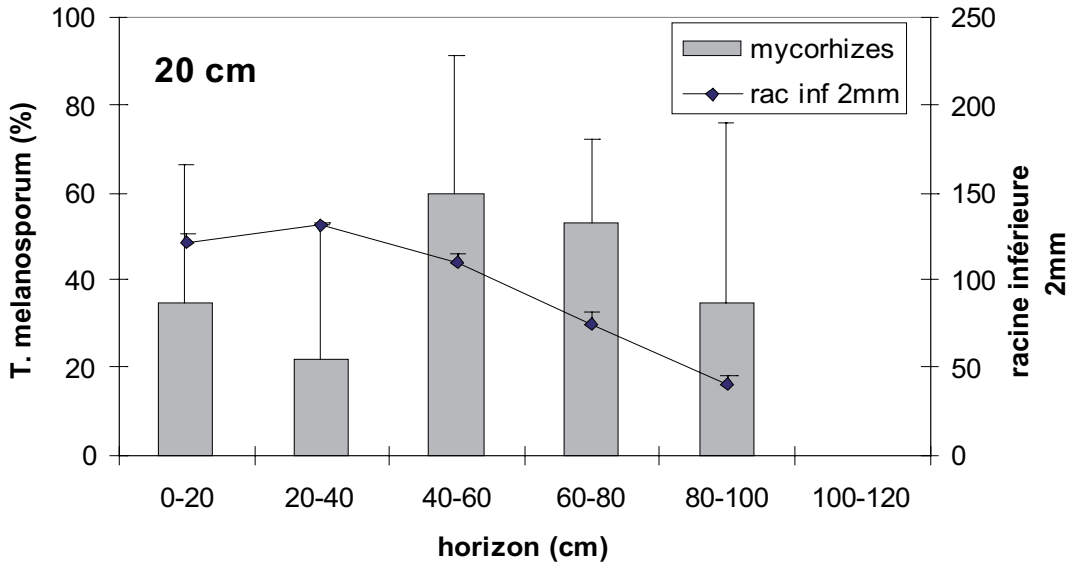
Figure 2 : Représentation de la densité en racines inférieures à 2mm (en haut) et supérieure à 2mm (en bas) sur des profils de 0 à 120cm de profondeur et à trois distances du tronc: 20cm, 50cm (limite extérieure du brûlé) et 120cm (hors brûlé). Pour chaque distance au tronc, les valeurs calculées sont une moyenne du nombre de racines comptées sur deux profils racinaires pour chacun des trois arbres.

20cm du tronc
La densité en racines fines est plus importante dans les deux horizons superficiels (0—20cm et 20-40cm de profondeur) (Fig. 2A). Une zone particulièrement dense en racines fines se situe près du tronc.
La densité en racines moyennes est plus importante dans les horizons superficiels. A la verticale du tronc, les racines moyennes sont plus nombreuses (Fig. 2B)

50cm du tronc
A 50cm du tronc qui correspond à la limite du brulé, la densité en racines fines est plus importante sur les premiers 40cm de profondeur (Fig. 2A). Ce profil montre que les racines fines sont moins fréquentes sur les horizons les plus profonds (inférieur à 60cm de profondeur).
Les racines moyennes ont fortement colonisé les horizons supérieurs entre 0 et 40cm de profondeur. A 50cm du tronc, pour les mêmes horizons, leur densité est plus forte que celle mesurée à 20cm du tronc (Fig. 2B).

120cm du tronc
Sur le profil le plus éloigné du tronc et qui est hors brûlé, la zone la plus dense en racines fines se situe entre 20 et 60 cm de profondeur. De plus, à cette distance du tronc, les racines fines ont colonisé tout le profil. L'arbre âgé de 13 ans a colonisé le sol profondément (Fig. 2A).
A 120 cm du tronc, la densité en racines moyennes est plus importante dans les horizons supérieurs du sol (Fig. 2B).

Le nombre moyen de racines inférieures à 2mm et racines supérieures à 2mm par horizon exprimé en racine/m^2 (Fig. 3) confirment les images des profils d'enracinements. La densité en racines fines est significativement plus importante (195 RF/m^2) dans l'horizon 20-40cm à la limite du brûlé (50cm du tronc).



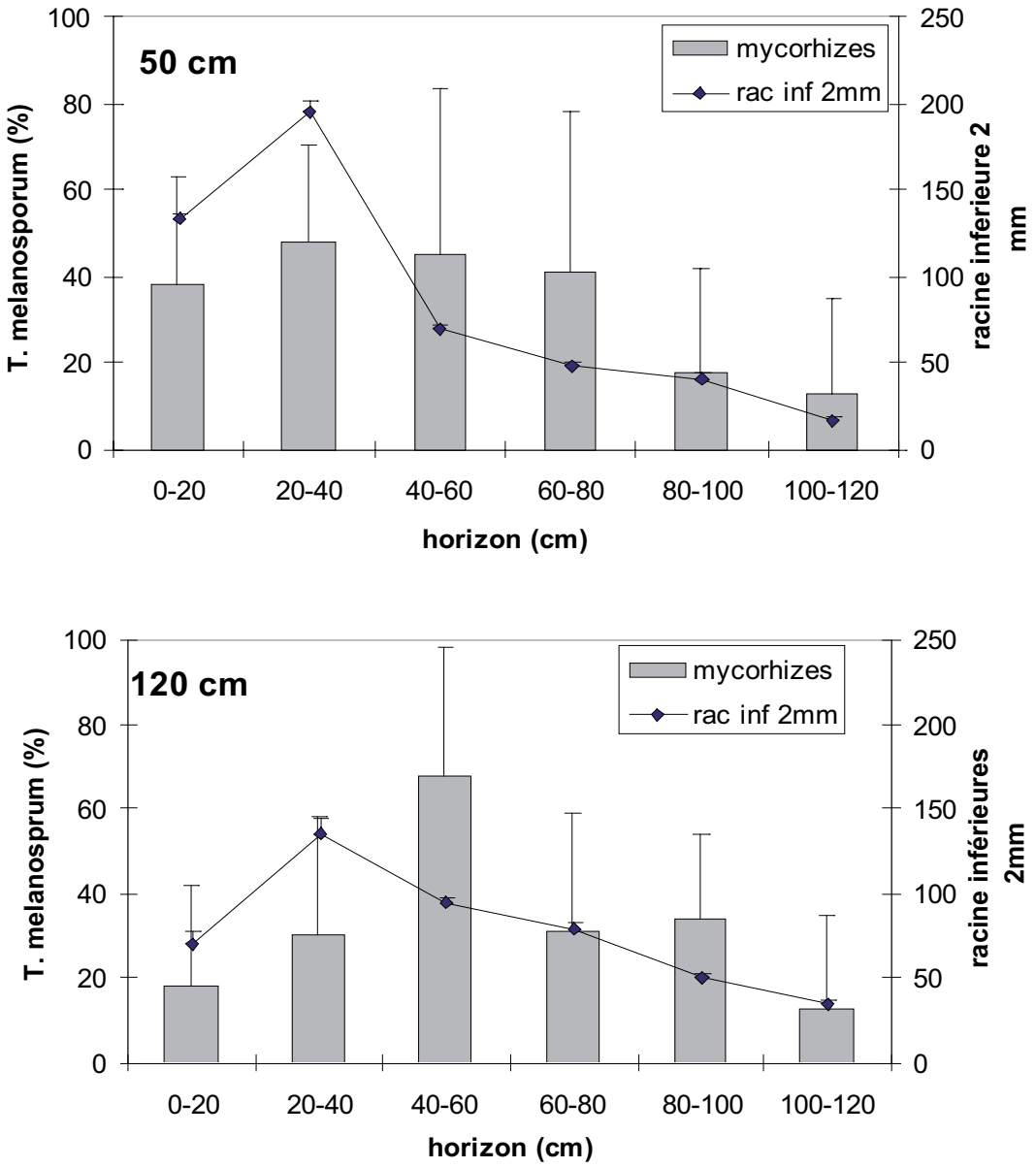


Fig. 3 Représentation du nombre de racines inférieures à 2mm (RF/m²) et de la fréquence en mycorhizes de *Tuber melanosporum* dans chaque horizon de 20cm d'épaisseur étudié en fonction de la distance au tronc: 20cm, 50cm (limite extérieure du brûlé) et 120cm (hors brulé).

Répartition des mycorhizes de *Tuber melanosporum*

Les mycorhizes de *T. melanosporum* ont été trouvées à toutes les distances au tronc et à toutes les profondeurs étudiées. Pour le profil situé à 120cm du tronc, des mycorhizes de *T. melanosporum* ont été identifiées à 140cm de profondeur (résultat non présenté).

1- Comparaison de la fréquence en mycorhizes par profondeur d'horizon à distance fixe au tronc

20cm du tronc

A cette distance, la fréquence en mycorhizes n'est pas significativement différente entre les différents horizons sauf pour l'horizon 20-40cm de profondeur qui est significativement moins riche en mycorhizes que les horizons 40-60 et 60-80cm de profondeur (Fig. 3). Sur l'horizon supérieur 0-20cm d'autres champignons sont présents comme *Scleroderma sp.* (résultat non présenté).

50cm du tronc
A cette distance du tronc qui correspond à la limite du brûlé, la fréquence en mycorhizes de *T. melanosporum* n'est pas significativement différente entre les horizons étudiés sauf pour l'horizon situé entre 20-40cm de profondeur où la fréquence en mycorhizes de *T. melanosporum* est significativement plus élevée que dans l'horizon 80-100cm (Fig. 3).

120cm du tronc
A 120cm du tronc, les mycorhizes de *T. melanosporum* sont présentes sur tout les horizons des profils étudiés. L'horizon situé entre 40-60cm de profondeur est significativement plus riche en mycorhizes de *T. melanosporum* (68,6 %) que les autres horizons (Fig. 3).

2- Comparaison de la fréquence en mycorhizes par distance au tronc à profondeur fixe
Pour l'horizon 20-40cm de profondeur, la fréquence en mycorhizes est significativement plus importante à 50cm du tronc qu'à 20cm. Pour tous les autres horizons étudiés, la fréquence en mycorhizes n'est pas significativement différente à 20, 50 et 120cm du tronc.

Comparaison des profils densité racinaire avec les fréquences de mycorhizes de *T. melanosporum*.
20cm du tronc
A 20cm du tronc, les deux horizons les plus riches en racines fines (0-20cm et 20-40cm de profondeur) ne correspondent pas à l'horizon où les mycorhizes de *T. melanosporum* sont les plus fréquentes (40-60cm de profondeur) (Fig. 3).

50cm du tronc
A 50cm du tronc, l'horizon où le nombre de racines fines par m^2 de profil est le plus important (195 racines <2mm / m^2) correspond à l'horizon où la fréquence en mycorhizes de *T. melanosporum* est est significativement plus élevée (48,0%) pour cet horizon. Cet horizon se situe entre 20 et 40cm de profondeur (Fig. 3).

120cm du tronc
A 120cm du tronc, l'horizon 40-60cm où la fréquence en mycorhizes de *T. melanosporum* est significativement plus forte (68,8%) correspond à un horizon où le nombre de racines fines par m2 est moyennement important (95,83 racines <2mm / m^2) (Fig. 3).

Discussion
Cette étude a pour objectif d'apporter des éléments sur la connaissance de la répartition des mycorhizes de *T. melanosporum* sur un système racinaire de chêne vert en production, en vue de mettre au point un plan d'échantillonnage pour le diagnostic de potentialité truffière d'un arbre.

Individuellement, les trois arbres étudiés présentent des profils de nombre de racines de diamètre inférieur et supérieur à 2mm assez proches. Les profils indiquent que l'arbre colonise le sol sur tous les horizons étudiés jusqu'à au moins 120cm du tronc de l'arbre et jusqu'à 140cm de profondeur.
Toutefois, les racines les plus fines se développent préférentiellement dans les horizons supérieurs, puisque selon la distance au tronc 43 à 65% du nombre total de racines fines se situent entre 0 et 40cm de profondeur. Ces résultats rejoignent ceux déjà observés pour les chênes. En effet, sur *Quercus douglasi,* Millikin et Bledsoe (1999) montrent que la biomasse en racines fines diminue avec la profondeur et que 70% de la biomasse se situait dans les 50 premiers centimètres.
De même, sur *Quercus ilex*, Lopez *et al.*, (2001) ont montré que 59% de la biomasse en racine fine se situait dans les 30 premiers centimètres du sol. L'arbre coloniserait les horizons du sol les plus riches en éléments nutritifs (Lopez *et al.*, 2001).

La répartition des mycorhizes

La répartition des mycorhizes de *T. melanosporum* sur le système racinaire n'est pas homogène puisque les pourcentages de mycorhizes varient selon la profondeur et la distance au tronc. Toutefois, le champignon de *T. melanosporum* sous forme de mycorhizes est présent dans tous les horizons, à toutes les distances au tronc et dans toutes les directions étudiés. Ces résultats rejoignent ceux de Suz *et al.* (2006 et 2008) sur l'abondance de mycélium de *T. melanosporum* dans le sol. Le champignon sous forme mycélienne colonise préférentiellement les 35 premiers centimètres de sol et est peu fréquent en dessous de 60cm de profondeur. De plus, comme pour la forme mycorhizienne, le mycélium de *T. melanosporum* est assez fréquent en dehors de la zone du brûlé d'arbres producteurs. L'ensemble de ces observations indiquent que le champignon en colonisant le sol en profondeur sous forme de mycorhize ou de mycélium, peut résister aux conditions climatiques lorsqu'elles deviennent défavorables (sècheresse ou gel) comme l'indiquait Ricard (2005) qui avait observé des mycorhizes de *T. melanosporum* à 80cm de profondeur.

T. melanosporum ne représente ni la totalité ni la majorité des mycorhizes des systèmes racinaires étudiés, 42% des mycorhizes analysées étaient du *T. melanosporum* et 6% du *T. brumale*. Les études sur la répartition et la nature des mycorhizes montrent qu'il peut y avoir jusqu'à plusieurs dizaines d'espèces de champignon associées à un arbre (Richard *et al.*, 2005; Morris *et al.*, 2008). Malheureusement, nos analyses ne nous permettent pas de déterminer quels autres champignons ectomycorhiziens étaient présents sur les racines fines étudiées. Seules des mycorhizes typiques de Scléroderme ont été visuellement déterminées. Dans le cas de la truffe, la présence d'autres espèces de champignon ne semble pas empêcher la fructification, mais l'apparition de certaines espèces peuvent être aussi le signe d'une modification des conditions du milieu et d'une future disparition de la truffe (Sourzat, 2002).

Les observations de Callot (1999) et Sourzat (2002) sur la répartition des mycorhizes de *T. melanosporum* autour d'arbres truffiers, indiquaient que les racines étaient peu mycorhizées par *T. melanosporum* prés du tronc et en dehors du brûlé. Or, nos résultat montrent que dans ces zones les mycorhizes sont bien présentes mais en dessous de 40cm de profondeur. Il semblerait que le champignon sous forme de mycorhize colonise préférentiellement les horizons les moins riches en matières organiques comme les horizons plus profonds et la zone externe du brûlé. En effet, c'est dans cette zone que nous avons mis en évidence une fréquence en mycorhizes assez forte (40%) sur toute la hauteur du profil. Il semblerait que le champignon colonise différemment le sol sous sa forme mycélienne (Suz *et al*., 2006 et 2008) et sous sa forme mycorhizienne en fonction de ses besoins biologiques notamment au moment de la fructification (Suz *et al*., 2008).

Mode opératoire pour un échantillonnage

L'ensemble de nos résultats ont permis de mettre en évidence une zone où le nombre de racines fines est important et où les mycorhizes les plus fréquentes sont celles de *T. melanosporum.* Cette zone, située à la limite extérieure du brûlé, répond aux critères d'une zone préférentielle pour le prélèvement de racines fines mycorhizées en vu du diagnostic de potentialité truffière d'un arbre.

Depuis 3 ans, nous avons développé des méthodes afin d'améliorer le diagnostic du potentiel truffier. Pour les arbres, cette étude nous a permis de répondre à notre problématique de départ concernant l'efficacité et la reproductibilité de l'échantillonnage. Aujourd'hui, grâce à cette étude, nous avons pu déterminer pour nos prestations une méthode d'échantillonnage facile à mettre en oeuvre, peu couteuse et qui permet d'obtenir des résultats fiables et répétables.

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ROLE OF *TUBER BORCHII* MANNITOL DEHYDROGENASE IN KEY ENVIRONMENTAL AND METABOLIC RESPONSES

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Abstract
Mannitol plays a role in fungi as a storage or translocated carbohydrate and it is important in spore germination and utilization under starvation conditions. Mannitol also quenches reactive oxygen species (ROS) leading to the hypothesis that it can play an antioxidant role in host-pathogen or plant-fungus interactions. In the plant symbiotic ascomycete fungus *Tuber borchii* we confirmed the presence of all enzymes involved in mannitol cycle. In particular, a novel $NADP^+$-dependent mannitol dehydrogenase (TbMDH) and the corresponding gene were identified and characterized. The distinctive properties of the protein, respect to other fungal mannitol dehydrogenases characterized so far, including the molecular weight, high substrate specificity and the coenzyme binding motif, show TbMDH to be the first example of a fungal mannitol dehydrogenase belonging to the medium-chain dehydrogenase/reductase. The carbon responsive transcriptional pattern showed that *Tbmdh* is up-regulated when mycelia are transferred to a culture medium containing the preferred substrates, mannitol or fructose. In osmotic stress conditions, the expression data showed that *Tbmdh* messenger decreased following transfer on NaCl medium for 48 h and the same down regulation response was also evident in temperature stressed mycelia. These findings show that the down regulation of TbMDH in stressed mycelium is important to permit the accumulation of mannitol in the hyphae which acts as osmoregulator.
Key words: mannitol cycle, mannitol dehydrogenase, stress, *Tuber borchii* mycelium.

Introduction

Polyols or sugar alcohols are acyclic, polyhydric alcohols formed by the reduction of the carbonyl group of an aldose or ketose monosaccharide to a hydroxyl group. Inside these compounds, the acyclic hexitol known as mannitol is one of the most abundant polyols occurring in nature and it is found in large quantities in spores, fruiting bodies, sclerotia and mycelia of many fungi (Lewis and Smith, 1967).
In filamentous fungi, a multitude of roles, including carbon storage or translocation of carbohydrate, reservoir of reducing power, stress tolerance and spore dislodgement and/or dispersal has been ascribed to mannitol (Solomon *et al.*, 2007). Furthermore, it has been suggested that mannitol has two roles in phytopathogenic fungal-plant interactions. First, it quenches reactive oxygen species (ROS) produced by plants in response to attack; second, the fungus sequesters fructose from host as mannitol, following cleavage of sucrose by host and/or fungal invertase. This latter role depends on the fact that only a small number of plants have the ability to metabolize mannitol and the fungus becomes a carbohydrate sink within the host (Solomon *et al.,* 2006).
Mannitol metabolism in fungi is thought to occur through a mannitol cycle first described in 1978 (Hult and Gatenbeck, 1978). In this cycle, mannitol 1-phosphate 5-dehydrogenase was proposed to reduce fructose 6-phosphate into mannitol 1-phosphate, followed by dephosphorylation by a mannitol 1-phosphatase resulting in inorganic phosphate and mannitol. Mannitol would be converted back to fructose by the enzyme mannitol dehydrogenase. Although mannitol 1-phosphate 5-dehydrogenase was proposed as the major biosynthetic enzyme and mannitol dehydrogenase as a degradative enzyme (Vélëz *et al.*, 2007), both enzymes catalyze their respective reverse reactions.

The ascomycetous fungus *Tuber borchii* accumulates mannitol as endogenous storage pools. In particular, mannitol is present during the active growth of mycelium and it is continually synthesized and metabolized in the hyphae (Ceccaroli *et al.*, 2003). More recently, a novel $NADP^+$-dependent mannitol dehydrogenase and the corresponding gene from *T. borchii* was identified and characterized. The enzyme, called TbMDH, is a homotetramer with two zinc atoms per subunit. It catalyzes both fructose reduction and mannitol oxidation, although it showed the highest substrate specificity and catalytic efficiency for fructose (Ceccaroli *et al*., 2007).
In this work we reported the regulation pattern of MDH in different stress conditions and nutrient supply.

Materials and methods

Growth conditions

Strain MYA-1019 was isolated from fresh *T. borchii* fruitbody growing on *Pinus pinea* L. roots (Zambonelli *et al.,* 1995). The mycelia were grown in the dark at 24 °C, with no agitation, in 70 ml of modified Melin–Norkrans liquid nutrient (MMN) (pH 6.6) (Molina, 1979) containing 10 mM glucose for 30 days. For carbon source shift experiments, the mycelia, precultured for 21 days in MMN liquid medium containing 10 mM glucose, were washed and transferred for 3 days to MMN liquid medium containing 3 mM of one of the following carbon sources: glucose, fructose, mannose or mannitol. For salt stress conditions the mycelia were transferred for 2 days to MMN liquid medium containing 150, 300 or 400 mM NaCl. For temperature stress conditions the mycelia were transferred at 4 or 37 °C.

Quantitative real-time PCR (qRT-PCR)

One microgram of DNase (Ambion)-treated total RNA extracted from *T. borchii* mycelia, was reverse transcribed as described by Guescini *et al*. (2003). *T. borchii* 18S rRNA (tb18S) was used as an internal standard. Specific primers for *Tbmdh* were designed to amplify under the same cycling conditions and procedure reported in Guescini *et al.* (2003), generating products with sizes ranging from 142 to 167 bp, respectively. Each sample was tested in triplicate by quantitative PCR, and samples obtained from at least six independent experiments were used to calculate the means and standard error. The Kruskal-Wallis test was used to compare the DCt medians, and results were considered significant if P values were <0.05.

Phylogenetic analysis

The amino acid deduced sequence of TbMDH was used for extensive database searching for both homolog sequences and sequences that were closely related phylogenetically. Protein sequence data were taken from SWISSPROT and EMBL protein databases and the GenBank non-redundant protein database. Sequence comparisons with the members of the MDR superfamily were performed using the method of Riveros-Rosas *et al.* (2003). A progressive multiple protein sequencing alignment was applied using the CLUSTALX package (Thompson *et al*., 1997). Phylogenetic analyses were performed with the PHYLIP software package, version 3.63 (Felsenstein, 2004) and inferred using Neighbour Joining, Maximum-Parsimony and Maximum-Likelihood methods. Bootstrap analyses were based on 1000 re-samplings of the sequence alignment. The TreeView program was used to plot the tree files (Page, 1996).

Enzyme assay

After growth, the mycelia were washed with distilled water to remove traces of the growth medium and homogenized using a Potter homogenizer with a glass pestle (Steroglass, Italy) in 50 mM MOPS and 5 mM dithiothreitol (DTT), pH 7.5. The suspension obtained was then centrifuged at 20,000g for 15 min to remove broken hyphae and the supernatant was used for the enzymatic assay of all enzymes of mannitol cycle according the methods described in (Ramstedt *et al*., 1987).

Results and Discussion

A mannitol cycle was first proposed by Hult and Gatenbeck (1978) when examining polyketide formation in *Alternaria alternata* (Fig. 1). The cycle comprises four enzymes: mannitol 1-phosphate dehydrogenase (MPD), $NADP^+$-mannitol 2-dehydrogenase (MDH), mannitol

1-phosphate phosphatase (MPP) and hexokinase (HX). These enzymes have been purified from several fungal sources, including ascomycetes, basidiomycetes and fungi imperfecti, and their kinetic parameters have been determined (Solomon *et al.,* 2007) The mannitol cycle pathway branches off from glycolysis at fructose 6-phosphate. Mannitol biosynthesis traditionally occurs the dephosphorylation of mannitol 1-phosphate. The formed mannitol is then consumed through oxidation to fructose, thus completing the cycle (Jennings, 1984).

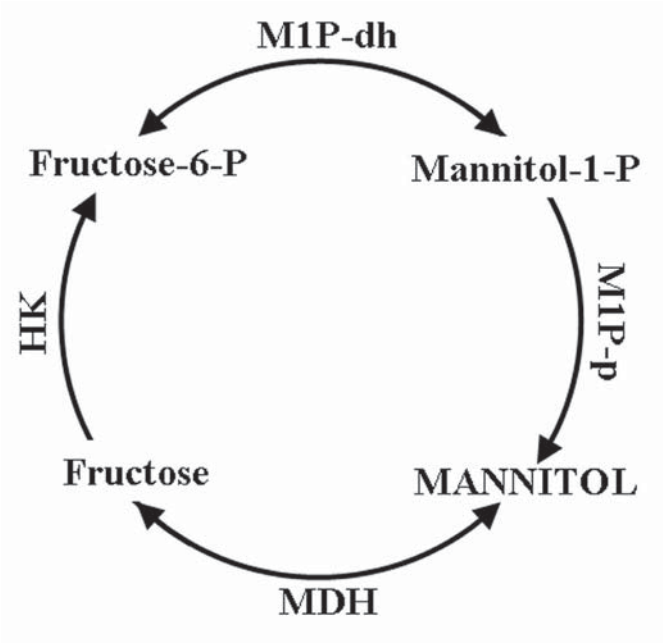

Fig. 1 Proposed mannitol cycle in fungi (Hult and Gatenbeck, 1978).

The advancement of genetic manipulation techniques in filamentous fungi has rapidly accelerated the understanding of the roles and metabolism of mannitol. Recently, Solomon *et al*. (2006) and Vélëz *et al*. (2007) using different mutant strains of *Stanogospora nodorum* and *A. alternata* suggest that the mannitol metabolism does not operate as a cycle. Although Grimaldi *et al.* (2005) report the transient transformation of *T. borchii*, the transformed hyphae are present in low amount. For this reason the cycle has not been yet confirmed in *T. borchii* through genetic analysis, but we showed all the enzymes involved in mannitol cycle using a biochemical approaches (Table 1).

Tab. 1 Enzymes of mannitol cycle in *Tuber borchii*

Enzymes	Specific activity (U/mg of proteins)	
	Mannitol	Glucose
HK (EC 2.7.1.1)	0.73 ± 0.25	0.24 ± 0.06
MPD (NADH-dependent) (EC 1.1.1.138)	0.031 ± 0.0077	0.068 ± 0.0077
MDH (NADP-dependent) (EC1.1.1.17)	1.25 ± 0.34	0.74 ± 0.12
MPP (EC 3.1.3.22)	1.26 ± 0.36	1.11± 0.39

As reported in Table 1 the activities of mannitol dehydrogenase, mannitol-1-phosphate dehydrogenase, and mannitol-1-phosphatase were assessed in mycelium grown either on glucose or mannitol. The specific activities of all these enzymes were determined, thus we can hypothesize that in the vegetative *T. borchii* mycelium the mannitol biosynthesis can occur as a cycle.

Among these enzymes a novel $NADP^+$-dependent mannitol dehydrogenase and the corresponding gene from *T. borchii* was identified and characterized (Ceccaroli *et al.,* 2007). The enzyme, called TbMDH, presents distinctive properties respect to mannitol dehydrogenases from other fungi such *Agaricus bisporus*, *Uromyces fabae*, *Fusarium graminearum* and *S. nodorum* (Hörer *et al*., 2001; Hahn and Mendgen, 1997; Trail and Xu, 2002; Solomon *et al*., 2006). The enzyme is a homotetramer with two zinc atoms per subunit, catalyzed both fructose reduction and mannitol oxidation, although it showed the highest substrate specificity and catalytic efficiency for fructose.

In a commonly used sequence-based classification, the dehydrogenase reductases fall into three main superfamilies that are referred to as short-chain dehydrogenase/reductase (SDRs), medium-chain dehydrogenase/reductases (MDRs) and long-chain dehydrogenase/reductases (LDRs). The fungal mannitol dehydrogenases characterized so far belong to SDR superfamily and these proteins have subunits typically of 250-odd residues and not contain zinc. TbMDH, a protein with 345 residues, represents the first example of a fungal mannitol dehydrogenase belonging to MDR superfamily. Among the MDR superfamily, in which at least eight families can be distinguished (Riveros-Rosas *et al.* 2003), the protein gives rise to a new subfamily inside polyol dehydrogenase (PDH) family. In fact, as shown in Figure 2, all methods of phylogenetic reconstruction (Neighbour Joining, Maximum Parsimony and Maximum Likelihood), based on the sequences selected, have shown the same tree topology and unambiguously placed the TbMDH sequence in a new cluster among the polyol dehydrogenase (PDHs) family of MDRs (COG1063). The TbMDH new branch results closely related to putative zinc-binding dehydrogenases or hypothetical proteins of the Ascomycetes Phylum such as *Aspergillus fumigatus*, *A. nidulans*, *Cryptococcus neoformans*, *Magnaporthe grisea* (Dean *et al*., 2005; Galagan *et al*., 2005; Loftus *et al.,* 2005) described in genome projects and to an arabitol dehydrogenase from *U. fabae* able to use also the D-mannitol as substrate (Link *et al*., 2005). The TbMDH protein is also closely related to previously characterized eubacterial mannitol dehydrogenases (Sasaki *et al*., 2005).

The stability of this new cluster was verified by bootstrap analysis and a confidence level of 96-98% was obtained with all criteria used also after complete exclusion or inclusion of gaps.

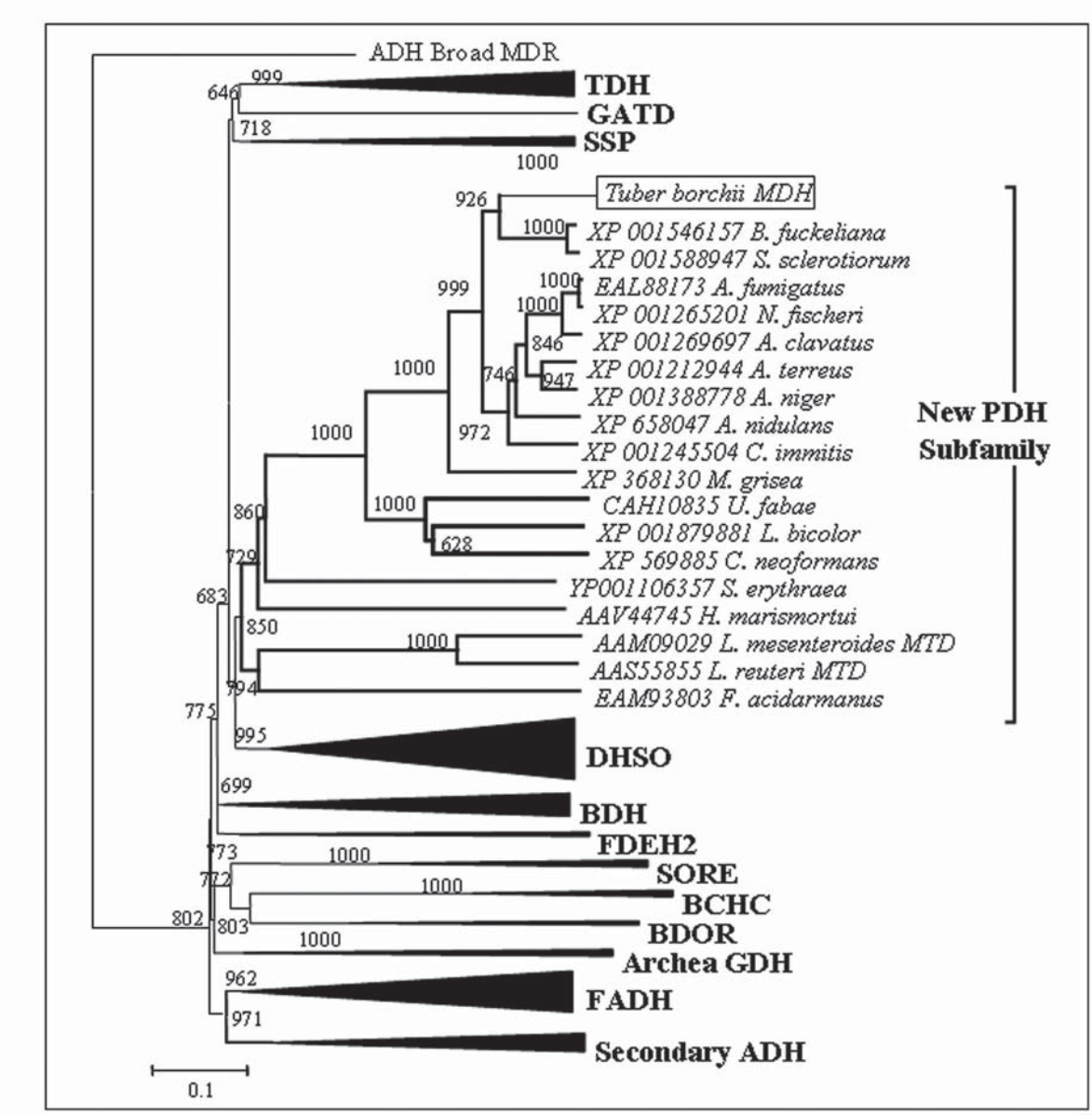

Fig. 2 Neighbour joining phylogenetic analysis of the TbMDH sequence compared with the representative members of the PDH family of MDR (COG1063) and homolog sequences obtained by BLASTP algorithm. The figure is displayed as tree topology to highlight the candidate cluster for new PDH Subfamily. Bootstrap values have been obtained by 1000 nj replicated. Out group is represented by a Broad ADH sequence (AAK41537 MDR- COG 1064 - Macrofamily II) (Riveros-Rosas et al., 2003).

It has been demonstrate that mannitol provides protection against different stressors. In order to understand the role of mannitol in *T. borchii* we evaluated the mannitol dehydrogenase regulation at the molecular level. The quantitative real-time PCR experiments were set up to determine whether TbMDH transcription levels was up or down regulated in salinity stress, low temperature condition and in different nutrient.

As first approach, immunoblot experiments were performed to evaluate TbMDH regulation in mycelia transferred to fresh media containing glucose, fructose, mannose or mannitol, respectively. As shown in Figure 3A, *Tbmdh* gene responds positively to mannitol and fructose, whereas gene expression remains at the basal levels in mycelia shifted in glucose or mannose. The response to mannitol is accompanied by parallel variation of TbMDH protein (Fig. 3B) and enzyme activity (Fig. 3C) levels indicating that TbMDH overexpression under mannitol growth condition takes place mainly at the transcriptional level. This upregulation of TbMDH in

presence of mannitol could allow large amounts of mannitol to be utilized in central metabolism. In fact, in a previous study the ^{12}C chase experiments showed that mannitol, in addition to being a storage compound, is also metabolically active and used to feed the Krebs cycle and the amino acid biosynthesis (Ceccaroli *et al.,* 2003).

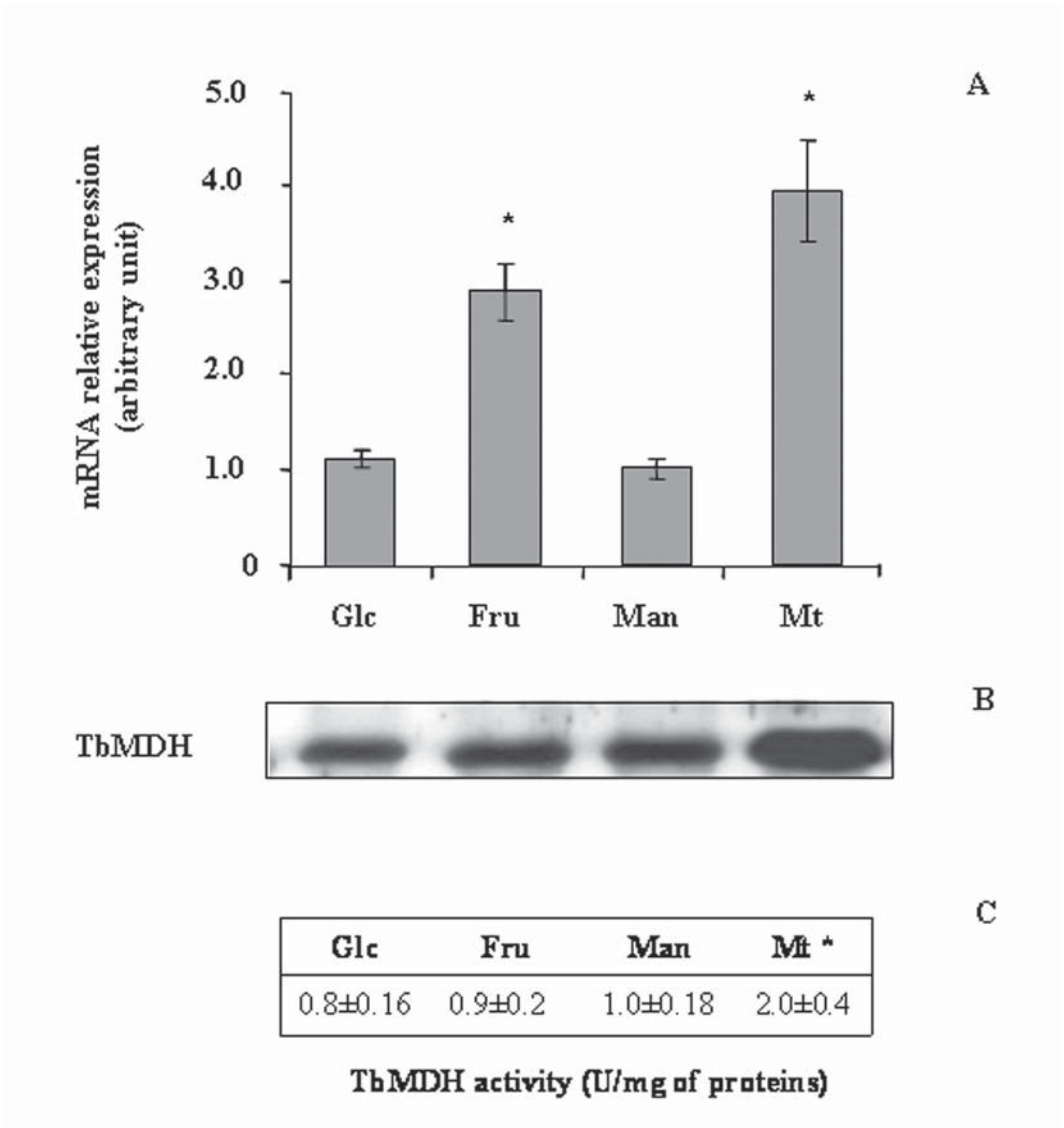

Fig. 3 TbMDH modulation in different sugar nutritional regimens. (A) *Tbmdh* mRNA expression level, (B) TbMDH Western blot analysis and (C) TbMDH activity. The immunoblot experiments were performed loading 20 µg of crude extract per line. *Tbmdh* expression levels were normalised against 18S rRNA levels. Glc, glucose; Fru, fructose, Man, mannose; Mt, mannitol. Values are the means ±SE of six independent determinations. *P<0.05, non-parametric Kruskal-Wallis test.

In osmotic stress conditions, the expression data showed that *Tbmdh* messenger decreased following transfer on NaCl medium for 24 h and such down-regulation is still evident in a NaCl treatment prolonged to 48 h. Figure 4 showed the *Tbmdh* messenger levels on mycelia transferred in 0.3 M and 0.4 M NaCl concentrations respect to the mycelia grown in a control liquid media at 48 h of treatment. As shown in the figure the most down-regulation was detected at 0.4M NaCl concentration.

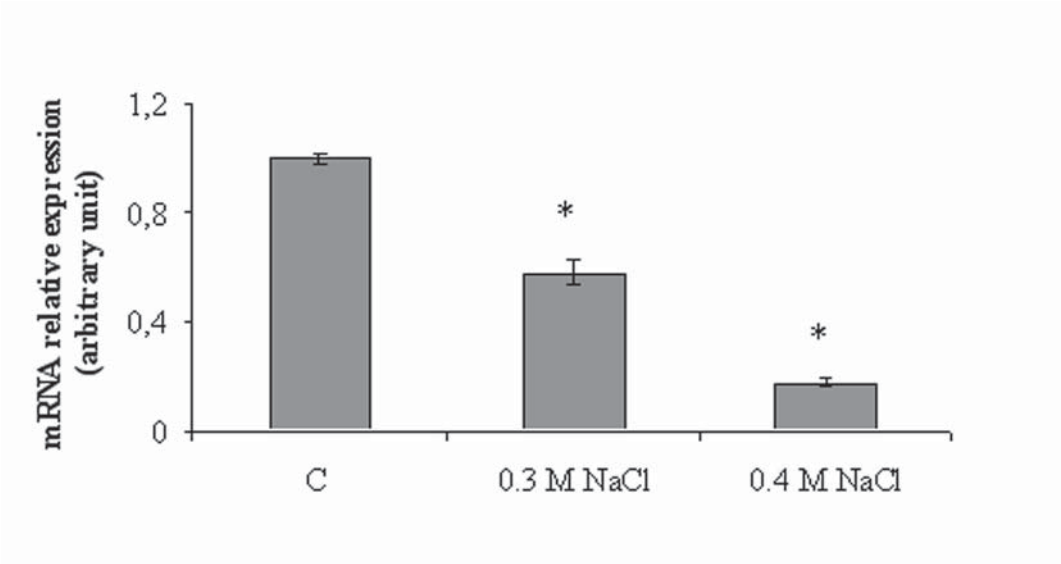

Fig. 4 Effect of NaCl on the *Tbmdh* expression level.

This result suggests that salt repression of MDH could determine a drastic decrease in mannitol use and this type of regulation could allow stressed mycelia to accumulate mannitol as an osmoprotectant.
The same down-regulation response is also evident in temperature stressed mycelia. In fact, the mycelium transferred at 4°C for 72 h showed the *Tbmdh* messenger transcript four fold lower than the control growth at 24°C whereas the *Tbmdh* messenger transcript remained almost constant in the mycelia shifted at 37°C (Fig. 5).

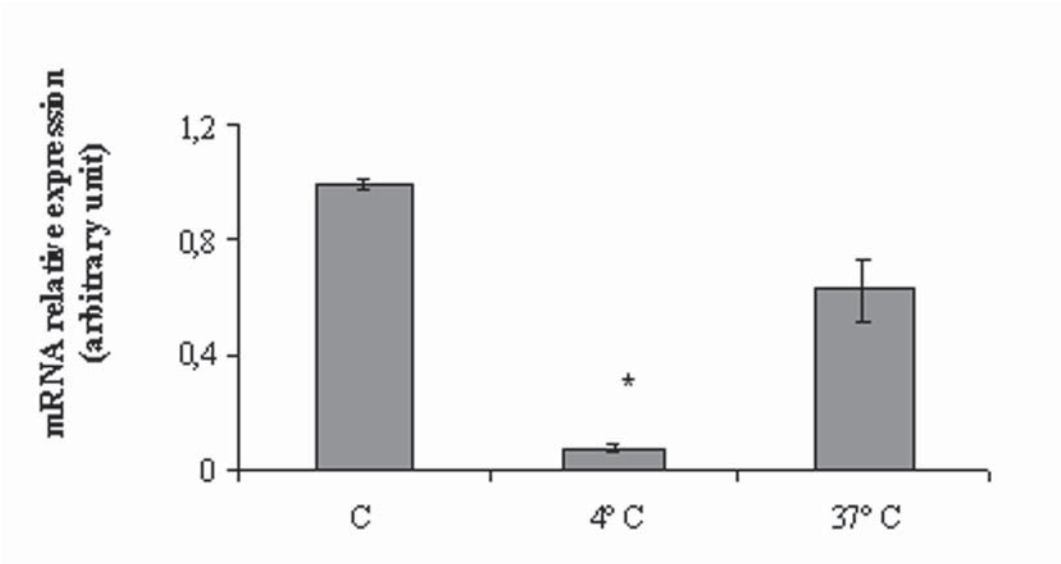

Fig. 5 Effect of temperature on the *Tbmdh* expression level

This response could be of particular importance for growth and survival of vegetative mycelium in environments during the autumn/winter season when the external temperature is very low. In fact, in the literature is reported that mannitol provides protection against heat, cold and drought (Dijksterhuis and De Vries, 2006).
These findings show that in osmotic and temperature stress conditions the down-regulation of MDH suggest that mannitol can act as osmoprotectant. The characterization of this new protein and its regulation deepens our fundamental understanding of *Tuber* biology and enhances our knowledge of fungi which have not been thoroughly investigated.

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HYPOGEOUS FUNGI FROM SLOVENIA

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Abstract
The tradition of black truffles in Slovenia exists for a longer time. It is undoubtedly documented since 18th century when J.A. Scopoli in his 2nd edition of Flora Carniolica (Vienna, 1762) under the name *Lycoperdon gulosorum* described a truffle with black interior and dark brown surface, being excavated by patiently trained dogs. The first attempt to look at hypogeous fungi from Slovenia was made only recently. It gives the insight into the locally poorly known world of hypogeous fungi and widens the knowledge about it. Special emphasis was put on truffles (*Tuber*), among them 16 different taxa from Slovenia. These are: *Tuber aestivum* incl. *T. uncinatum*, *T. borchii, T. brumale, T. brumale* f*. moschatum, T. hiemalbum, T. excavatum, T. excavatum* var. *sulphureum, T. fulgens, T. macrosporum, T. magnatum, T. melanosporum, T. mesentericum, T. nitidum, Tuber puberulum, T. rufum and T. rufum* sensu lato. The group of *Tuber rufum* appears to be more diverse macroscopically and ecologically as under the microscope. This group should therefore need further attention and critical evaluation.
Key words: truffles, taxonomy, tradition, Slovenia.

Introduction

Hypogeous fungi with their subterranean way of life differ significantly from their epigeous relatives. Hidden to usual mushroom collector they could be seen only occasionally or by chance. To attract animals for vectors for spore dispersal, many of them developed most incredible scents that can hardly be compared to anything alike. These scents attract humans as well and are, together with their famous reputation, responsible for incredible, ever growing popularity of truffles. They do represent a fungal world of its own and do really astonish every newcomer having the occasion to watch and see the real truffle hunt for the first time. Even more it is exiting to see your own dog, patiently trained for months, digging the first truffle in nature. The price of most valuable commercial species of truffles exceeds any other food or vegetables. Truffles are important source of income in rural communities especially in demographically endangered areas. Their cultivation could become important factor for the future sustainable development. But before all, basic knowledge of the diversity of hypogeous fungi including truffles and their distribution as well as potential habitats should be studied.
The article is the result of the work of the first author, being to some extent familiar with the taxonomy of higher (epigeous) fungi and the second author, an experienced truffle hunter and keen observer of nature.

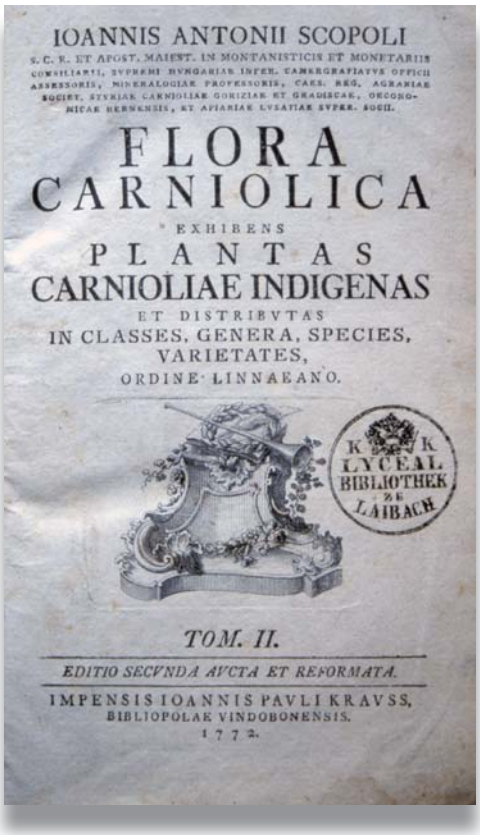


Fig. 1 Front page of Flora Carniolica (J.A.Scopoli, 1762)

Materials and methods

Most samples were collected with the help of trained Labrador retrievers. For local conditions, especially for prospecting dense spiny bushes of abandoned arable land and slopes of rough terrain Labradors seem to make it better as any other dogs breed. They can relatively easily be trained as truffle hunt is entered through retrieving game and this training brings higher motivation as training through force and obedience only.

It should be noted that some truffle hunters train their dogs for digging commercial truffle species only forcing them to reject anything else. By training for the inventory of diversity dogs should be encouraged to widen their search in a manner to get a reward and be praised for every single find. Collector should pay attention to minor reactions by dogs and check every sign mark by careful digging. Truffle hunt relies on the dog's nose and hunter's patience. There is no alternative as to believe the dog´ s nose. Even then some hypogeous species go undiscovered by dogs as is the case of *Elaphomyces granulatus* for example.

The majority of studied samples was gathered in Slovenia, some of them also in neighbouring countries. Some material was obtained from imported Chinese truffles from Far East without known provenance. Samples were determined according to morphological method. Material for microscopic examination of *Ascomycota* was stained in Methyl blue (C.I. 42780 Merck) in lactophenol and observed in aqueous solution of chloralhydrat, for *Basidiomycota* Cyanosine C.I. 45410 in tap water was used (Erb & Matheis, 1983). Microscopic examination was done using binocular optical microscope MOTIC B1-223A with oil immersion at the magnification of 1000X.

The monograph Truffes d'Europe et de Chine (Riousset *et al.,* 2001) was used for the genus *Tuber* and Funghi Ipogei d'Europa (Montecchi et Sarasini, 2000) for other genera incl. *Basidiomycota*. For some ripe samples, where only mature spores without tissue structures could be observed, the Key to Spores of the Genera of Hypogeous Fungi of North Temperate Forests (Castellano *et al*., 1989) was also consulted. All analysed samples were gently dried and preserved for further study and sent for further molecular analyses to the Institute of forestry in Ljubljana.

Results

From the material gathered 35 different taxa could be determined, 6 of them belonging to *Basidiomycota* and the rest to *Ascomycota*. The genus *Tuber* was represented with 22 different taxa. *Tuber uncinatum* was included with *Tuber aestivum* as one taxon. Imported material is also listed. Although spore dimensions, shape and ornamentation are the most important taxonomical features for truffle determination, it was not always possible to come to conclusive results.

The group of *Tuber rufum* was found to have high ecological range and high macroscopic diversity but no conclusive microscopic differences.

Some rare taxa as *Picoa lefebvrei* and *Tuber hiemalbum* deserve to be confirmed in further study yet.

List of fungi

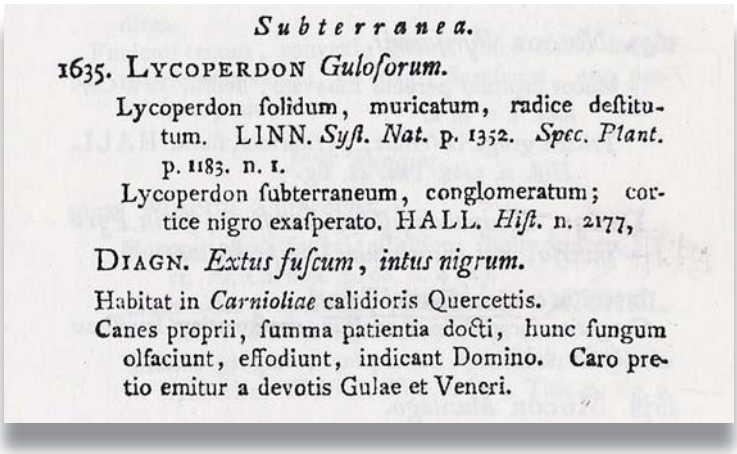
Taxa are listed in the following text. Provenance is stated separately only for taxa of non Slovenian origine. Those taxa, which are marked with asterisk, are first finds for Slovenia (Jurc *et al*., 2005)

Balsamia polysperma Vittadini 1831 *
Balsamia vulgaris Vittadini 1831
Choiromyces meandriformis Vittadini 1831
Elaphomyces granulatus Fries 1821
Gautieria morchelliformis Vittadini 1831
Genea verrucosa Vittadini 1831 *
Hysterangium stoloniferum Tulasne & C. Tulasne 1843 *
Melanogaster broomeianus Berkeley apud Tulasne & C. Tulasne 1843
Melanogaster variegatus (Vittadini) Tulasne & C. Tulasne 1843
Octavianina asterosperma (Vittadini) Kuntze 1898 *
Pachyphloeus sp.
Picoa lefebvrei (Patouillard) Maire 1906 *
Tuber aestivum Chatin 1887 incl. ***T. uncinatum*** Chatin 1887
Tuber borchii Vittadini 1831
Tuber brumale* f. *moschatum (Ferry) Montecchi & Lazzari 1993
Tuber brumale Vittadini 1831
Tuber excavatum var. sulphureum G. & L. Riousset 1998
Tuber excavatum Vittadini 1831*
Tuber fulgens Quelét 1879 *
Tuber hiemalbum Chatin 1869 *
Tuber himalayense B.C. Zhang & Minter 1988 Imported
Tuber indicum Cooke & Massee 1892 Imported
Tuber macrosporum Vittadini 1831 *
Tuber maculatum Vittadini 1831 Serbia
Tuber magnatum Pico 1788
Tuber melanosporum Vittadini 1831
Tuber mesentericum Vittadini 1831
Tuber nitidum Vittadini 1831 * 1 SLO
Tuber oligospermum (Tulasne & Tulasne) Trappe 1979 Imported
Tuber pseudohimalayense G. Moreno et al. 1997 Imported
Tuber puberulum Berk. & Broome (1846) *
Tuber rufum Pico ex Fries 1823
Tuber rufum sensu lato
Tuber sp.
Zelleromyces sp. Serbia

Tradition of truffles in Slovenia

The tradition of truffle hunt and truffle use in Slovenia exists. The best evidence for that can be found in the second edition of Flora Carniolica (1772), written by famous18th century botanist, naturalist and medicine J.A. Scopoli (1723-1788). During his appointment as a medicine doctor in mercury mine in Idria (1754-1769) he investigated a big part of Slovenia, at that time belonging to Austrian empire. On page 491 of Flora Carniolica, under the name of *Lycoperdon gulosorum,* we can find a vivid description of a black truffle. There we can recognise a truffle with black interior (*T. melanosporum, T. brumale* or *T. macrosporum*), being collected in SW part of Slovenia: … "dig by dogs, trained with utmost patience, and sold for big money to those, devoted to good food and love" ….

So, nothing new from today´ s perspective!



Subterranea.

1635. LYCOPERDON *Guloſorum.*

Lycoperdon ſolidum, muricatum, radice deſtitutum. LINN. *Syſt. Nat.* p. 1352. *Spec. Plant.* p. 1183. n. 1.

Lycoperdon ſubterraneum, conglomeratum; cortice nigro exaſperato. HALL. *Hiſt.* n. 2177,

DIAGN. *Extus fuſcum, intus nigrum.*

Habitat in *Carnioliae* calidioris Quercettis.

Canes proprii, ſumma patientia docti, hunc fungum olfaciunt, effodiunt, indicant Domino. Caro pretio emitur a devotis Gulae et Veneri.

Fig. 2 J.A. Scopoli description of black truffles in Flora Carniolica

Modest knowledge of hypogeous fungi

At the other side, however, Slovenia has long been a white spot on the European truffle distribution map. The knowledge about hypogeous fungi was scarce until recently and it was the cooperation of mycologists and truffle hunters needed to make a breakthrough. All small tuber like "odds", measuring sometimes only a fraction of a cm, instead of being thrown away, when already collected, were preserved and studied.

Fig. 3 Second author demonstrating a truffle hunt

Fig. 4 Truffle hunter dreams, two white truffles (*Tuber magnatum*), twinns, growing closely together. Both weighted over 1400 g

About the truffle legislation in Slovenia

Truffles (in natural habitats) in Slovenia are protected by law. They are not allowed to be picked without special permission. There were more proposals to change this legislation as it is inappropriate. It does not encourage the development of trufficulture in broader sense nor does it protect natural truffle grounds from devastating digging. New legislative in preparation will hopefully change this.

To conclude

Present inventory has widened the knowledge of hypogeous fungi in Slovenia. It has shown, that:

- Slovenia is not a blank spot on the European truffle distribution map
- Truffles are neither rare nor endangered
- practically all important commercial *Tuber* species can be found in Slovenia
- formal status of truffles being protected by law as rare and endangered fungal species should be and reconsidered
- further inventory is needed to evaluate the species distribution and to get the answers to some unresolved taxonomical problems.

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SCOPOLI J.A., 1772 – *Flora Carniolica*, Viennae, 496 p.

ACTIVITE ANTIBACTERIENNE ET CARACTERISATION CHIMIQUE DE DIFFERENTS EXTRAITS DE *TERFEZIA BOUDIERI*

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Abstract: Chemical characterization and antibacterial activity of some *Terfezia boudieri* Chatin extracts.
In traditional medicine, the aqueous extracts of desert truffles are used to treat some ocular infections such as blepharits and conjunctivitis. This property has prompted us to extract natural substances from carpophores of *Terfezia boudieri*, and to characterize some chemical families from these extracts and finally to test, *in vitro*, their inhibitory activities on reference bacterial strains involved in ocular infections. Extracts were made from fresh and freeze-dried fruitbodies. The preliminary characterization tests of a few chemical families on several extracts showed the presence of tannins, saponosids, free quinons, amino acids, anthraquinons and phenolic compounds. The spectrum of activities shows a good antibacterial activity of aqueous and methanolic extracts against most studied strains (*Staphylococcus aureus, Salmonella arizonae, Pseudomonas aeruginosa*, *Escherichia coli, Enterococcus faecalis)*. This activity may be due to the presence of major compounds identified in the aqueous extracts especially tannins, heterosids, phenolic compounds and saponosids, but no formal conclusion can be advanced except that dealing with the responsible agent for this activity which is a polar compound.
Key words: Valorisation, desert truffles, chemical characterization, antibacterial activity.

Résumé
En médecine traditionnelle, les extraits aqueux des truffes du désert sont utilisés pour traiter certaines infections oculaires comme la blépharite et la conjonctivite. Cette propriété nous a incité à extraire les substances naturelles à partir des carpophores de *Terfezia boudieri*., et de caractériser certaines familles chimiques présentes dans ces extraits et enfin de tester, *in vitro*, leurs activités inhibitrices sur des souches bactériennes de référence qui peuvent être à l'origine de certaines infections oculaires.
Les extractions ont été effectuées à partir de carpophores frais et lyophilisés. La caractérisation de quelques familles chimiques présentes dans les différents extraits montre la présence de tanins, de saponosides, de quinones libres, d'anthraquinones et de dérivés phénoliques. Les spectres d'activités antibactériennes montrent une bonne activité des extraits aqueux et méthanoliques contre la majorité des souches étudiées (*Staphylococcus aureus*, *Salmonella arizonae*, *Pseudomonas aeruginosa*, *Escherichia coli* et *Enterococcus faecalis)*. Cette activité peut être due à la présence des composés majoritaires identifiés dans les extraits aqueux en particulier les tanins, les hétérosides, les dérivés phénoliques et les saponosides, mais aucune conclusion formelle ne peut être avancée sauf que l'agent responsable de cette activité est un composé polaire.
Mots clefs: Truffes du désert, caractérisation chimique, activité antibactérienne.

Introduction

En Afrique du nord et dans toute la zone semi désertique et désertique du bassin méditerranéen, les champignons symbiotiques, les truffes du désert ou terfess, sont consommées depuis des millénaires et présentent sans doute une grande importance économique. Ces truffes également appelées ''Kameh'' ou ''Kamé'' désignent les carpophores de certains ascomycètes symbiotiques avec frutification hypogées de l'ordre des Pezizales et la famille de Pezizaceae. Les truffes du désert les plus connues appartiennent aux genres *Picoa*, *Balsamia*, *Tuber*,

Tirmania et *Terfezia* et vivent en association symbiotique mycorhizienne avec des espèces annuelles et pérennes du genre *Cistus* et *Helianthemum* de la famille des Cistacés. Le rôle bénéfique de ces champignons auxiliaires a surtout été mis en évidence ces dernières décennies et a conduit à un foisonnement d'études biologiques, écologiques, morphologiques et moléculaires des terfess ainsi que des plantes hôtes. Les études qui visent à rechercher des substances naturelles issues des ces organismes et qui peuvent être efficaces contre certains agents pathogènes pour l'homme sont rares, bien que plusieurs travaux dans ce domaine ont été réalisés sur plusieurs familles de champignons. Le présent travail porte sur l'étude chimique et les activités antibactériennes des extraits d'une truffe du désert de la Tunisie méridionale, il s'agit de *Terfezia boudieri*.

Matériels et méthodes

Matériel biologique

Les échantillons de *Terfezia boudieri* Chatin ont été prélevés à partir de la région de Bougrara située dans l'étage aride inférieur avec une pluviométrie annuelle moyenne de l'ordre de 180 mm et une température moyenne de 19.9°C. La température minimale moyenne du mois le plus froid est de 7°C, et la température maximale moyenne du mois les plus chaud est de 50°C. Les échantillons ont été caractérisées par détermination des trais morphologiques propres à cette espèces (couleur du péridium, ornementation des spores…).

Extraction des substances naturelles à partir des ascocarpes

Différents types d'extraits ont été préparés à partir du matériel fongique frais et de la poudre des carpophores lyophilisés.

a- Décoction. Pour préparer une décoction aqueuse à 10%, 10 g du matériel fongique frais ont été mis dans 100ml d'eau distillée et portés à ébullition pendant 1 heure. Le décocté refroidi a été filtré et concentré au Rotavapor, puis lyophilisé.

b- Macération. Une macération aqueuse a également été effectuée sur 10 g de matériel frais avec 100 ml d'eau pendant 24 heures. Après filtration, l'extrait est concentré au Rotavapor et lyophilisé.

c- Digestion à 50°C. 10 g de matériel fongique frais ont été mis dans 100 ml d'eau distillée et portés à 50°C pendant 3 heures. Après filtration, le filtrat a été concentré sous vide à 50°C au Rotavapor et lyophilisé.

Les extraits lyophilisés ont été conservés dans des dessiccateurs.

d- Extraction par des solvants organiques à polarité croissante

L'extraction par les solvants organiques se fait selon deux modalités:

Extraction à froid. 10 g de poudre des carpophores ont été extraits avec 3 x 100 ml d'éther de pétrole et placés sous agitation pendant 3 x 24 heures. Après filtration sur papier wattman, le marc est mis en agitation avec 100 ml de dichlorométhane pendant 3 x 24 heures, puis 100 ml d'acétate d'éthyle et enfin 100 ml de méthanol. Les solutions ont été filtrées sous vide à l'aide de filtres wattman, et les extraits sont concentrés sous vide à 40°C au Rotavapor. Après concentration, les extraits d'éther de pétrole, de dichlorométhane et d'acétate d'éthyle sont laissés à l'air libre pour permettre l'évaporation de tout le solvant. A la fin de l'extraction par les solvants organiques, le marc a été séché pendant 24 heure, puis porté à 50°C puis 100°C avec 100 ml d'eau distillée, pendant trois heures à chaque fois, après quoi, l'extrait à été filtré. Les extraits méthanoliques et aqueux ont été ensuite lyophilisés.

Extraction par l'appareil du soxhlet (extraction à chaud). L'extracteur de soxhlet est un appareil spécialement conçu pour l'extraction continue solide-liquide. Le solvant d'extraction est porté à ébullition. Quatre extraits ont été préparés à partir de 20 g de poudres des ascocarpes des terfess. Ces extraits sont obtenus grâce à des épuisements successifs par l'appareil à l'aide des solvants de polarités croissante: l'éther de pétrole, le dichlorométhane, l'acétate d'éthyle puis le méthanol.

Après extraction par les solvants organiques, le résidu est additionné de 200 ml d'eau distillée et porté à ébullition pendant 3 h, puis filtré et lyophilisé.

Caractérisation des principaux constituants chimiques des extraits

Les principaux constituants chimiques recherchés ont été choisis parmi ceux qui ont le plus de chance de posséder des propriétés thérapeutiques. Ils ont été caractérisés dans les extraits par des réactions colorées. Les réactifs de caractérisation classiques ont permis de tester la présence des groupes chimiques suivants: les alcaloïdes (réactifs de Dragendorff (tétraiodobismuthate de potassium) et de Mayer), les Cardénolides (réaction de Keller-Kiliani), les flavonoîdes (réaction de cyanidine), les dérivés phénoliques ($FeCl_3$ à 5%), les quinones libres (NaOH à 10%), les saponosides (indice de mousse), les anthraquinones (KOH à 10%), les tanins (chlorures ferriques), les acides aminés (réaction de ninhydrine), les iridoides (l'acide chlorhydrique concentré) et les stérols (réaction de Liebermann Buchard).

Etude de l'activité antibactérienne

Choix des bactéries. Les souches bactériennes qui ont été retenues pour ce travail sont celles qui ont tendance à causer des infections oculaires. Il s'agit notamment de *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* CIP 106510, *Pseudomonas aeruginosa* LMBA, *Salmonella arizonae* ATCC 25922, *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* DH5α. Ces souches ont été fournies par le Laboratoire des Microorganismes et des Biomolécules Actives de la Faculté des Sciences de Tunis.

Préparation des souches bactériennes et des extraits. Les extraits aqueux et méthanoliques (polaires) sont dilués dans l'eau pure alors que les extraits organiques sont dissous dans une solution de DMSO (diméthylsulfoxyde) dont l'activité antibactérienne ne se manifeste plus lorsqu'il est dilué de 1/50 (German-Fattal, 1987). Tous les extraits ont subit une désinfection par filtration.

Au cours de ces essais, toutes les souches bactériennes ont été préparées de façon à avoir un inoculum standard de 5.10^6 bactéries/ml où les germes sont en phase exponentielle de croissance.

Etude de l'activité antibactérienne des extraits: méthode de diffusion à partir des puits (aromatogramme). L'aromatogramme est basé sur une technique utilisée en bactériologie médicale, appelée antibiogramme ou méthode par diffusion en milieu gélosé ou encore méthode des puits; il s'agit d'une méthode en milieu gélosé réalisée dans des boîtes de Pétri décrite par Tagg et Mc Given, (1971) modifiée par Cherif et *al.*, (2001). Le contact se fait par l'intermédiaire des puits contenant les différents produits à tester. Les puits sont creusés dans la gélose uniformément ensemencée avec une suspension de la bactérie à étudier. Chaque produit diffuse à partir du puit au sein de la gélose et y détermine un gradient de concentration. Les souches microbiennes sont cultivées jusqu'à la phase stationnaire de croissance (12 à 18 h, à 37°C) en bouillon nutritif. Un inoculum bactérien titrant environ 104 bactéries / ml est préparé en diluant la culture. 50 µl de cet inoculum sont ajoutés à 5 ml de gélose molle. Le mélange est étalé dans des boites de Pétri contenant de gélose Muller Hinton solidifié de façon à recouvrir presque entièrement la surface gélosée (ensemencement en nappe). Des mouvements de rotation imprimés par la main accélèrent le recouvrement. Ces boites sont incubées à 37°C pendant une demi-heure pour la polymérisation de la gélose mole et la formation d'un tapis bactérien. Des puits de 6 mm de diamètre sont creusés dans la gélose. Chaque puit est rempli par 20 µl d'une dilution d'extrait. Au bout de 24 heures d'incubation à 37°C, on observe l'effet sur la croissance bactérienne autour des puits. La boîte de contrôle négatif, réalisée pour chaque expérience, est une boite ensemencée dont les puits déposés au centre de la gélose ne sont pas imbibés d'extrait à tester. Le témoin est une boite de Pétri ensemencée dans les conditions de l'expérience, sans puits. Elle nous renseigne sur l'homogénéité du tapis bactérien.

Résultats et discussion

Rendement des extraits préparés. Les extraits préparés des carpophores de terfess ont été caractérisés par leurs couleurs et leurs rendements par rapport à la drogue sèche. Ces descripteurs sont présentés dans le tableau 1.

Tab. 1 Aspect, couleur et rendements des extraits des carpophores de *T. boudieri*

Extraits		**Couleur- aspect**	**Rendement (%)***
Décocté		Blanc (poudre)	0,15
Macéra		Jaune (poudre)	0,22
Digésté à 50°C		Blanc (poudre)	0,19
Extraits aux solvants organiques (à froid)	Ether de pétrole	Jaune (pâteux)	0,02
	Dichlorométhane	Marron (pâteux)	0,01
	Acétate d'éthyle	Jaune (pâteux)	0,01
	Méthanol	Marron foncé (pâteux)	0,1
Extraits aux solvants organiques (à chaud)	Ether de pétrole	Jaune (pâteux)	0,1
	Dichlorométhane	Rouge foncé (pâteux)	0,07
	Acétate d'éthyle	Rouge brique (pâteux)	0,03
	Méthanol	Marron foncé (pâteux)	0,15
* en poids par rapport à la matière sèche			

D'après les résultats consignés dans ce tableau, on constate que chaque extrait se caractérise par une couleur et un rendement (par rapport à la matière sèche) appropriés. Les fractions extraites avec l'eau (les extraits aqueux: décoction, digestion et macération) présentent les rendements les plus élevés avec une valeur de 0,1% et une coloration blanche. Ces rendements restent toujours très faibles comparativement à ceux des végétaux supérieures (Bouquet et Paris, 1967; Dialo *et al*., 2004).

Pour les extraits obtenus par des solvants organiques, le rendement le plus élevé est celui du méthanol (extrait le plus polaire) avec une valeur de 0,15. Les rendements les plus faibles sont ceux des solvants apolaires ou peu polaires (l'acétate d'éthyle, l'éther de pétrole et le dichlorométhane) (tableau 1). Tous les extraits organiques présentent une couleur allant du jaune au marron.

Caractérisation des principaux constituants chimiques des extraits

Réactions de caractérisation des différents extraits.

Les résultats relatifs aux études chimiques effectuées sur les extraits des carpophores de terfess permettent de mettre en évidence plusieurs familles chimiques qui sont consignées dans les tableaux 2 et 3.

Tab. 2 Résultats de la caractérisation des groupes chimiques dans les extraits de carpophores de *T. boudieri*

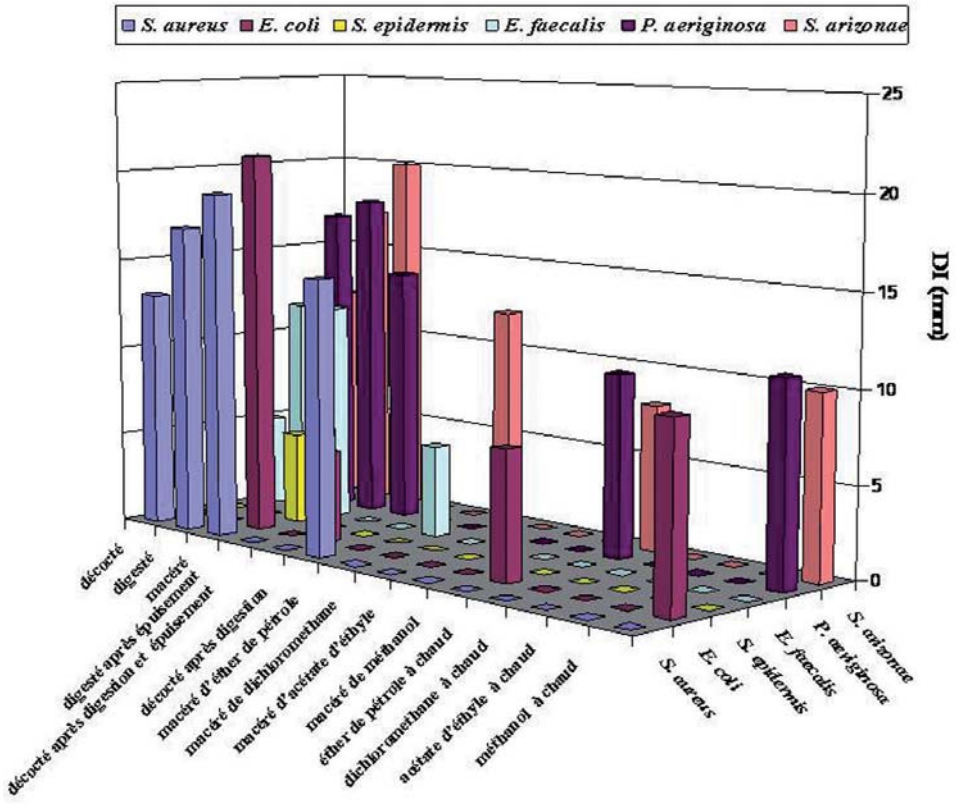
Familles chimiques	**Réactif de caractérisation**	**Types des extraits**	**Caractérisations**
Quinones libres	NaOH	Extrait d'éther de pétrole	Couleur jaune de la phase aqueuse: présence de quinones libres
flavonoïdes	HCl	Extrait de méthanol	Test négatif
tanins	HCl	Extrait de méthanol	Changement de couleur
		Digestion après épuisement par les solvants organiques	Une coloration rouge brique: présence des tanins catéchiques
		Décocté	Une coloration rouge brique: présence des tanins catéchiques
	$FeCl_3$ à 1%		Test négatif
stérols	Réactif de liebermann-Burchard	Ether de pétrole	Test négatif
alcaloïdes	Réactif de Dragendorff	Extraits méthanol	Test négatif
	Réactif du Mayer	Extraits méthanol	Test négatif
iridoides	HCl	Décocté	Test négatif
anthraquinones	KOH	Extrait dichlorométhane	Couleur rouge de la phase aqueuse: existence des anthraquinones
Acides aminés	ninhydrine	Décoction	Couleur rouge brique: présence d'acides aminés
		Macération méthanol	coloration violette avec précipité: présence d'acides aminés
		Macération d'éther de pétrole	Test négatif
		Dichlorométhane (soxhlet)	Couleur jaune: test négatif
Les dérivés phénoliques	$FeCl_3$ à 5%	Décocté	Une coloration verdâtre montrant la présence des dérivés phénoliques
Hétéroside cardiotonique	Sulfate ferrique et H_2SO_4 à 5%	Méthanol (soxhlet)	Une coloration rouge fluorescent: présence de digitoxigénine

Tab. 3 Détermination de l'indice de mousse des extraits aqueux (décoctés) de *T. boudieri*

Type d'extrait	Indice de mousse
Décocté	500
Décoction après épuisement par les solvants organique	450

Les essais de caractérisation des groupes chimiques ont révélé la présence de tanins, d'anthraquinones, d'acides aminés, des dérivés phénoliques et des quinones libres dans les différents extraits. Les tests de recherche des flavonoïdes, des alcaloïdes, des stérols et des iridoides ont été négatifs pour tous les extraits étudiés.
Les indices de mousse déterminés dans les décoctés après épuisement par les solvants organiques sont toujours inférieurs à ceux des décoctés après élimination d'une partie de saponosides lors des extractions successives des substances naturelles par ces solvants (tableau 3).

Activités antibactériennes des différents extraits.
L'activité antimicrobienne des extraits a été déterminée par la méthode des puits contre des cellules microbiennes en croissance (tableau 3). Cette méthode a permis de mettre en évidence l'activité de plusieurs extraits contre les souches testées. Les bactéries les plus sensibles vis à vis de la plupart des extraits sont les souches *Staphylococcus aureus* et *Salmonella arizonae* alors que *Staphylococcus epidermis* s'est avérée la plus résistante vis à vis de ces mêmes extraits. Il serait très probable que les extraits testés exercent leur activité en agissant sur les parois cellulaires. Les extraits polaires des carpophores entre autre le digésté, le décocté et le macéré présentent le spectre d'activité le plus large essentiellement contre les souches *Staphylococcus aureus*, *Escherichia coli*, *Salmonella arizonae*, *Enterococcus faecalis* et *Pseudomonas aeruginosa* avec des diamètres d'inhibition allant de 12 jusqu'à 20 mm pour une concentration de 125 mg/ml (figure 1).



Ces résultats nous ont paru assez intéressants dans la mesure où ces souches provoquent des problèmes préoccupants en raison de leur résistance aux antibiotiques et la gravité des affections tant oculaires que générales (Richards et *al*., 2000; Grare et *al*., 2006).
En ce qui concerne les extraits organiques des carpophores, il s'est avéré que seuls les extraits méthanoliques se sont montrés actifs vis à vis de *Pseudomonas aeruginosa*, *Escherichia coli* et *Salmonella arizonae* avec des diamètres d'inhibition de 10 à 11 mm. Les autres extraits organiques sont inactifs puisqu'aucune inhibition n'est observée vis à vis de l'ensemble des souches testées. Ces résultats sont en accord avec ceux d'Al Marzooky (1981), de Rougieux (1963) et de Chellal et Lukasova (1995). Une étude approfondie de l'activité anti-microbienne des extraits aqueux et methanoliques de *Terfezia claveryi*, exploitant notamment des méthodes quantitatives de mesure d'activité, conduite en 2005 par Janakat *et al*. contre la souche bactérienne *Pseudomonas aeruginosa* a montré que seul l'extrait aqueux peut inhiber la croissance bactérienne de 40.9%.
La forte activité des extraits polaires vis à vis de la plupart des bactéries testées, peut être expliqué par le fait qu'ils sont plus riches en composés actifs tel que les tanins et les dérivés phénoliques (Bruneton, 1999), ou bien en hydrates de carbone et en acides aminés (Janakat et *al*., 2005).
Il y a lieu toutefois de mentionner que l'activité antibactérienne de ces extraits peut être influencée par plusieurs facteurs tels que le stade de maturation des échantillons analysés et les techniques d'extraction utilisées.

Conclusion

Les essais de caractérisation de quelques familles chimiques effectués pour la première fois sur les différents extraits de *Terfezia boudieri* par des réactions colorées montrent la présence de tanins, de saponosides, de quinones libres, d'acides aminés, d'anthraquinones et de dérivés phénoliques ayant diverses activités biologiques. D'autres familles sont absentes telles que les alcaloïdes, les stérols, les flavonoïdes et les iridoides. L'analyse quantitative des extraits des carpophores montre que les extraits polaires (extraits aqueux et méthanoliques) présentent les rendements les plus élevés comparativement aux extraits apolaires ou peu polaires. Il sera utile ultérieurement de chercher d'autres familles chimiques susceptibles d'être présentes dans les extraits de terfess, et d'effectuer une analyse plus fine des familles présentes. Le spectre d'activité antimicrobienne de ces extraits montre une bonne activité des extraits aqueux et méthanoliques vis-à-vis de la majorité des souches étudiées ce qui explique l'utilisation de terfess en médicine traditionnelle comme remède oculaire. Cette activité peut être due à la présence des composées majoritaires identifiées dans les extraits aqueux en particulier les tanins, les saponosides et les dérivés phénoliques, mais aucune conclusion formelle ne peut être avancée sauf que l'agent responsable de cette activité est un composé polaire. Il sera utile de bien identifier le ou les composés responsables de cette activité ainsi que les mécanismes d'action des ces agents.

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NATURE DE L'ASSOCIATION SYMBIOTIQUE ENTRE *TERFEZIA BOUDIERI* CHATIN DE LA TUNISIE ET SA PLANTE HÔTE DANS DIFFÉRENTES CONDITIONS DE CULTURE

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Abstract: Effect of culture conditions and soil type in the mycorrhiza between *Terfezia boudieri* Chatin and its host plant in Tunisia
The objective of this work is to study the mycorrhization between *Terfezia boudieri* Chatin and *Helianthemum sessiliflorum* Desf.. Samples of fungal specie and seed were taking from a truffle area situated in South of Tunisian. This mycorrhization was done in three conditions: inoculation of plants by *Terfezia boudieri* ascosporous; transplantation of plants on an experimental field and a seedling and inoculation of *Helianthemum sessilifloum* directly in the field.
The survey of the shape and type of mycorrhizes under binocular and under optic microscopic (after coloration by the acidic Fuschine according to the protocol of Phlippe and Hayman (1970)) showed the presence of no branched and short mycorrhiza grouped generally and localized at the secondary roots. These mycorrhizas are colorless when they are young and become dark when they are aged. All plant tested contain endomycorrhiza.
Key words: *Terfezia boudieri*, *Helianthemum*, mycorrhization, culture Tunisia.

Résumé
Un essai de synthèse mycorhizienne entre *Terfezia boudieri* Chatin et *Helianthemum sessiliflorum* Desf., de la Tunisie présaharienne, a été effectué sur deux types de sols (sol gypseux et sol sablo-limoneux) et dans trois conditions:

- inoculation gnotoxénique des plantes par les ascospores du champignon; ces plantes ont été maintenues ensuite dans une chambre de culture;
- transplantation d'un lot de ces plantes sur une parcelle expérimentale;
- et un semis et inoculation directe sur place d'Hélianthèmes au niveau de la même parcelle.

L'étude de la forme et de type de mycorhizes par observation sous loupe binoculaire et sous microscopique optique après coloration à la Fuschine acide selon le protocole de Phlippe et Hayman (1970) a montrée la présence de mycorhizes courtes non ramifiées groupées généralement en amas au niveau des racines secondaires, ces mycorhizes sont incolores lorsqu'elles sont jeunes et deviennent marrons foncés lorsqu'elles sont plus âgées. Il s'agit dans tous les cas d'endomycorhizes.

Matériel et Méthodes

1. Plante hôte
La plante testée à travers cette étude est mentionnée dans la littérature comme étant une plante hôte des Terfez, il s'agit d'*Helianthemum sessiliflorum* en provenance de Ben Guardane (Ismaîliette) 2003.
Des semences d'*Helianthemum sessiliflorum* ont été récoltées au moi de Mai à partir de ce site prospecté.

2. Substrats de culture
Deux types de sol ont été choisis pour les synthèses mycorhiziennes. Le premier substrat est

un sol en provenance de Dar Edhaoui (terrain de mise en défens situé dans la région de Ben Guardane, à pH neutre (7.1), pauvre en matière organique (1.17%) et dépourvu de phosphore assimilable), c'est à partir de ce site que les prélèvements des ascocarpes de *Terfezia boudieri* et des graines d'*Helianthemum sessiliflorum* ont été effectués.
Le deuxième sol est prélevé d'une parcelle expérimentale située au siège de l'Institut des Régions Arides (IRA) de Médenine, il s'agit d'un sol gypseux à pH neutre faiblement basique (7.7), dépourvu de phosphore assimilable, assez riche en matière organique (3.45%).

3. Inoculation des plantules
La synthèse mycorhizienne a été réalisée par des graines d'*Helianthemum sessiliflorum* scarifiées. Ces graines ont été mises à germer directement dans le substrat de culture.
Les ascocarpes de *Terfezia boudieri* Chatin ont été desséchés au soleil.
L'inoculation a été effectuée avec des suspensions sporales, selon la technique utilisée pour la production de plants mycorhizés par *Tuber melanosporum* (Chevalier et *al.*, 1973; Chevalier et Grente, 1978).
Les synthèses en conditions gnotoxéniques et dans le cas des plantes qui seront ensuite transplantées ont été réalisées dans des pots en plastique préalablement désinfectés.
Les plantes témoins ont été semées de la même façon que les inoculées avec la seule différence que les substrats sont dépourvus de la suspension sporale de *Terfezia boudieri*.

4. Conditions de culture
Des plantes inoculées et témoins ont été placées dans une chambre de culture (Température=23±1°C; 40µmolm-2s-1 Growlux, 16h photopériode; Humidité= 60%). Ces plantes seront ensuite divisées en deux lots le premier reste en chambre de culture et le deuxième sera transplanté sur une parcelle expérimentale. L'irrigation a été effectuée deux fois par semaine par remontée capillaire.

5. Conditions de transplantation des plantes d'*H. sessiliflorum* témoins et inoculées
Une parcelle expérimentale située dans le siège de l'Institut des Régions Arides de Médenine a été préparée pour la transplantation des plantes d'*H. sessiliflorum* témoins et inoculées. Cette parcelle est formée de deux types de sols: le sol gypseux de l'Ira et le sol sablo-limoneux de Dar Edhawi. Pour ce second sol on a procédé à éliminer le sable (2m de profondeur de) et le remplacer par le sol prélevé de Dar Edhawi.
Les plantes ont été transplantées avec une densité de 5 plantes/m^2. L'irrigation a été assurée par l'eau de robinet deux fois par semaine.

6. Conditions de culture des plantes semées directement (inoculées et témoins)
Sur la même parcelle d'expérimentation et sur les deux types de sol on a procédé à un semis direct et à l'inoculation de même lot de graines d'*H. sessiliflorum* et selon le même protocole adopté pour l'inoculation en chambre de culture. Les plantes témoins sont semées sans addition d'ascospores. La densité de plantation a été de l'ordre de 5 plantes/m^2
L'irrigation de ces plantes a été assurée par l'eau de robinet deux fois par semaine.

7. Etude morphologique des mycorhizes
Le matériel utilisé pour l'étude des mycorhizes est constitué de racines des plantes entières d'*Helianthemum sessiliflorum* cultivées sur les deux types de sols en conditions gnotoxéniques en chambre de culture, transplantées et d'autres semées directement sur le champs.
L'étude morphologique de l'association mycorhizienne a été réalisée via deux techniques:
*** <u>par observation sous loupe</u>**
Dans chaque traitement le système racinaire de dix plantes d'*H. sessiliflorum* témoins et inoculées a été observé sous loupe binoculaire à l'état frais pendant la phase de fructification (mois de Mars). Pour les plantes cultivées en chambre de culture, l'analyse du système racinaire a été effectuée chaque mois.

Cette observation vise à mettre en évidence la présence ou l'absence des mycorhizes et de déterminer leurs formes et couleurs si elles existent.

***par observation microscopique après coloration**

Dans le cas de présence de mycorhizes au niveau de racines par observation sous loupe binoculaire, on procède à leur coloration par la fuschine acide selon le protocole de Philippe et Hayman (1970).

Résultats

1. Mycorhization entre *Helianthemum sessiliflorum* et *Terfezia boudieri* dans les conditions contrôlées

1.1. Aspect macroscopique des mycorhizes

L'observation sous loupe binoculaire des racines de 10 plantes inoculées en condition gnotoxéniques sur deux types de sols (sol gypseux prélevé de la station de l'IRA et sol sablo-limoneux prélevé de mise en défens de Dar Edhaoui) à l'état frais a révélé la présence au niveau des racines secondaires de mycorhizes lisses incolores lorsqu'elles sont jeunes et marrons foncés à maturité (Figs.1 et 2);

Les mycorhizes observées aussi bien sur sol gypseux que sur sol sablo-limoneux sont lisses simples, courtes et non ramifiées seulement que leurs nombre est plus grand sur le sol de Dar Edhaoui où elles se présentent généralement en amas. Ceci prouve que l'aspect des mycorhizes formées entre *Terfezia boudieri* et *Helianthemum sessiliflorum* est indépendant du type de sol.

1.2. Aspect microscopique des mycorhizes

♣ Cas du sol sablo-limoneux de Dar Edhaoui

L'observation mensuelle des systèmes racinaires de 10 plants témoin et inoculés sur le sol sablo-limoneux, en conditions gnotoxéniques et placées en chambre de culture, a permis de conclure que:

- après 1 mois et 2 mois de la date d'inoculation, *Terfezia boudieri* demeure sous formes d'ascospores et de certains hyphes qui commencent à s'associer aux systèmes racinaires des plantes d'*Helianthemum sessiliflorum*. Les plantes témoins dans ces mêmes conditions sont dépourvues d'infection fongique;
- à l'âge de 3 mois, l'infection devient intracellulaire; certaines ascospores sont aussi présentes;
- après 4 mois il a été observé que l'association symbiotique entre le champignon et sa plante hôte est de type endomycorhize;
- à l'âge de 5 et 6 mois les mêmes formes d'endomycorhize ont été mises en évidence (Fig. 3);
- toujours l'infection est localisée au niveau du parenchyme cortical racinaire;
- les plantes témoins sont toujours dépourvues de contamination fongique (Fig. 4).

♣ Sol gypseux de l'IRA

L'observation mensuelle des systèmes racinaires de 10 plants témoin et inoculés sur le sol gypseux prélevé de la parcelle de l'IRA, en conditions gnotoxéniques et placées en chambre de culture, a permis de conclure que:

- durant les deux premiers mois de la date d'inoculation, *Terfezia boudieri* demeure sous formes d'hyphes qui s'associent aux cellules du parenchyme.
- à partir de l'âge de 3 mois, la colonisation traverse les cellules, il s'agit d'endomycorhize (Fig. 5).

2. Mycorhize entre *Terfezia boudieri* et *Helianthemum sessiliflorum* après transplantation

2.1. Aspect macroscopique des mycorhizes

L'observation sous loupe binoculaire des racines d'*H. sessiliflorum* inoculées et transplantées sur les 2 types de sol (sol sablo-limoneux et sol gypseux) à l'état frais a révélé la présence

de mycorhizes lisses au niveau des racines secondaires. Ces mycorhizes sont incolores lorsqu'elles sont jeunes et deviennent ensuite marron sombre (Figs. 6 et 7).
Les mycorhizes des plantes d'*H. sessiliflorum* transplantées aussi bien sur le sol de l'IRA (gypseux) que sur le sol de Dar Edhaoui (sablo-limoneux) sont simples, courtes et non ramifiées. Leur nombre est toujours plus important sur le sol de Dar Edhaoui où elles se présentent généralement en amas.
Après 2 ans de la date de transplantation, l'observation sous loupe binoculaire a révélé les mêmes structures de mycorhizes trouvées dès la première année aussi bien pour les plantes cultivées sur le sol de Dar Edhaoui que celles cultivées sur le sol de l'IRA.

2.2. Aspect microscopique des mycorhizes

L'observation microscopique du système racinaire des plantes d'*H. sessiliflorum* inoculées et témoins transplantées sur sol gyspseux et sur sol sablo-limoneux a permis de conclure que:

- les racines des plantes inoculées observées après une et deux années de la date de transplantation montrent que *T. boudieri* est toujours présent au niveau des racines des plantes transplantées sur les deux types de sols;
- dans le cas des deux sols, le champignon forme avec sa plante hôte des endomycorhizes (Fig. 8a et 9).
- des ascospores attachées aux racines des plantes transplantées sur le sol de l'IRA ont été observées après deux ans de cette transplantation (Fig. 8b);
- les plantes témoins transplantées sur le sol de Dar Edhaoui sont dépourvues d'infection fongique après 1 et 2 ans de cette transplantation;
- après une année de leur transplantation sur le sol de l'IRA, les plantes témoins ne présentent aucune forme d'association mycorhizienne avec *T. boudieri*. Alors qu'après 2 ans, des hyphes fongiques ont été détectés au niveau du système racinaire de certaines plantes et on a même pu identifier l'infection intracellulaire du champignon. Cette infection est peut être en provenance du sol original de la parcelle ou engendrée par un passage d'hyphes à partir des autres plantes inoculées.

3. Mycorhize entre *Terfezia boudieri* et *Helianthemum sessiliflorum* semées et inoculées directement

3.1. Aspect macroscopique des mycorhizes

L'observation sous loupe binoculaire des racines d'*H. sessiliflorum* (inoculées et témoins) semées directement sur les 2 types de sol (sol sablo-limoneux et sol gypseux) à l'état frais a révélé un résultat analogue aux plantes transplantées où on a remarqué la présence de mycorhizes au niveau des racines secondaires. Ces mycorhizes sont toujours lisses et incolores lorsqu'elles sont jeunes. Leurs couleur devient ensuite marron sombre (Fig. 10).
Les mycorhizes des plantes d'*H. sessiliflorum* semées directement sur les deux sols sont simples, courtes et non ramifiées seulement que leur nombre est toujours plus important sur le sol de Dar Edhaoui. Ces structures se maintiennent durant les deux années d'observation même si durant la première année les mycorhizes sont moins nombreuses à retrouver.

3.2. Aspect microscopique des mycorhizes

L'observation microscopique du système racinaire des plantes d'*H. sessiliflorum* semées inoculés directement sur le champs par les ascospores de *T. boudieri* et des témoins transplantées sur les deux types de sol a permis de conclure que sur les deux types de sols le champignon colonise toujours le système racinaire de sa plante hôte et forment ensemble une endomycorhize (Fig. 11a et 12a)
Les plantes témoins sont dépourvues d'infection fongique (Fig.11b et 12b).

Discussion

Les travaux portant sur la description des mycorhizes hélianthèmes avec le genre *Terfezia* sont peu nombreux cependant, selon certains auteurs, les hélianthèmes peuvent former avec les terfez des ectomycorhizes sans manteau (Chevalier *et al.*, 1984; Kovacs et Jakus, 2001). Les résultats de ce présent travail montrent que *Terfezia boudieri* Chatin du sud tunisien

forme avec *Helianthemum sessiliflorum* Desf. des endomycorhizes aussi bien dans le cas d'inoculation gnotoxénique suivie de culture en conditions contrôlées, qu'après transplantation et que dans le cas de l'inoculation et le semis direct sur le champ. Un résultat analogue a été déjà trouvé par Alsheikh (1984) et Gutièrrez *et al*. (2003) même s'il ne s'agit pas des mêmes espèces étudiées.

Ces différences morphologiques entre les mycorhizes de terfez montrent que le caractère ecto ou endomycorhizien pourrait être déterminé par les conditions externes (climat et nature des espèces impliquées) et les variations morphologiques des associations mycorhiziennes entre les terfez et leur plante hôte semblent jouer un rôle important dans l'adaptation des espèces annuelles ou pérennes d'*Helianthemum* dans les semi-arides et arides.

Certains auteurs ont indiqué que la morphologie des mycorhizes diffère avec les conditions de culture (Chevalier *et al.,* 1984; Dexheimer *et al.,* 1985; Roth-Bejerano *et al*., 1990; Fortas et Chevalier, 1990; Kagan-Zur *et al*., 1994; Gutièrrez *et al*., 2003). Les conditions étudiées par ces auteurs varient entre des situations gnotoxéniques, culture *in vitro* ou parfois le cas des conditions naturelles. Cette étude présente, par contre, la première recherche dans le but de comparer entre la nature des mycorhizes obtenues en conditions contrôlées (par inoculation gnotoxénique), après transplantation ainsi que celles trouvées dans le cas d'une inoculation et un semis direct sur le champs.

Conclusion

Cette étude a permis de mettre en évidence par une étude morphologique la nature et l'aspect de mycorhizes entre *Helianthemum sessiliflorum* et *Terfezia boudieri* en provenance des zones arides tunisiennes. Il s'agit de mycorhizes simples, lisses, courtes et non ramifiées. L'observation microscopique après coloration à la fuchine acide a révélée des endomycrohizes entre ces deux partenaires. La nature et l'aspect de ces mycorhizes semblent indépendants de type de sol (gypseux ou sablo-limoneux) et des conditions de culture (conditions contrôlées, transplantation ou semis directe sur le champs).

Le type et la nature de mycorhize entre *H. sessiliflorum* et *T. boudieri* apparaît donc spécifique à ces deux partenaires et se maintient sur les deux types de sols dans les conditions externes du champs à conditions de leurs optimiser les conditions d'irrigation.

Dans le cas des plantes témoins transplantées, ce travail a montré la possibilité de mycorhization sur le champ des plantes hôtes. Cette mycorhization peut être en provenance du sol original de la parcelle mais surtout peut être engendré par le passage d'hyphes fongiques présents au niveau des racines des plantes inoculées.

Cette dernière idée est confirmée par plusieurs auteurs (Chevalier et Grente, 1973; Cameleyre, 1996) et constitue l'une des méthodes de mycorhization utilisée par l'introduction dans le substrat de culture de fragments de racines de plantes déjà associées au champignon.

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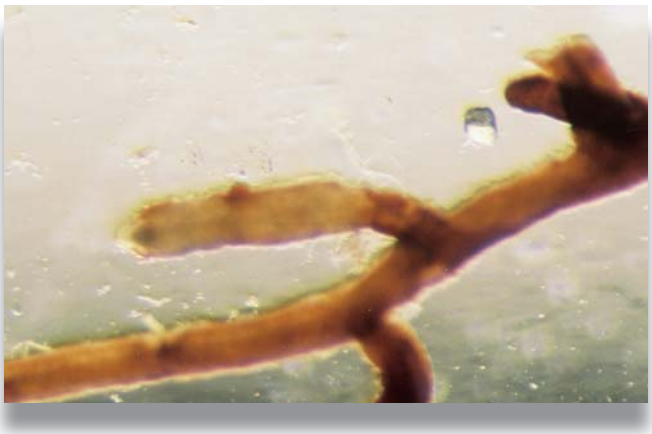

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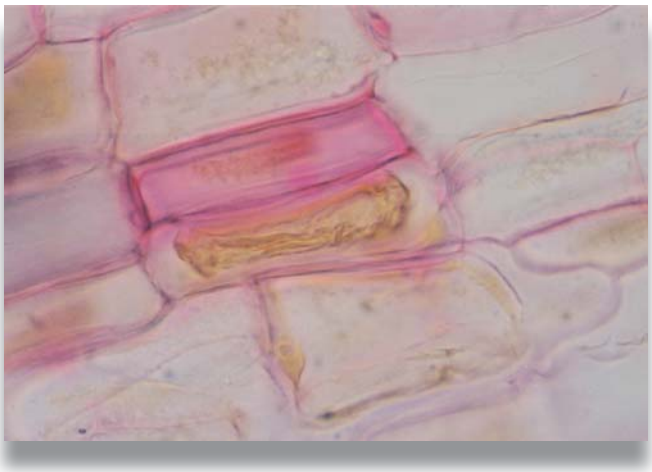

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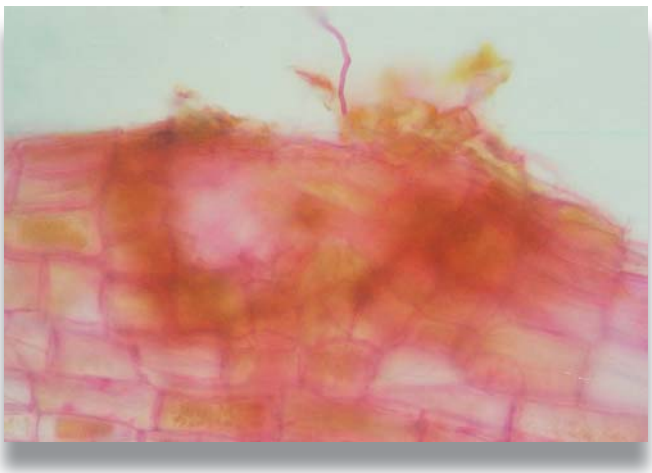

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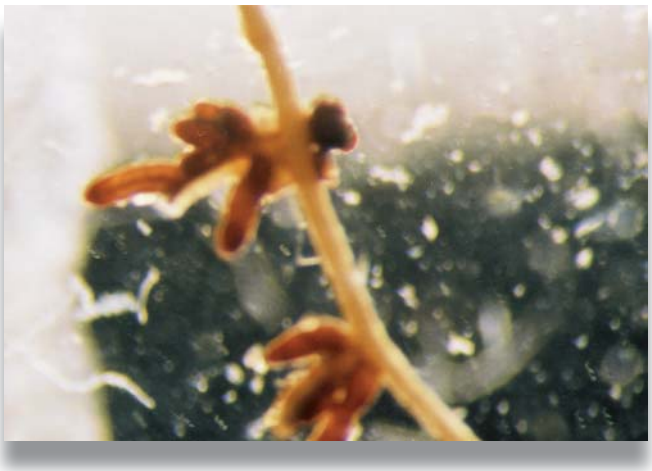

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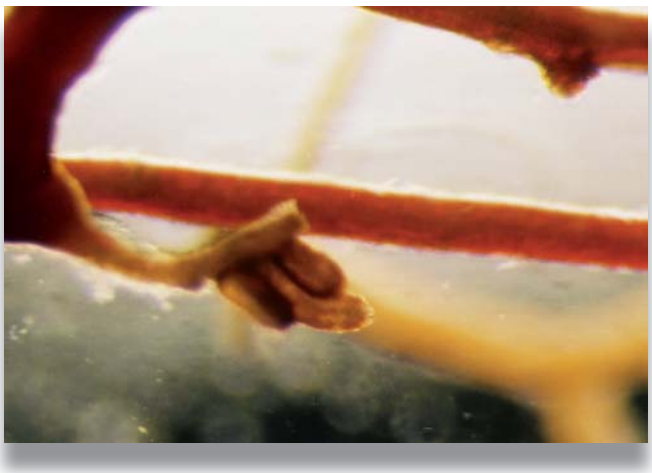

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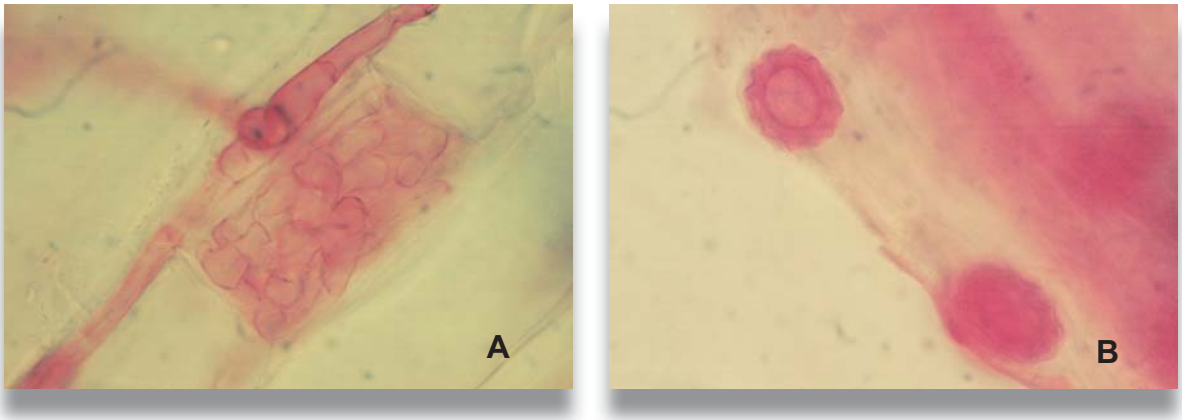

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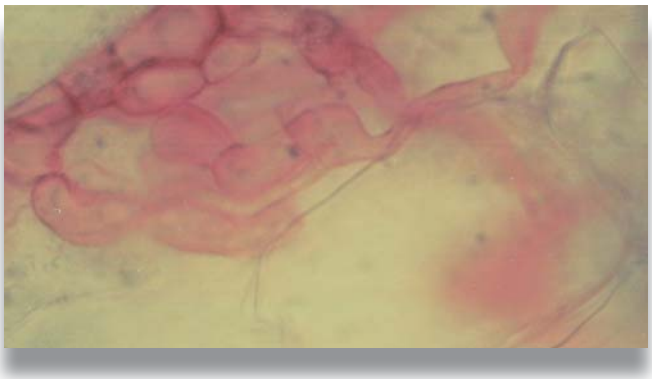

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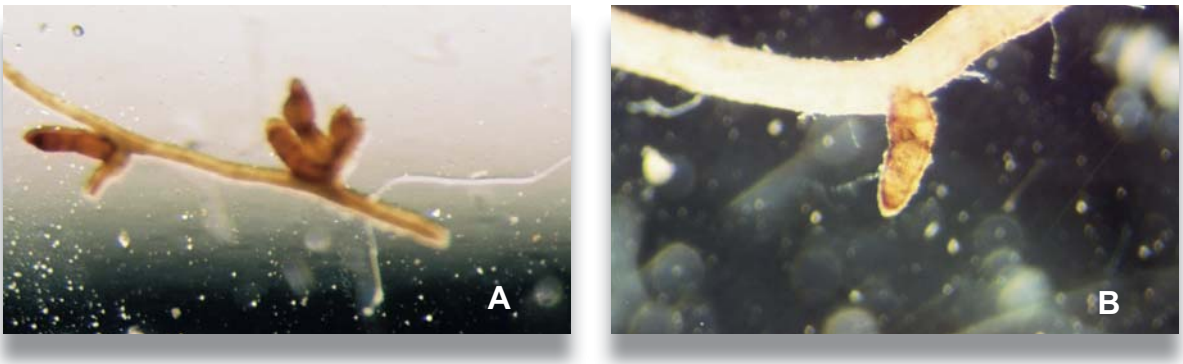

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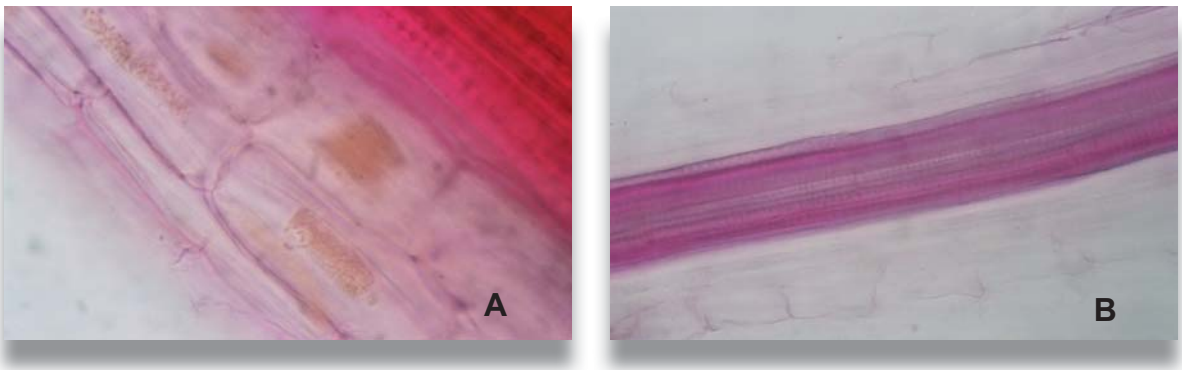

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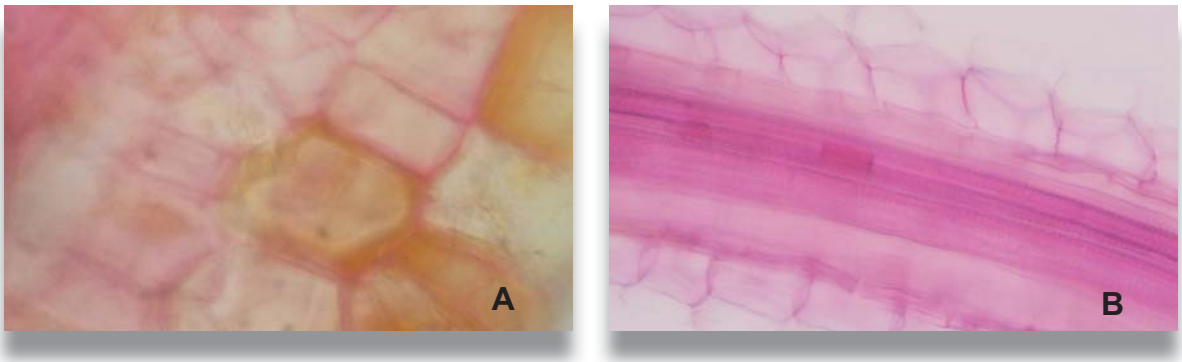

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IN SITU MICRO - AND ULTRASTRUCTURAL STUDY OF ASCOCARP DEVELOPMENT IN *TUBER MESENTERICUM*

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Abstract
Ascocarp development of *Tuber mesentericum* in its natural soil environment was studied, using light and electron scanning microscopy. The microenvironment and the soil interface are of great importance for the ascocarp. A continuum was found between the soil and the truffle with the development of many hyphae forming the peripheral tufts, during the ascocarp's spring growth phase. In autumn, the hyphae of the peripheral tufts were no longer differentiated and the truffle had reached maturity. During the growth phase of the ascocarp a high earthworm activity was found in the environmental soil, leading to the formation of aggregates in which truffle spores could be included. These aggregates, as well as the formation of many channels contributed to the increased microporosity of the soil thus facilitating the growth of the truffle. In addition to physical function, the aggregates participate in the nutrition of the ascocarps via the hyphae (in the tufts colonizing the aggregates).
Keys words: *Tuber mesentericum*, fruit body development, microscopy, soil structure, earthworm activity.

Résumé
Le développement de l'ascocarpe de *Tuber mesentericum* dans son milieu édaphique naturel a été étudié en microscopie photonique et en microscopie électronique à balayage. Le microenvironnement et l'interface avec le sol sont d'une grande importance pour l'ascocarpe. Au printemps, pendant de la phase de grossissement de la truffe, de nombreuses hyphes groupées en houppes périphériques autour de la truffe, établissent un continuum entre le sol et le champignon. En automne, les hyphes périphériques ne sont plus différenciées et la truffe a atteint sa maturité. Lors de la phase de croissance de l'ascocarpe, l'intense activité des vers de terre dans le sol environnemental, conduit à la formation d'agrégats dans lesquels des spores de truffe peuvent être incluses. Ces agrégats, ainsi que la formation de nombreuses galeries contribuent à l'augmentation de la microporosité du sol, favorable au grossissement de la truffe. En plus de leur fonction physique, les agrégats participent à la nutrition des ascocarpes par l'intermédiaire des hyphes des touffes colonisant les agrégats.
Mots clés: *Tuber mesentericum*, développement du corps fructifère, microscopie, structure du sol, activité des vers de terre.

Introduction

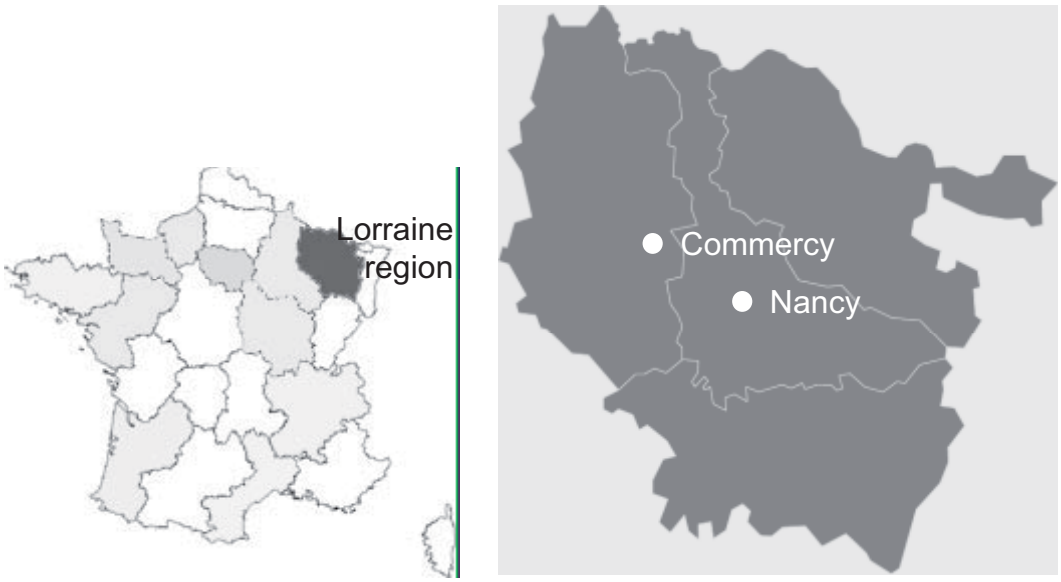
Lorraine is a north-eastern region in France favourable for the development and cultivation of truffles due to its climate and a strong presence of ground limestone in the soil. The climate is semi continental: neither too dry, nor too wet, with a relatively significant pluviometry (700 to 800 mm of water per annum, well distributed throughout the year). In the Lorraine region, and particularly in the Meuse department, climatic and edaphic conditions support the development of *Tuber uncinatum* Chatin and *Tuber mesentericum* Vittad. very well. Hence there are numerous truffle plantations in this region. These two species of autumn truffles come to maturity just before the beginning of the frosts which normally destroy them.
T. mesentericum is a pre-forest and forest species living often in ecosystems more closed than those required by the "Périgord" truffle (*T. melanosporum* Vittad.). Truffles under isolated trees in sunny places are more rare. The fungus is widespread in Europe. In France, it develops naturally in Lorraine, Burgundy and south-eastern areas under oak, hazel and beech trees. The maturity period is spread from the end of September to December.
T. mesentericum is found widely throughout the Meuse department, and especially between

Commercy and Verdun. However, the harvested quantity due to the transformation and disturbance of the ecosystems (agricultural extension and loss of sites). Although it represents an interesting model for the study of mushroom biology (underground mushrooms in particular), this truffle has not yet been the subject, until now, of any scientific research. The aim of this study is to analyze the soil-truffle interface by morphological and ultrastructural observations of the fungus in its microenvironment.

Material and Methods

Study Site

The studies were carried at a the truffle experimental site located near the town of Commercy 40 kilometres to the west of Nancy (Lorraine region, Northeaster France).



The site is on a land slope facing north/north-east, formerly cultivated with field crops until 1980. The soil is rich in alterite, an iron-rich limestone, and can be classified as a brown Renzina. In addition, the soil is approximately 80 cm in depth, with a profile schematised as: LA Ca/Sca /Cca. It is calcareous on all the profiles, and comprises an anthropic higher horizon. It presents a significant high hydrous reserve (about 100 mm) and it is rather rich in stones (Chevalier & Frochot, 1997).

Ascocarp Sampling

T. mesentericum ascocarps were collected from soils under hazel trees, at two periods of the year, in June and November and were located with a trained dog. Each truffle was taken with its surrounding soil using an aluminium box with a double bottom, called a “Kubiena box” (50 x 90 x 60 mm). This sampling technique only slightly disturbs the soil structure. The boxes were then brought to the laboratory for observation and preparation.

Observation Techniques

In order to carry out observations on various scales, several techniques were used.

Observation techniques of samples extracted from soil

Fragments of truffle cortex in contact with soil were carefully taken, as not to disturb the contacts between the fungus and soil. They were fixed by 4% osmium tetroxyde vapour 4% for 48h at 5°C and placed in a desiccator containing silicagel for 2 days. After carbon metallization, the samples were observed by scanning electron microscopy using a Hitachi S2500 SEM.

In situ observation techniques

After sampling, soil blocks, containing truffles, were soaked in acetone, renewed three times a week in order to eliminate water from the soil and the truffle. The samples were then drained for one hour and infiltrated under vacuum with Norsodyne's resin for 4h (Fitzpatrick & Gudmunsson, 1978). Polymerization of the resin was carried out in a ventilated drying oven at 30°C for 4 to 5 weeks. 2 to 3 mm thick sections were cut with a diamond saw. One of the faces of the section was polished and then fixed on a glass slide. A diamond disk gradually decreased the thickness of the section to about 25 µm thick. Sections were either (i) covered with a cover glass and observed with a binocular lens and by light microscopy using normal or polarized light with or without Nomarski's lighting, or (ii) not covered and examined by scanning electron microscopy using a Hitachi S2500 associated with a dispersive energy spectrometer.

Results

Structure of the Ascocarps

At the two sampling periods, *T. mesentericum* ascocarps displayed a black peridium with small warts and a characteristic basal cavity (fig. 1 and 2).

In June, the ascocarp is still immature. Its inner part, called the gleba, is white and is crossed by small whitish veins (fig. 1). The gleba contains ascospores which appear translucent in light microscopy (fig. 3). In November, the ascocarp is mature. The gleba becomes brown and shows a network of white veins (fig. 2). The ascospores have a clear brownish colour and are ellipsoid (fig. 4). Their wall is well developed and has réticulate-alveolate ornamentations (fig. 5 and 6). However, in a single gleba, the spores are not all at the same stage of development and maturity. Sections observed by SEM from autumn samples show that few spores were transversely damaged during preparation. Most of the spores were uncut and remained intact in spite of the sectioning (fig. 5 and 6). This emphasizes resistance heterogeneity of the spore walls and the spores themselves appear highly differentiated. In autumn, the ascocarps present cavities within their gleba. These cavities result from the movement of predators which consequently displaced spores within the gleba (fig. 21).

Hyphal Peripheral Tufts

Hyphae emerge at the tops of the warts by forming peripheral tufts (fig. 7). Hyphal peripheral tufts develop around the ascocarp and explore the surrounding soil (fig. 8). They are numerous and dense on the June samples (fig. 7 and 9), but they are scarcer and less dense on the ascocarps sampled in November (fig. 10). The tufts result from hyphae of the gleba localized under the peridium (fig. 11). When the surrounding soil is removed from the ascocarp under a binocular lens, many hyphae, from the tufts, are retained in the ore and are separated from the peridium (fig. 8) indicating their fragility and their intimate cohesion with the soil. Any disturbance of the soil appears to be detrimental to the development of the ascocarps. In June, it was found that many hyphal peripheral tufts penetrated the soil aggregates and colonized them. Some hyphae show intimate contact with mineral fragments in the soil (fig. 12) and/or the colonization of dead roots and organic materials included a in the aggregates (fig. 13 and 14). From a number of sections, it was observed that hyphae adopt an oriented growth directed towards organic debris located as far as 4-5 cm away from the ascocarps. In autumn, there are fewer peripheral tufts and the remaining hyphae colonize little of the soil around the ascocarps.

Microenvironment of the Ascocarps

Extruded from the soil, ascocarps are surrounded by a peripheral soil ore (fig. 15 and 16), which strongly adheres to their surfaces where various roots of trees and/or other herbaceous plants develop (fig. 17). These roots can also penetrate the ascocarp cavity, which is characteristic of the ascocarp of *T. mesentericum* (fig. 18). The soil not adhering to the ore is made up of aggregates (fig. 19) which have a length of a few mm and are more or less spaced (fig. 19 and 20). They consist of fine mineral grains, organomineral substances (clay associated with organic matter) and fragmented organic debris (provided mainly by the unfinished degradation

of roots, leaves, branches, bud scales and flowers). Our results show that earthworms ingest mineral fragments of the soil as well as organic material (fig. 19 and 20).
Soil aggregates are composed of earthworm faeces (Toutain et al. 1988). The loose organization of the soil around the ascocarps is due to the individualization of the faeces and to a lesser extent to the channels formed by the earthworms (fig. 19 and 20). This particular organization with wellindividualized units corresponds to an air-containing environment, which prevents any compaction of the soil, the latter being detrimental to the ascocarps (fig. 19 and 20). Truffle fragments (fig. 20) and scattered, isolated spores (fig. 23 and 24) were observed in aggregates located 1-2 cm from the ascocarps. The wall of such spores does not appear deteriorated and their réticulo-alveolate ornamentation indicates that they belong to the genus *T. mesentericum* (fig. 23 and 24).

Synthesis and Discussion
The two developmental stages of sampling correspond to two physiological stages of truffle ascocarps development. In June, immature ascocarps are characterized by their small size, absence of scent and rapid growth. Hyphal peripheral tufts are well developed and the immature spores have developed a wall in the process of differentiation. In November, ascocarps have enlarged, have became mature and their growth phase has ceased. Hyphal peripheral tufts were rare and the spores are characterized by a well differentiated wall.

Hyphal Peripheral Tufts
Hyphal peripheral tufts have many, particularly long, filaments around the ascocarp of *T. mesentericum* while those of *T. melanosporum* and other species, are generally shorter (Callot *et al.,* 1999). In this investigation, it was shown that the hyphal peripheral tufts develop in and around the aggregates close to the ascocarps, thus exploring this specific environment. They reveal the potential for colonization of dead plant fragments located up to several centimetres away from the ascocarp. In favourable observations one can follow the way of groups of very many filaments since ascocarp of *T. mesentericum* until organic residues located at more 4 cm. The invasion of organic fragments close to the surface of the peridium by peripheral tufts hyphae has already been observed in the species *T. melanosporum* but never reported at such long distances from the ascocarp (Callot *et al.,* 1999). The colonization of the organic elements shows that the truffle mycelium has the capacity to behave like a saprophytic hyphae. This type of behaviour has already been observed in various phases of the truffle cycle (Pargney *et al.,* 1999 *a* & *b*). Hyphal peripheral tufts are abundant during the growth phase of the ascocarp but become less common during maturity. This mycelium develops when the immature ascocarp becomes independent of the roots of the host tree, thus acquiring its autonomy (Barry *et al*., 1993; Callot *et al*., 1999). However, the fruit body becomes dependent on its environment to ensure its growth. The hyphae of peripheral tufts can then take in the nutrients necessary for the development of the ascocarp. The organic residues in the soil constitute a nutritional source not only of carbon and nitrogen, but also of minerals associated with organic substances.

Aggregates of the Surrounding Soil
The morphological observations show that almost all the aggregates around the ascocarps are faeces from earthworms. One specific earthworm, *Allolobophora chlorotica,* was frequently found in this type of soil during truffle extraction. The organization of particularly spaced and channels separated organomineral aggregates contributes to maintain the best structure of the environmental soil for the development of the ascocarp. Spaces between the aggregates, resulting from the channels made by worms, also contribute to better circulation of fluids (water, gases) around the developing ascocarp. A rapid recycling of water avoids stagnation causes early and rapid degradation of the fruit body by rot. Circulation of gases also allows for improved oxygenation of the developing ascocarp and accompanying aerobic bacteria. Furthermore, the aggregate organization of the soil avoids the compaction of the soil and allows for the enlargement of the ascocarp (Boumaza *et al.,* 2002). These observations are in agreement

with the findings of Callot *et al*. carried out on *T. melanosporum* and *T. aestivum* in 1999. Aggregate minerals are of varied nature. The present study revealed the presence of clays, which are minerals with great specific surfaces fixing exchangeable ions. Earthworms feed on minerals and plant remains found in the soil. In their digestive tracts, the mixture of these substances is within an active enzymatic environment (Lavelle & Spain, 2001). Progressively, with their movement in the ground and under the contraction of their intestinal muscles, the earthworms produce faeces that can be taken up by enchytraeids, collembolans or by others worms. Once into the soil, the enzymes fixed to the clays remain active (Gobat *et al.,* 2003). The faeces around truffles can be colonized by the hyphae of the peripheral tufts and are absorbed by other earthworms. The worms are attracted to the ascocarps in response to the presence of hyphae. The presence of gleba fragments and spores in the faeces indicates that earthworms have absorbed them. Worms do not consume ascocarps directly. Predatory animals that feed on truffle (such as liodes, slugs, molluscs, phytophagous, coleopters larvae, field mice, etc) then digest and defecate gleba fragments containing spores (Callot *et al.,* 1999). These truffle fragments are absorbed later by earthworms and are mixed with the other elements during their gut transit. This process potentially allows for the displacement of the spores for from the ascocarp of origin.

Associated Roots

Truffle environments contain living or dead roots belonging to host tree or to various plants associated with the truffle ecosystem. They preexist in the development of the ascocarp or they grow after development of the ascocarp. Living roots and mycorrhizas can develop within the soil ore around the ascocarp and enter to the characteristic basal cavity of the *T. mesentericum*. Other truffles with scaly peridium are also wrapped by a layer of adherent soil, which implies that they need to be brushed before consumption. Living roots are rare in the immediate environment of the *T. melanosporum* ascocarp and normally only some dead roots are present (Callot *et al.,* 1999). This likely suggests different behaviours between the *Tuber* species with respect to their environment and to other components in the truffle ecosystem. Death of roots can be the consequence of a development of the ascocarp, as it has already been observed in *T. melanosporum* (Callot *et al*., 1999). *T. melanosporum* and *T. mesentericum* both show a saprophytic behaviour. When in comparison, the saprophytic behaviour of *T. melanosporum* is more marked than that of *T. mesentericum.* In all examples investigated truffle environments contain dead roots that are more or less finely fragmented. Some fragments are still of large size (visible to the naked eye). Others are of reduced size (mm) and after having been digested by earthworms they appear again in the faeces. These root fragments appear to create a trophic influence for the mycelium coming from the peripheral tufts and capable of colonizing the aggregates. Different root types surround *T. mesentericum* ascocarp. However living roots are not found in the environment of the *T. melanosporum* ascocarp. This emphasizes some problems of the differential biodynamics at the level of root development and formation of the ascocarp according to the *Tuber* species.

Conclusions

The observations and the present results show that the microenvironment and the soil interface have a great importance for ascocarp formation. A hyphal continuum is established between the soil and the fruit body with the development of the numerous hyphae forming the peripheral tufts during the ascocarp growth phase. These hyphae show significant conquest potentiality for the edaphic environment. Their functional dynamics merit further study. In the spring, the hyphal peripheral tufts explore the aggregates of the local soil and this activity depends on the presence of the edaphic fauna, in particular, that of earthworms. The numerous organic residues form a potential material in which the hyphae of the peripheral tufts are able to develop. By this saprophytic behaviour, fungal cells can take up nutrients (carbon, nitrogen and/or mineral elements) necessary for growth of the ascocarp. In autumn the hyphae of the peripheral tufts are no longer differentiated and the truffle ascocarp has reached maturity. Earthworm activity

in the ascocarp environment leads to the formation of aggregates in which truffle spores can be included. Such soil, structured with individualized units and in addition, the formation of many channels, has a high degree of porosity. This emphasizes the ecological impact of earthworms. In their digestive tract, earthworms mix mineral and organic components. Thus, they significantly contribute to the production of a fertile soil as well as to its structure. For hypogeal mushrooms, animals are necessary for the dispersal of the spores. Various predators of the mushrooms themselves ensure this role. Earthworms are not truffle predators. However they contribute to the dissemination of the spores as soon as they are released from the gleba. Worms thus take part in spore dispersion. Their role in dispersing spores could be important for the colonization of host tree seedlings in nurseries.

In *T. mesentericum*, ascocarp maturity is accompanied by a modification of the juxtaposed microenvironment. Roots of host plants and/or accompanying herbaceous plants develop rapidly in this favourable medium without being disturbed by the truffle. The comparison with other truffle species suggests that *T. mesentericum* may have a tolerance behaviour with respect to the neighbouring roots, which would not be the case with *T. melanosporum*. The behaviour of *T. melanosporum* is aggressive. These two species can thus represent interesting models for the study of truffle ecosystems dynamics.

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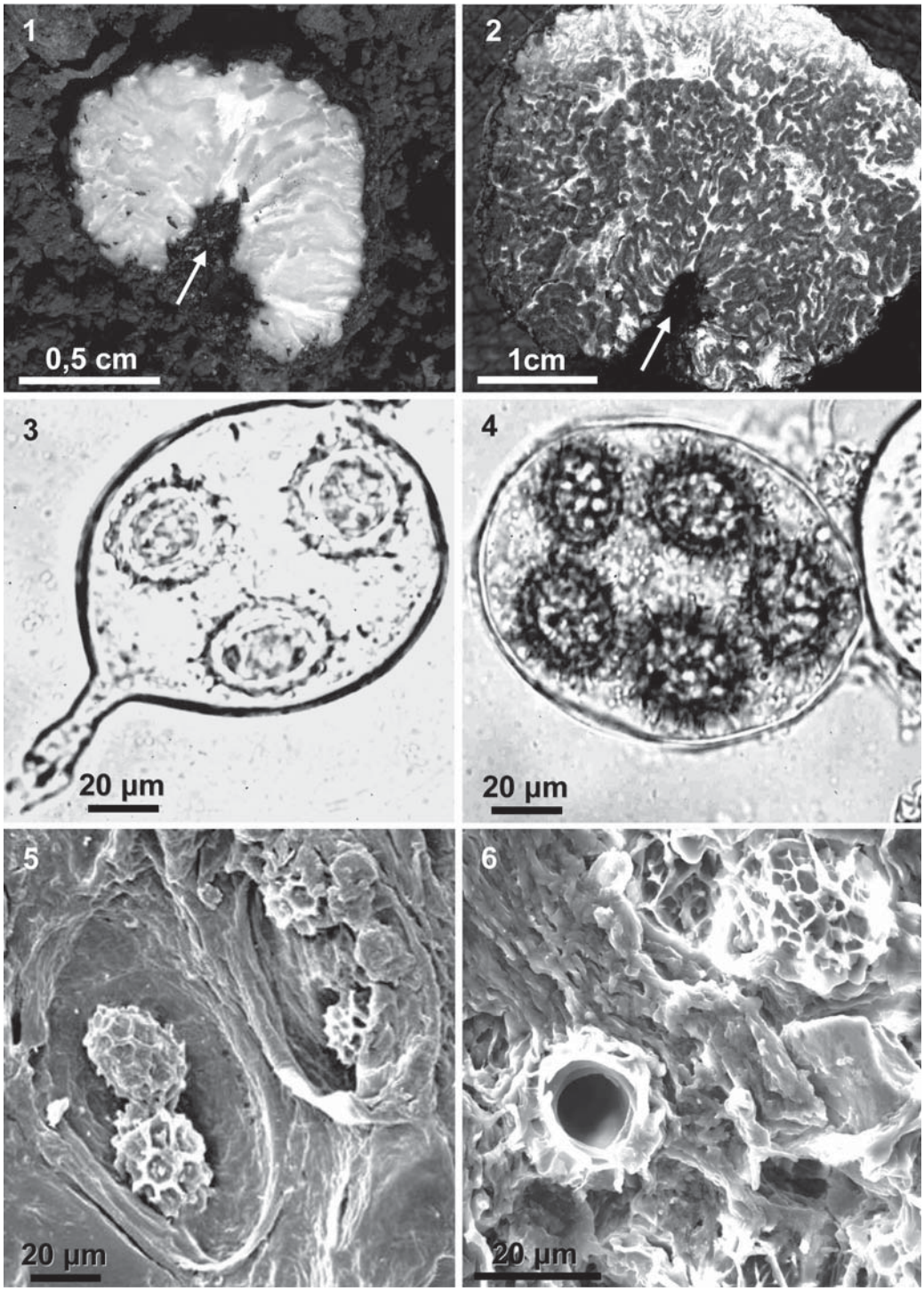


Figure 1. Section of immature truffle and its characteristic basal cavity (arrow) in June.
Figure 2. Section of mature truffle and its cavity (arrow) in November.
Figure 3. Immature ascospores in June. Light microscopy.
Figure 4. Mature ascospores in November. Light microscopy.
Figure 5. Sections of immature gleba in June. SEM.
Figure 6. Sections of mature gleba in November. SEM.

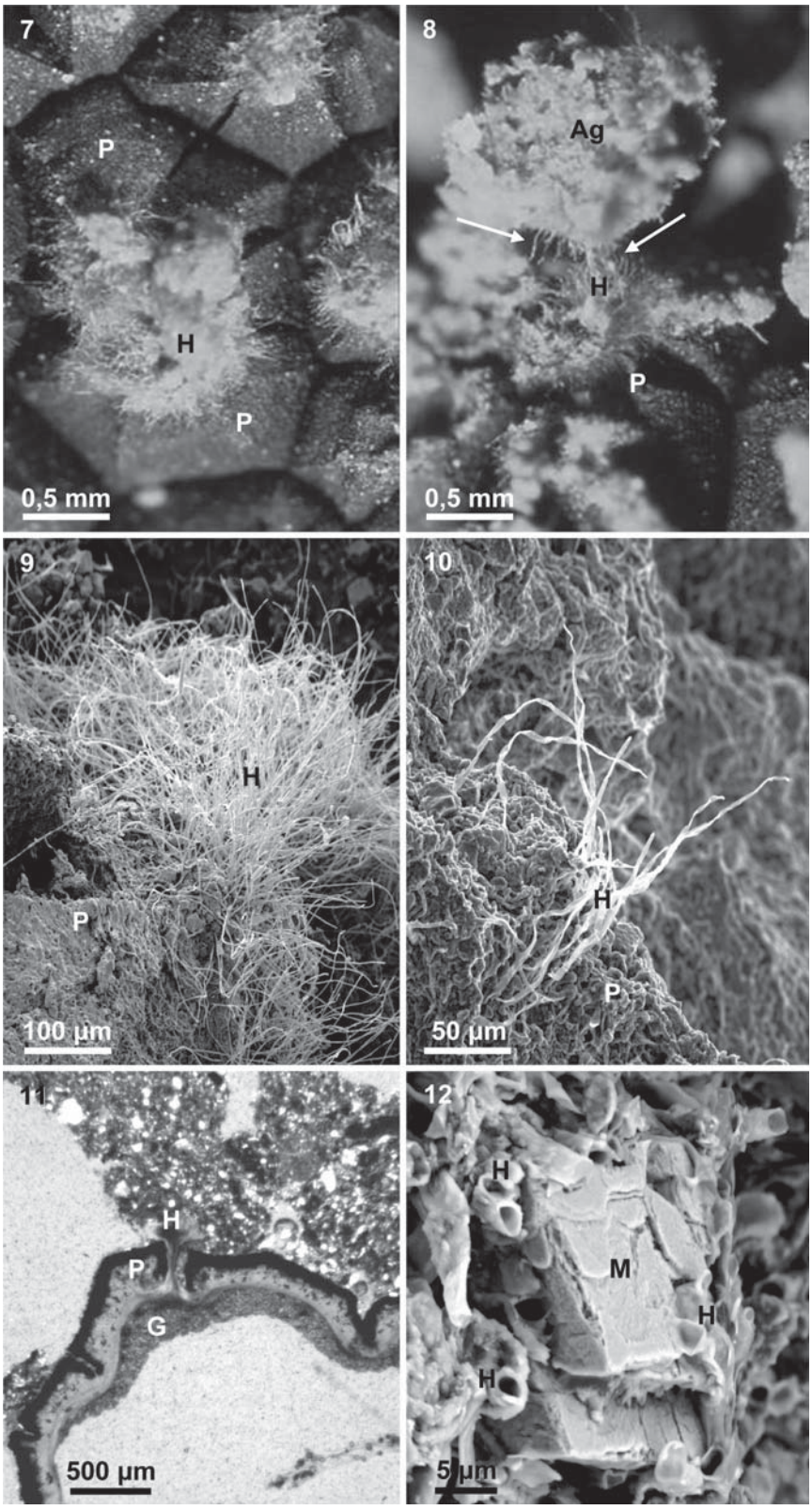


Figure 7. Peripheral tuft of hyphae (H) at a wart top of peridium (P) in June. Binocular lens.
Figure 8. Hyphae of a peripheral tuft (H) penetrating (arrows) in a soil aggregate (Ag) in June. Binocular lens.
Figure 9. Peripheral tuft (H) in June. SEM.
Figure 10. Peripheral tuft (H) in November. SEM.
Figure 11. Tuft (H) coming from the gleba (G) through the peridium (P) and penetrating the surrounding soil. *In situ* observation in June. Light microscopy.
Figure 12. Contacts between hyphae of a tuft (H) and a mineral fragment of the soil (M). SEM.

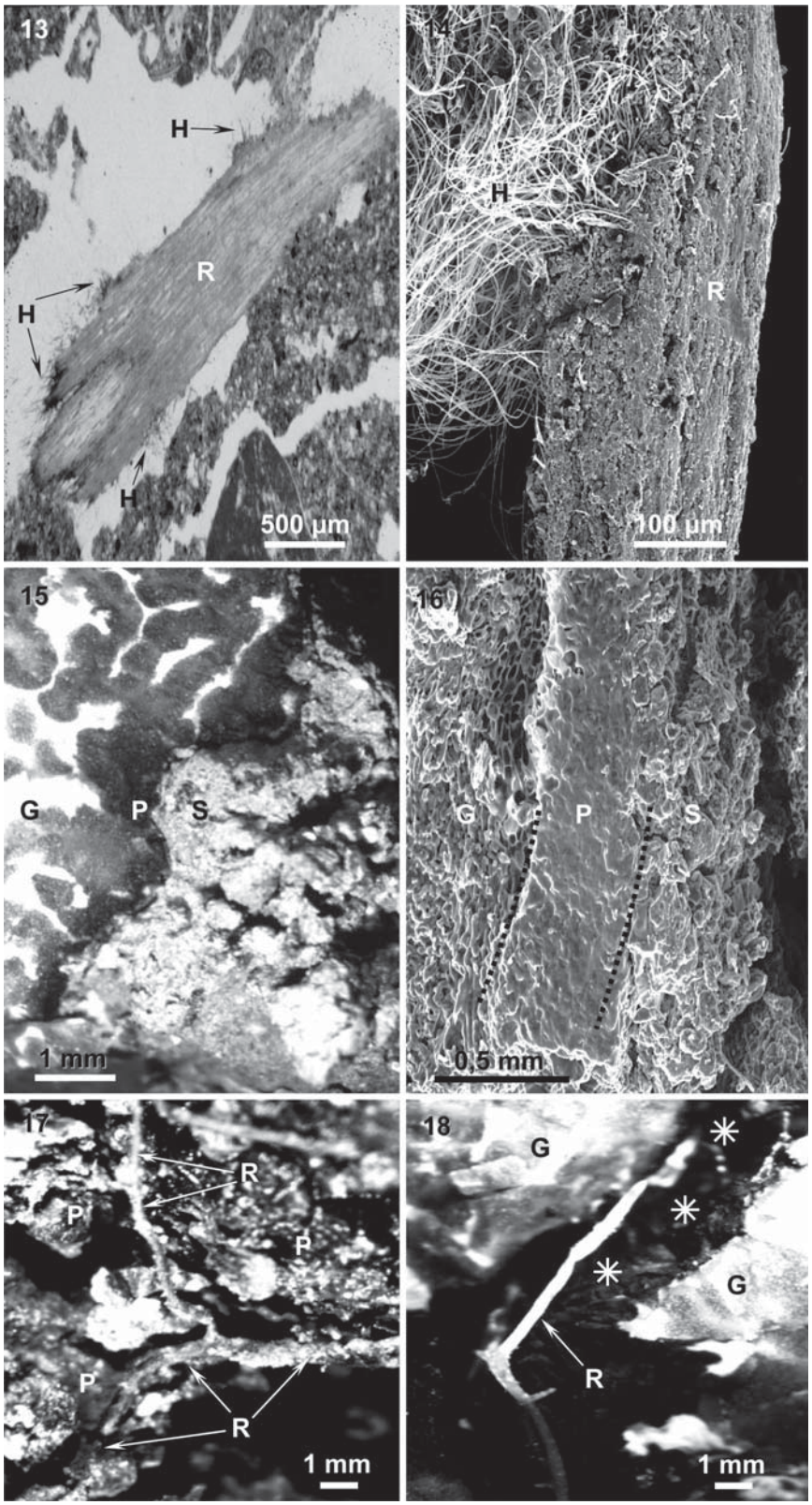
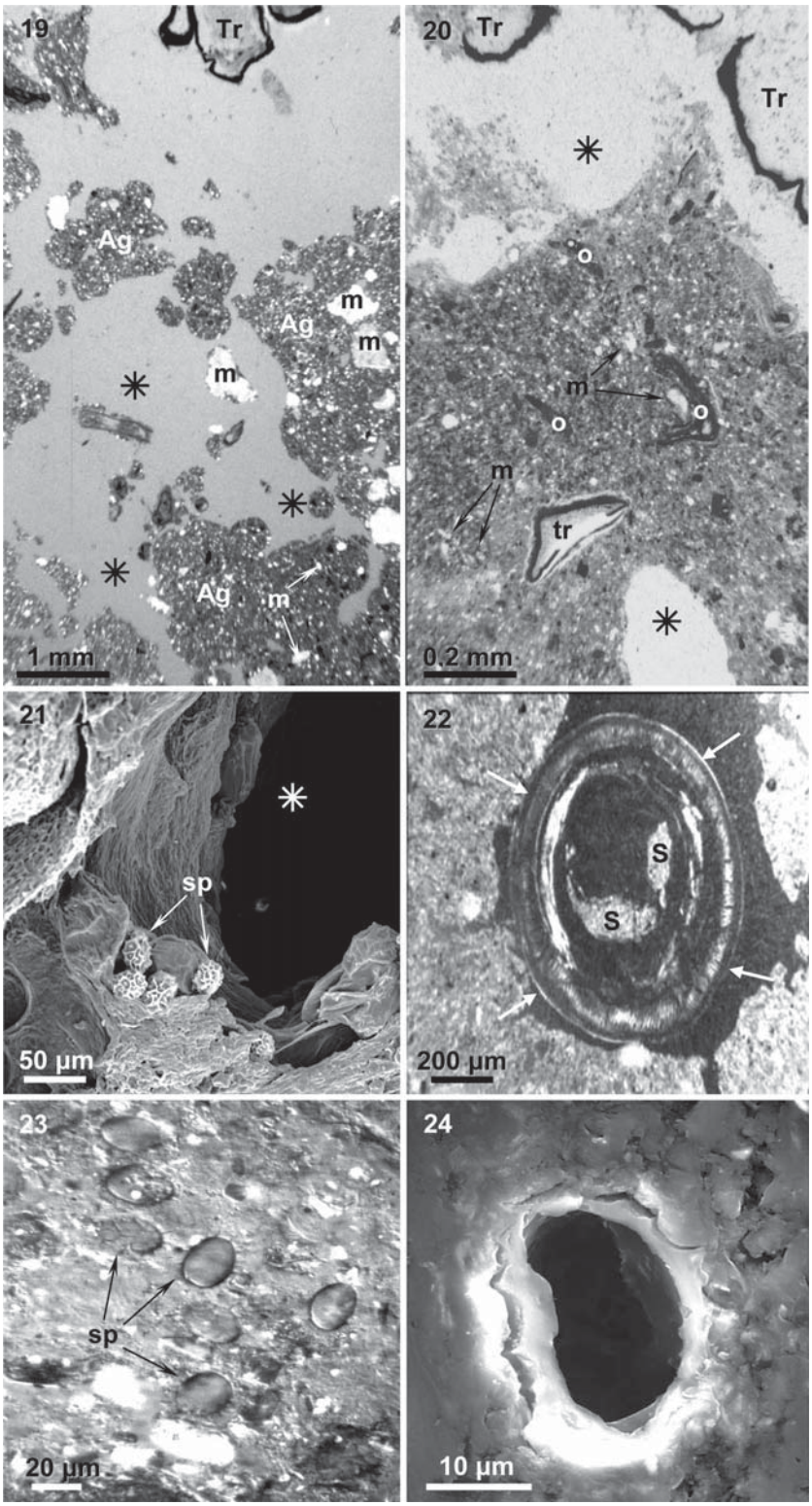


Figure 13. A dead root fragment (R) colonized by hyphae (H) coming from an ascocarp nearly. Observation on thin section of soil. Light microscopy.
Figure 14. Contacts between hyphae of a tuft (H) and a dead root fragment (R). SEM.
Figure 15. Sections of gleba (G), peridium (P) and soil peripheral ore (S). Binocular lens on thin section of soil. *Figure 16.* Sections of gleba (G), peridium (P) and soil peripheral ore (S). SEM.
Figure 17. Roots (R) on the surface of a fruit body (P). Binocular lens.
Figure 18. Section of ascocarp (G) and root (R) penetrating in the cavity (Ä). Binocular lens.



Figures 19-20. Soil not adhering to the ascocarp (Tr) with aggregates (Ag) of mineral fragments (m), organic remains (o) and fragments from truffle (tr) and spaces between the aggregates (Ä). Observation on thin section of soil. Light microscopy.

Figure 21. Section of a predator cavity (Ä) containing spores (sp) in November. SEM.

Figures 22. Transversal section of a earthworm (arrows) with soil in its intestine (S). Observation on thin section of soil. SEM.

Figures 23. Spores of *T. mesentericum* (sp) into an aggregate corresponding to faeces of earthworm. Observation on thin section of soil. Microscopy with Normaski's lighting.

Figure 24. Section of spore into an aggregate. Observation on thin section of soil. SEM.

COMPETITION BETWEEN *PISOLITHUS ARHIZUS* (SCOP.) RAUSCHERT AND *SCLERODERMA VERRUCOSUM* (BULL.) PERS. AND DIFFERENT SPECIES OF *TUBER*

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Abstract
The present work aims to verify whether *Pisolithus arhizus* (Persoon) Rauschert and *Scleroderma verrucosum* (Bull.: Pers.) Pers. compete for mycorrhization with other *Tuber* species in the soil. In spring 2004 some seedlings of *Quercus pubescens* Willd. were inoculated with *P. arhizus* and *S. verrucosum* alone and/or with different species of *Tuber* (*T. melanosporum* Vittad., *T. aestivum* Vittad., *T. borchii* Vittad., *T. brumale* Vittad.) and in all possible combinations. 17 different test inoculums were inoculated in the same conditions and using the same quantity of inoculating material. Two types of substrates were used to raise the plants: natural (natural soil and sand) and artificial (agriperlite, vermiculite and peat). In fall 2004, spring 2005 and spring 2006, the percentage of mycorrhization for each of the species of fungi involved was assessed in 32% of the plants for each test inoculum. Root tips were counted and percentages were calculated for mycorrhizae of each species.
The presence of mycorrhizae was quite low some months after inoculation, especially in the artificial substrate (To). In 2005 and 2006 almost all test inoculums were mycorrhized, mainly with *Tuber* species, in both the natural and artificial substrates. *P. arhizus* shows a positive trend during three years in To, whereas in Te the opposite occurs. The presence of *S. verrucosum* was low and constant at 7.7%, but it doubled in To during the last analysis (2006); the two species are not, therefore, very competitive.
Key words: mycorrhization, fungal competition, inoculums substrate.

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Introduction

Many researchers have studied the question of competition between ectomycorrhizal fungi present in the productive and non productive truffle orchards (Chevalier *et al.*, 1982; Bencivenga *et al.*, 1992; Granetti e Angelini, 1992; Donnini e Bencivenga, 1995; Baciarelli Falini e Granetti, 1998). In some cases the exclusive morphological type of *Tuber melanosporum* mycorrhiza was found in sites where truffles of *T. melanosporum* Vittad. were produced (Bencivenga *et al*., 1992), but, generally, in a natural truffle bed, up to 10 different types of mycorrhizae can be observed (Donnini e Bencivenga, 1995). Moreover, after about 15 years of plant root sampling and observation, in both natural and planted truffle beds, 6 known fungal species and 26 unknown fungal morphotypes have been detected along with the various well known *Tuber* species (Donnini *et al.,* 2005). Some of these species have also been observed in root samples collected from specialised Garden Centres, when contamination occurred during *Tuber* inoculation and plant breeding (Bencivenga *et al*., 2005). The known fungal species are: *Sphaerosporella brunnea* (A. & S.: Fr.) Svrcek & Kubicka, *Hymenogaster citrinus* Vittad., *Cenococcum geophilum* Fr., *Scleroderma verrucosum* (Bull.: Pers.) Pers., *Pisolithus arhizus* (Persoon) Rauschert, *Pulvinula constellatio* (B. & Br.) Boud.. The 26 unknown morphotypes are mainly observed on symbiotic plants present in natural and planted beds of various truffle species in Central Italy. It is noteworthy that during the morphological analyses of root samples, collected in both natural and artificial truffle beds, many other morphological types

of mycorrhizae were observed, but their description was not possible. It would be useful to identify the fungal species associated to this biodiversity, in order to evaluate their actual competitiveness against *Tuber* spp. It is in fact important to know the ecology of these fungal species, to recover and improve unproductive natural and planted truffle beds.
Two species of fungi, *Pisolithus arhizus* (Scop.) Rauschert and *Scleroderma verrucosum* (Bull.) Pers., are often present in cultivated truffle beds and seem to obstruct mycorrhization with *Tuber* species. The present work aims to verify whether *P. arhizus* and *S. verrucosum* compete for mycorrhization with other *Tuber* species in the soil.

Material and methods
In spring 2004 some seedlings of *Quercus pubescens* Willd. were inoculated with *P. arhizus* and *S. verrucosum* alone and/or with different species of *Tuber* and in all possible combinations.

Tuber melanosporum + *Pisolithus arhizus*
Tuber melanosporum + *Scleroderma verrucosum*
Tuber melanosporum + *Tuber aestivum* + *Pisolithus arhizus*
Tuber melanosporum + *Tuber aestivum* + *Scleroderma verrucosum*
Tuber melanosporum + *Tuber brumale* + *Pisolithus arhizus*
Tuber melanosporum + *Tuber brumale* + *Scleroderma verrucosum*
Tuber melanosporum + *Scleroderma verrucosum* + *Pisolithus arhizus*
Tuber aestivum + *Pisolithus arhizus*
Tuber aestivum + *Scleroderma verrucosum*
Tuber aestivum + *Scleroderma verrucosum* + *Pisolithus arhizus*
Tuber brumale + *Pisolithus arhizus*
Tuber brumale + *Scleroderma verrucosum*
Tuber borchii + *Pisolithus arhizus*
Tuber borchii + *Scleroderma verrucosum*
Pisolithus arhizus
Pisolithus arhizus + *Scleroderma verrucosum*
Scleroderma verrucosum

17 different test inoculums were inoculated in the same conditions and using the same quantity of inoculating material. Two types of substrates were used to raise the plants. The first (To) was made up of artificial components available on the market, namely agriperlite, vermiculite and peat in equal proportions. The second substrate (Te) was made up of a mixture of natural soil and sand in equal proportions. In fall 2004, spring 2005 and spring 2006, the percentage of mycorrhization for each of the species of fungi involved was assessed in 32% of the plants for each test inoculum. Root tips were counted and percentages were calculated for mycorrhizae of each species.

Results and discussion
In this case, the data of mycorrhizae were considered as a total mycorrhization for each species, but not separated for each test.
The presence of mycorrhizae of *Tuber* and other fungi was quite low some months after inoculation, especially in the artificial substrate (To; Fig. 1).

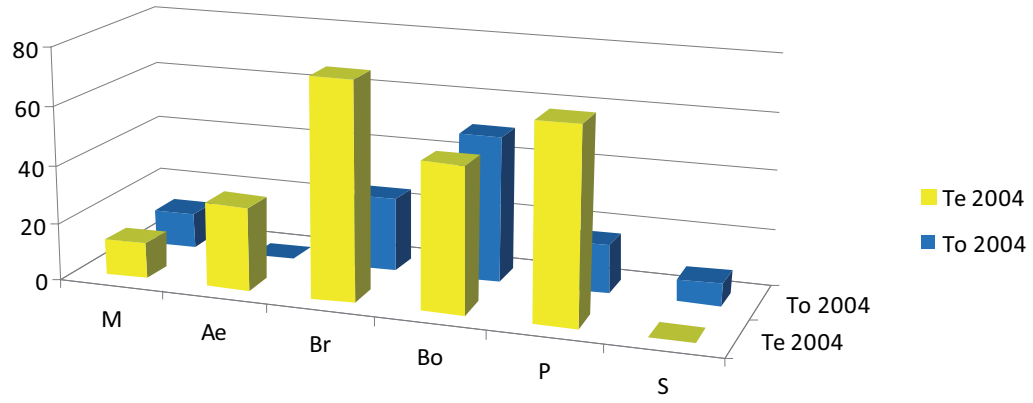

Fig. 1 Percentage of mycorrhized plants some months after inoculation
(M: *T. melanosporum* Vittad.; Ae: *T. aestivum* Vittad.; Br: *T. brumale* Vittad.; Bo: *T. borchii* Vittad.; P: *P. arhizus* (Scop.) Rauschert; S: *S. verrucosum* (Bull.) Pers.)

With *Tuber brumale* and *T. borchii* mycorrhization is very early, as it is with *Pisolithus arhizus*, they are very competitive at the first stage of the process, care is needed in order to avoid contamination with these fungi.
In 2005 and 2006 almost all test inoculums were mycorrhized, mainly with *Tuber* species, in both the natural (Fig. 2) and artificial (Fig. 3) substrates.

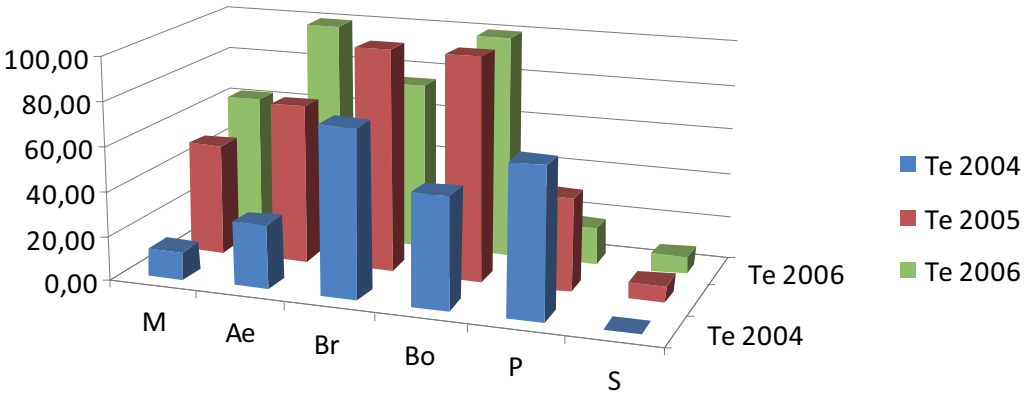

Fig. 2 Percentage of mycorrhized plants in natural substrate (Te)

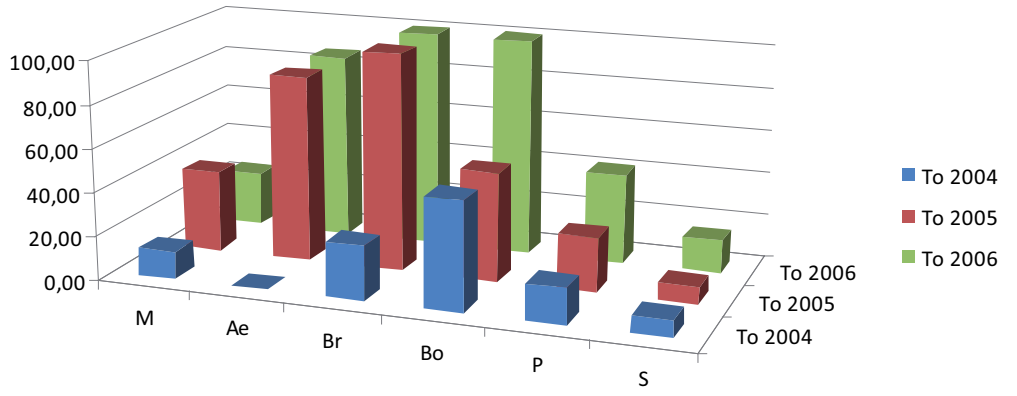

Fig. 3 Percentage of mycorrhized plants in artificial substrate (To)

The artificial substrate, however, seems to limit mycorrhization (Fig. 3), especially with the various species of *Tuber*. For each type of substrate the temporal process of mycorrhization is shown: in Te (Fig. 2) the trend is positive for *Tuber* spp., reaching some very high percentages, while the artificial substrate (To, Fig. 3) shows a positive trend for *P. arhizus* and *S. verrucosum*, which however only reach quite limited percentages of mycorrhization (max 40%). The third year is of course the last opportunity to evaluate mycorrhization in pot; after that the percentage could decrease due to problems with root development and/or polluting substances, even thought the plants are kept in a limited, protected environment.
With regard to the two fungi studied, it can be noted that *Pisolithus arhizus* shows a positive trend during three years in To, whereas in Te the opposite occurs (Fig. 4).

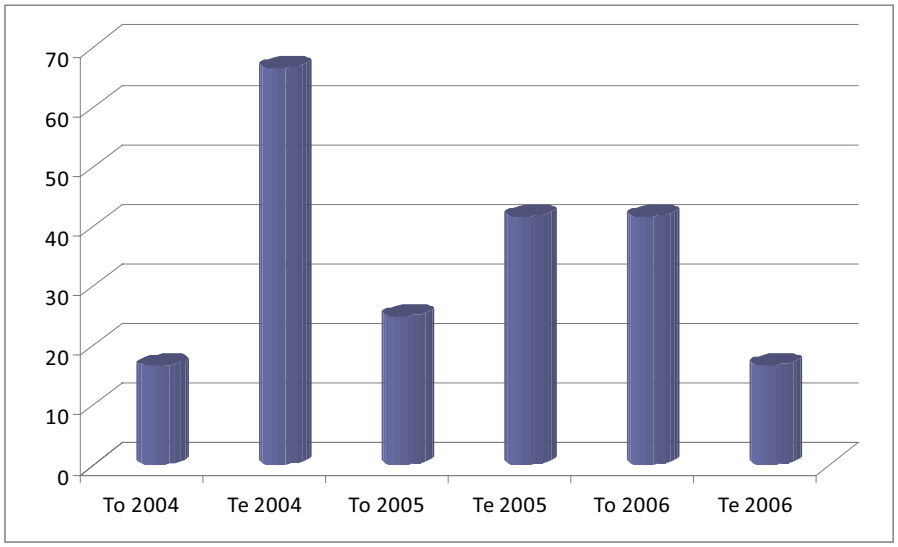

Fig. 4 – Percentage of mycorrhized plants with *Pisolithus arhizus*

The presence of *Scleroderma verrucosum* was low and constant at 7.7%, but it doubled in To during the last analysis (2006; Fig. 5): it seems slower than *Pisolithus arhizus*; the two species are not, therefore, very competitive.

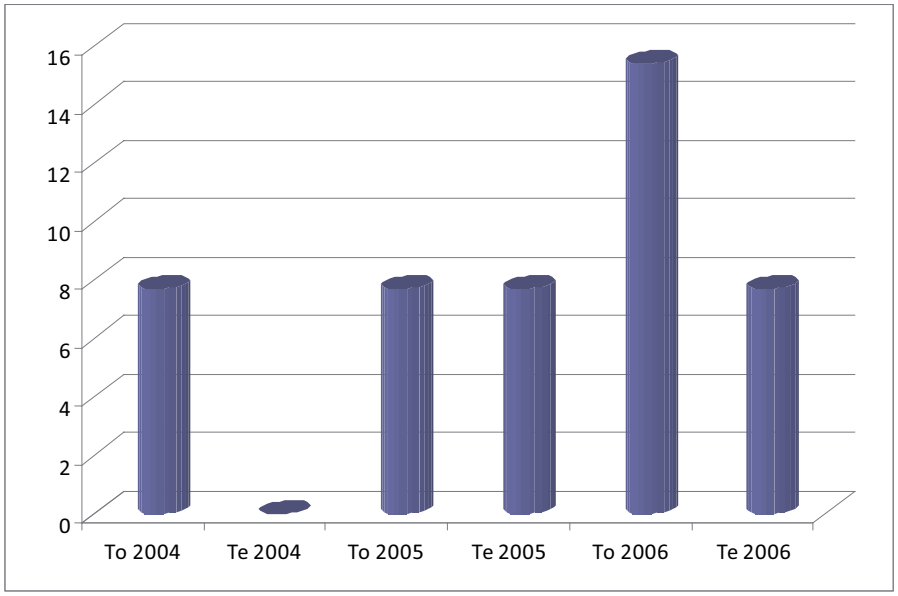

Fig. 5 – Percentage of mycorrhized plants with *Scleroderma verrucosum*

The presence of other *Tuber* species is more significant: the trend is always positive (Fig. 6).

T. borchii and *T. brumale*, in Te and To respectively, are very competitive.
Conversely, *T. melanosporum* is hardly competitive at all when in contact with the other species of *Tuber* and other fungi. Even in Te the percentage of mycorrhization does not exceed an average of 40% in the second year, and subsequently decreases in the third, probably because of the difficult growth conditions being in the same container. In the first year *T. aestivum* is quite slow, but then reaches high and constant levels of mycorrhization in the second year, especially in To.

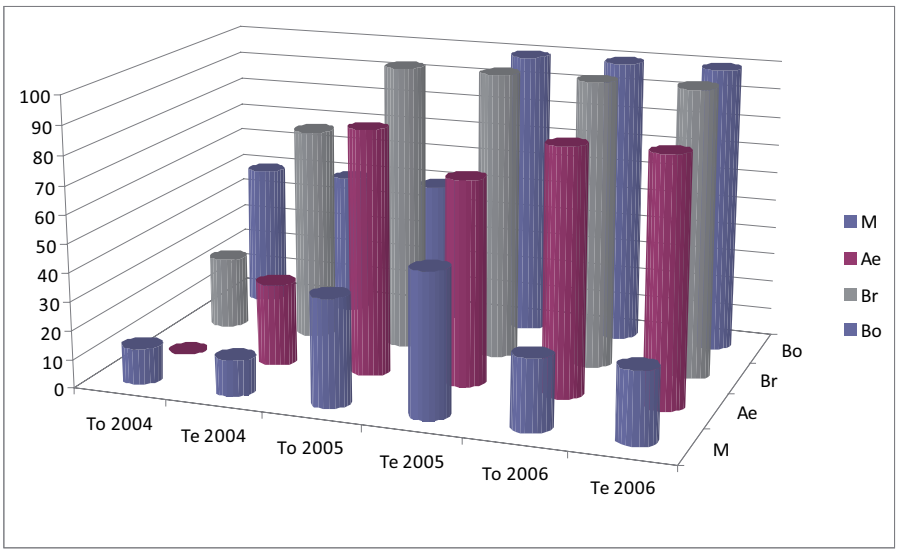

Fig. 6 – Percentage of mycorrhized plants with different species of *Tuber*

Conclusions

The process of mycorrhization with *Tuber melanosporum* and *Tuber aestivum* is rather slow, furthermore the best performance is in Te. This slowness means that, at first, mycorrhization with other competitive fungi is favoured.
Pisolithus arhizus e *Scleroderma verrucosum* are competitive in the initial phases of mycorrhization of young roots, which leads to the hypothesis that they may be able to occupy ecological niches available in the field. However the presence of these fungi in the productive truffle beds concords with the theory by which in field an equilibrium is established between the species present, which is not necessarily harmful for truffles. Despite this, in order to put into practice a truffle cultivating rationale, during the process of mycorrhization in nursery it is very important to work in limited, protected environments, so as to avoid any possible contact between other fungi and the roots of the seedlings. The substrate to choose is preferably the natural one; on the basis of the results obtained and the experimental conditions, this substrate limits mycorrhization with the other fungi and favours the development of mycorrhizae of *Tuber*.

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CARACTERIZACIÓN DE ECTOMICORRIZAS EN ENCINARES PRODUCTORES DE TRUFA NEGRA DEL NORESTE DE SORIA

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Abstract: **Truffled holmoak (*Quercus ilex*) stands in the north-west of Soria (Spain): present ectomycorrhizae**

Black truffle sporocarp production is determined, amongst many other factors, by an optimum mycorrhization degree in the roots of the host-tree. This process is triggered in balance with other fungal species that does not inhibit its sporocarp production. So, there is an ectomycorrhizal fungal community associated with the black truffle sporocarp productive host-trees that runs as any other living beings community, producing a certain fungal biodiversity and establishing connections in dynamic balance that will evolve. The absence of black truffle sporocarps production in some host-trees will be determined by its ectomycorrhiza absence, or because between the fungal community there are one or more species that inhibit this process or displace it. With the aim of study those topics, the ectomycorrhizae present in 23 adult holmoak trees (*Quercus ilex* subsp. *ballota* (Desf.) Samp.) of seven *Tuber melanosporum* Vittad. productive areas in the North-West of Soria (inner Spain) are characterized and quantified. During the spring and the autumn of 1999 and 2000, black truffle productive and non-productive holmoaks were studied following the global method (Verlhac *et al*., 1990, La truffe guide pratique). Ectomycorrhizal types were characterized following the guidelines of Agerer (1999). *Tuber melanosporum*, *T. aestivum* Vittad., *T. brumale* Vittad., *Cenococcum geophylum* Fr., *Pisolithus arrhizus* (Scop.) Rauscher, *Cantharellus tubaeformis* (Bull.) Fr., *Hebeloma* cf. *sinapizans* (Fr.) Sacc., *Tomentella galzinii* Bourdot, AD type, *Cortinarius* sp., *Hebeloma* sp. and *Scleroderma* sp. and many others Telephorales, Tuberales and Boletales ectomycorrhizal types were found. *Tuber melanosporum* mycorrhizae are present both in productive and non-productive host-trees, as it happens for *T. aestivum*, while *T. brumale* ectomycorrhizae are only present in non-productive holmoaks. The rest of identified ectomycorrhizal types are present in productive and non-productive host-trees.

Key words: ectomycorrhizae, ecology, population biology.

Introducción y Objetivos

La producción de trufas negras se determinada, entre otros muchos factores, por un grado de micorrización optimo entre las raíces del árbol hospedante y el hongo. Éste proceso se desencadena en equilibrio con otras especies de hongos que no inhiben la producción de los carpóforos. La comunidad de hongos ectomicorrícicos asociados a las raíces de los árboles hospedantes productores de trufas negras se comporta como cualquier otra comunidad de seres vivos, evolucionando en su grado de biodiversidad y estableciendo conexiones en equilibrio dinámico.

La ausencia de producción de trufas negras en algunos árboles hospedantes puede estar determinada por la ausencia de sus ectomicorrizas, o porque entre los hongos que forman la comunidad existe una o más especies que inhiben este proceso o lo interrumpen.

La zona de estudio se sitúa en la comarca del Noreste de Soria, cuya superficie potencialmente trufera es 22318 ha, el 42% del total de su superficie forestal. Estos montes carecen de otro tipo de aprovechamiento forestal, considerándose en los mapas de productividad potencial

como no productivos. La superficie potencialmente trufera para el total de la provincia de Soria es de 65000 ha (Mapa, 1995), lo que representa casi el 20% de la superficie forestal provincial.
En la primavera de 2000 se comenzaron a estudiar las ectomicorrizas presentes en estos encinares paralelamente a la ejecución de diversos trabajos selvícolas experimentales, con el objeto de obtener porcentajes de micorrización de *Tuber melanosporum* Vittad. que indiquen la presencia de este hongo en las raíces de las plantas y las especies ectomicorrícicas competidoras. Además, se han muestreado plantas no productoras de trufa con el objeto de conocer su composición micorrícica y contrastarla con la de las productoras.

Material y Métodos
La comarca del Noreste de Soria se encuentra a una altitud media de 1024 m, las truferas aparecen en zonas de pendiente variable y orientación predominante de solana, y los terrenos sobre los que se encuentran se caracterizan por ser poco evolucionados, con abundante pedregosidad y poco contenido en arcillas, situados sobre litología caliza o areniscas con cemento calizo.
El clima, según la clasificación de Allué (1990), corresponde al tipo IV(VI), meso-xerofítico de inviernos frescos, con precipitaciones anuales entre 425 y 600 mm, con 54 mm en la época estival. La temperatura media anual oscila entre 9 y 12 ºC, con un invierno muy riguroso y veranos muy calurosos, con fuertes variaciones de temperatura a lo largo del día.
Las series de vegetación potencial según Rivas Martínez (1987) son *Cephalantero longifoliae-Querceto faginae sigmetum*, serie supramediterránea castellano-alcarreño-manchega basófila de *Quercus faginea* o quejigo, y *Junipero thuriferae-Querceto rotundifoliae sigmetum*, serie supramediterránea castellano-maestrazgo-manchega basófila de *Quercus rotundifolia* o encina.
La vegetación actual se corresponde con montes bajos de encina (*Quercus ilex* L. subsp. *ballota* (Desf.) Samp.), orientados, en el pasado, hacia la obtención de leñas y carbones. Las especies arbustivas más frecuentes son: jaras (*Cistus ladanifer* L.), romeros (*Rosmarinus officinalis* L.), majuelos (*Crataegus monogyna* Jacq.), escaramujos (*Rosa* sp.), aliagas (*Genista scorpius* (L.) DC.), espliegos (*Lavandula latifolia* Med.), y tomillos (*Thymus vulgaris* L.) (Oyaregui, 1994).
Se eligieron 23 árboles de la zona de estudio situados en los términos municipales de Alconaba, Esteras del Campo, Hinojosa del Campo, La Quiñonería, Tajahuerce, Tardajos de Duero y Pinilla del Campo. Para su elección se tuvieron en cuenta los resultados de producción de *Tuber melanosporum* durante el invierno de 1999-2000.
Se realizaron dos muestreos anuales, uno en primavera y otro en otoño, siguiendo el método global (Verlhac *et al.*, 1990), se toman dos muestras en direcciones opuestas, preferentemente con orientación Sur-Norte en la zona superficial (10-20 cm) cercana al árbol, sin dañarlo.
Tras su extracción las muestras se conservan en cámara de frío a 4ºC hasta su estudio en laboratorio, para ello, se lavan ligeramente y se introducen en el baño de ultrasonidos durante quince minutos, tras reposar durante 24 horas en frío se repite la operación. Si es necesario se completa la limpieza con ayuda de pinceles y agujas.
Bajo la lupa binocular se procede al conteo de los ápices, diferenciando entre los no micorrizados y los micorrizados, y de entre estos, los micorrizados con *Tuber melanosporum* y con otros hongos. Las micorrizas no identificadas se conservan con FAA (Verlhac *et al.*, 1990) para su posterior caracterización.

Resultados
Tras tres muestreos, realizados en la primavera y el otoño de 2000 y en la primavera de 2001, los resultados obtenidos en cuanto a porcentajes de micorrización se resumen en la Tabla 1.

Tab. 1 Porcentajes de micorrización según la categoría de producción en los muestreos de primavera de 2000 (P00), otoño de 2000 (I00) y primavera de 2001 (P01).

Categoría de producción	Fecha	% Micorrizas	% Micorrizas *T. melanosporum*	% Otras micorrizas
NO PRODUCTIVOS	P00	43,27	6,69	93,31
	I00	31,75	0	100
	P01	47,95	0,60	99,40
PRODUCTIVOS	P00	71,30	21,78	78,22
	I00	61,41	5,11	94,89
	P01	71,68	7,46	92,54
MUY PRODUCTIVOS	P00	76,10	45,52	54,48
	I00	49,16	3,95	96,05
	P01	72,45	5,03	94,97
TOTAL	P00	64,44	27,99	72,01
	I00	48,69	3,73	96,27
	P01	64,02	5,01	94,99

Se observa que si bien los porcentajes de micorrizas de *Tuber melanosporum* son superiores en las encinas productoras, también se encuentran estas micorrizas en encinas no productoras. Las ectomicorrizas identificadas en cada muestreo y su abundancia respecto al total de árboles se indican en la Tabla 2.

Tab. 2 Principales tipos de ECM identificados en los tres muestreos y grado de presencia.

	Tuber melanosporum	*Tuber aestivum*	*Tuber brumale*	*Cenococcum geophilum*	Tipo AD	Tipo *Hebeloma*	Tipo *Scleroderma*	Tipo *Cortinarius*	Tipo SB	OTRAS
Primavera 2000										
Otoño 2000										
Primavera 2001										

	> 80%		60-80%		40-60%		20-40%		< 20%

Se observa que del conjunto de las ectomicorrizas identificadas, la más frecuente es *Tuber melanosporum*.

Se describen las características de los morfotipos de las ectomicorrizas identificadas y no identificadas más frecuentes, siguiendo las directrices marcadas por Agerer (1991) y Agerer & Rambold (2004-2006). Para la posterior identificación de los morfotipos de ectomicorrizas se utilizaron, entre otros, los trabajos de Agerer (1987-2002), Agerer *et al.* (1996-2001), Agerer & Rambold (2004-2006), Bencivenga *et al.* (1995), De Román *et al.* (1999); De Román y De

Miguel (2000), De Román (2003), De Román & De Miguel (2005), Donnini & Bencivenga (1995), Etayo (2001), Goodman *et al*. (1996-2000), Ingleby *et al.* (1990), Jakucs *et al.* (2005), Tedersoo *et al.* (2006) y Voiry (1981).

Descripción de Ectomicorrizas

***Cantharellus tubaeformis* (Bull.) Fr.**
Color amarillento, que pasa a marrón oscuro con el tiempo, superficie lisa, ramificación irregular. Manto plectenquimatoso con hifas irregulares, a veces hasta epidermoides, con numerosas gotas de contenidos oleosos. Hifas que emanan escasas, rectas, cubiertas de sustancias gelatinosas, con fíbulas. Rizomorfos ausentes. Cistidios ausentes.

***Cenococcum geophilum* Fr.**
Color negro, superficie fibrosa brillante, raramente ramificada. Manto plectenquimatoso con hifas dispuestas en forma de estrella, correspondiente al tipo G. Hifas que emanan frecuentes, de color marrón muy oscuro o negro, rectas y septadas, sin fíbulas. Rizomorfos y cistidios ausentes. Esclerocios abundantes, negros, esféricos, que no se encuentran unidos a la micorriza.

***Hebeloma* cf. *sinapizans* (Fr.) Sacc.**
Color marrón-blanquecino, algodonosa, ramificación irregular. Manto plectenquimatoso, hialino, con algunas vesículas redondeadas anaranjadas. Hifas muy abundantes, hialinas, cortas, con algunas anastomosis de tipo a_1, con fíbulas muy gruesas y marcadas. Rizomorfos muy abundantes, muy lanosos, aplanados, con la cubierta muy semejante a la del manto. Cistidios ausentes.

***Pisolithus arhizus* (Scop.) Rauscher**
Color marrón anaranjado, superficie lanosa, ramificación simple a irregular. Manto plectenquimatoso, con hifas onduladas bastante uniformes. Hifas que emanan comunes, con muchas anastomosis de contacto y fíbulas. Rizomorfos abundantes, lanosos, muy diferenciados, con numerosas acumulaciones de células globosas en la superficie. Cistidios ausentes.

***Tomentella galzinii* Bourdot**
Color marrón claro, superficie espinosa, ramificación simple a monopodial pinnada. Manto pseudoparenquimatoso, con células poligonales de tipo L. Hifas que emanan y rizomorfos ausentes. Cistidios aciculares formados por dos células separadas por un septo con fíbula.

***Tuber aestivum* Vittad.**
Color marrón-dorado, superficie lanosa, ramificación simple o monopodial pinnada. Manto pseudoparenquimatoso, con células poligonales correspondiente al tipo L. Hifas que emanan y rizomorfos ausentes. Cistidios largos, sinuosos y enredados entre sí, con vesículas intercalares o terminales.

***Tuber brumale* Vittad.**
Color marrón-ámbar, superficie espinosa, ramificación simple o monopodial pinnada. Manto pseudoparenquimatoso, con células en forma de puzzle, correspondiente al tipo M. Hifas que emanan muy escasas, sinuosas, con algunos acodos, sin fíbulas. Rizomorfos ausentes. Cistidios abundantes en forma de aguja, con la base ensanchada, cortos con paredes gruesas, sin ramificar y, a veces, con un septo en la base.

***Tuber melanosporum* Vittad.**
Color marrón-dorado, superficie lisa, solitaria o, a veces, formando glomérulos. Manto

pseudoparenquimatoso, con células en forma de puzzle, correspondiente al tipo M. Hifas que emanan y rizomorfos ausentes. Cistidios escasos, ramificados en ángulo recto en la zona cercana al manto, hialinos, sin fíbulas.

Tipo AD (Angle Droit; Giraud, 1988)
Color ocre, superficie lanosa, ramificación simple o irregularmente pinnada. Manto pseudoparenquimatoso con células poligonales de tipo L. Hifas que emanan y rizomorfos ausentes. Cistidios muy abundantes, muy ramificados en ángulo recto, rígidos, sin fíbulas.

Tipo *Cortinarius*
Color blanquecino, superficie algodonosa, ramificación irregular. Manto plectenquimatoso con hifas formando rosetas. Hifas que emanan hialinas, muy abundantes, con numerosas anastomosis y con fíbulas. Rizomorfos gruesos, ramificados en Y. Cistidios ausentes.

Tipo *Hebeloma*
Color blanquecino, superficie algodonosa, simples o con ramificación irregular. Manto plectenquimatoso con las hifas hialinas. Hifas que emanan muy abundantes, largas, hialinas, acodadas en algunos casos, con fíbulas muy marcadas. Rizomorfos abundantes, gruesos e hialinos. Cistidios ausentes.

Tipo *Scleroderma*
Blanquecina o marrón muy claro, superficie algodonosa, ramificación piramidal. Manto plectenquimatoso con las hifas sin organizar. Hifas que emanan muy abundantes, curvadas, formando anillos, hialinas, a veces con fíbulas. Rizomorfos abundantes, gruesos, rodeados de hifas que se curvan formando anillos. Cistidios ausentes.

Tipo Tuberal
Dorada o marrón-anaranjada, superficie lisa, solitaria o con ramificación monopodial. Manto pseudoparenquimatoso, tipo L o M, con las células bien definidas. Hifas que emanan ausentes. Rizomorfos ausentes. Cistidios ausentes.

Morfotipo VALONSADERO-MYCORRHIZA 006 (Teleforoide; Águeda *et al.,* 2003)
Color marrón oscuro, superficie fibrosa y brillante, ramificación piramidal con los ápices algo curvados y bastante aglomerada por micelio. Manto externo pseudoparenquimatoso poligonal de tipo L, manto interno plectenquimatoso muy denso. Hifas que emanan comunes, ligeramente tortuosas, con la superficie un poco punteada, con fíbulas muy marcadas, pero no en todos los septos. Rizomorfos ausentes. Cistidios abundantes, muy largos, con la base muy dilatada y septos comunes sin fíbulas.

Morfotipo VALONSADERO-MYCORRHIZA 007 (Boletal; Águeda *et al.,* 2003)
Color marrón-anaranjado, superficie algodonosa, ramificación simple o irregular, en algunos casos aglomeradas por micelio y rizomorfos muy blancos. Manto plectenquimatoso muy laxo, hialino. Hifas emergentes muy abundantes, hialinas, lisas, con numerosas anastomosis de tipo a_1, los septos algo estrechados y siempre ramificadas en los septos. Sin fíbulas. Rizomorfos abundantes, hialinos, de tipo F, con hifas diferenciadas, en las ramificaciones presentan aglomeraciones de hifas enmarañadas. Cistidios ausentes.

Morfotipo VALONSADERO-MYCORRHIZA 008 (Teleforoide; Águeda *et al.,* 2003)
Color marrón-anaranjado, superficie afieltrado-lanosa, brillante, ramificación de irregularmente pinnada a piramidal, muy aglomeradas por micelio. Manto muy denso, confuso, con gotas de exudados. Hifas que emanan muy abundantes, gruesas, con espinas grandes muy abundantes, con fíbulas, pero no en todos los septos. Rizomorfos ausentes. Cistidios abundantes, hialinos, muy finos, algo ensanchados en la base.

Morfotipo VALONSADERO-MYCORRHIZA 009 (Teleforoide; Águeda *et al.,* 2003)
Color marrón-anaranjado, superficie afieltrada mate, ramificación piramidal, aglomeradas ligeramente por micelio. Manto pseudoparenquimatoso, de células muy claras, triangulares, formando hexágonos. Hifas comunes, tortuosas, con algunos acodos, paredes bastante gruesas y algo punteadas, con fíbulas muy marcadas. Rizomorfos ausentes. Cistidios de dos tipos, los primeros comunes, hialinos, con septos pero sin fíbulas, presentes solamente en ápices jóvenes; los segundos muy escasos, gruesos y cortos.

Morfotipo VALONSADERO-MYCORRHIZA 015 (Teleforoide; Águeda & Fernández Toirán, 2004)
Color marrón oscuro, superficie lanosa con muchas hifas que emanan. Manto plectenquimatoso, tipo I, cubierto de abundantes vesículas. Hifas que emanan muy abundantes, acodadas, con fíbulas. Rizomorfos ausentes. Cistidios comunes, cortos, en forma de botella, con el cuello ondulado.

Morfotipo VALONSADERO-MYCORRHIZA 016 (Teleforoide; Águeda & Fernández Toirán, 2004)
Marrón oscuro, ondulada, superficie algodonosa y brillante, simple, con rizomorfos lanosos. Manto pseudoparenquimatoso, con células poligonales de paredes muy gruesas. Hifas que emanan comunes, rectas, con la superficie ligeramente punteada, sin fíbulas. Rizomorfos comunes, muy diferenciados, cubiertos de los mismos cistidios que la micorriza. Cistidios largos, ondulados, lisos, algunos con vesículas terminales.

Morfotipo VALONSADERO-MYCORRHIZA 017 (Teleforoide; Águeda & Fernández Toirán, 2004)
Color naranja, superficie algodonosa, sin ramificar y con forma cónica. Manto pseudoparenquimatoso de tipo L, con células pentagonales y triangulares. Hifas que emanan muy abundantes, onduladas, con fíbulas muy poco marcadas. Rizomorfos ausentes. Cistidios largos y lisos, con la base ensanchada y el ápice redondeado.

Morfotipo VALONSADERO-MYCORRHIZA 018 (Teleforoide; Águeda & Fernández Toirán, 2004)
Color marrón negruzco, superficie granulosa, irregularmente ramificada, cubierta de hifas. Manto plectenquimatoso, muy grueso, cubierto de vesículas redondeadas. Hifas que emanan abundantes, lisas, tortuosas, algunas en forma de sacacorchos, con las fíbulas muy marcadas. Rizomorfos ausentes. Cistidios ausentes.

Morfotipo VALONSADERO-MYCORRHIZA 045
Marrón anaranjada, superficie algo granulosa, ramificación piramidal, hifas que emanan y rizomorfos abundantes. Manto plectenquimatoso, de tipo Q, cubierto de una red de hifas con protuberancias. Hifas que emanan gruesas, espinosas, rojizas, con gotas de exudados muy abundantes, con fíbulas. Rizomorfos gruesos, cubiertos de cistidios finos, curvados y largos. Cistidios muy finos, hialinos, largos y tortuosos.

Morfotipo VALONSADERO-MYCORRHIZA 046
Color marrón rojizo muy oscuro, superficie granulosa, ramificación simple, hifas que emanan y rizomorfos muy abundantes. Manto externo pseudoparenquimatoso, de tipo K, con montones de células redondeadas, manto interno plectenquimatoso muy denso. Hifas que emanan hialinas, gruesas, ramificadas en ángulo recto, con fíbulas muy marcadas. Rizomorfos muy gruesos, con hifas emergentes finas. Cistidios de tipo B, largos, recios, con la base muy engrosada.

Morfotipo VALONSADERO-MYCORRHIZA 047
Color marrón grisáceo, superficie lanosa, ramificación irregular, hifas que emanan y rizomorfos muy abundantes, blancos. Manto externo plectenquimatoso, muy laxo, formando anillos de hifas, de tipo A. Hifas que emanan muy abundantes, hialinas, lisas, muy ramificadas, a veces con fíbulas. Rizomorfos abundantes, sin hifas diferenciadas, hialinos, lisos, con hifas dilatadas en los septos. Cistidios ausentes.

Morfotipo VALONSADERO-MYCORRHIZA 048
Color marrón-rojizo, superficie fibroso-lanosa, algo tortuosa, ramificación irregular. Manto pseudoparenquimatoso, formado por células triangulares cubiertas de una matriz gelatinosa. Hifas que emanan hialinas, espinosas, acodadas, con fíbulas. Rizomorfos gruesos, sin hifas diferenciadas. Cistidios ausentes.

Morfotipo VALONSADERO-MYCORRHIZA 049
Marrón-rojiza muy oscura, superficie algo granulosa, ramificación piramidal. Manto plectenquimatoso con montones de células redondeadas, tipo F. Hifas que emanan ausentes. Rizomorfos lineales con muchas hifas que emanan. Cistidios comunes, con base esférica.

Discusión y Conclusiones
La producción de carpóforos de especies micorrícicas requiere de la presencia de un grado de micorrización óptimo en las raíces de un árbol. Éste, se produce en equilibrio con otras especies competidoras que, no inhiben su fructificación. Es decir, hay un cortejo de hongos ectomicorrícicos asociados a las encinas productoras de trufa negra que se comportan como cualquier comunidad de seres vivos, dando lugar a una determinada biodiversidad fúngica y estableciendo relaciones en equilibrio dinámico que evolucionarán a lo largo del tiempo. Por el contrario, la no fructificación de la trufa en algunas encinas vendrá determinada por la ausencia de sus micorrizas o porque entre los hongos competidores hay alguno que inhibe este proceso o acaba desplazándola.
Es destacable la presencia de *Tuber melanosporum* en la mayor parte de los árboles muestreados, independientemente de su categoría de producción de trufas negras. Se obtienen porcentajes de micorrización por *Tuber melanosporum* más elevados en los dos muestreos de primavera que en el de invierno, tal y como se aprecia en la Tabla 1. Solamente en uno de los árboles estudiados, una encina no productora en Pinilla del Campo, no aparecen micorrizas de este tuberal. El único morfotipo identificado en este árbol es el Tipo *Hebeloma*. Según algunos autores esta micorriza desplazaría a la de trufa (Águeda *et al.*, 2001). *Tuber aestivum* aparece en árboles productores y no productores, mientras que *T. brumale* aparece solamente en árboles no productores. Se considera que ambas especies provocarían el desplazamiento de *T. melanosporum* en los árboles en los que aparecen en las plantaciones, aunque el porcentaje encontrado, en este caso, sea muy bajo.
El Morfotipo VALONSADERO-MYCORRHIZA045 presenta un manto grueso, hifas con fíbulas muy marcadas, y cistidios abundantes lo que, unido a las coloraciones oscuras con tonalidades rojizas, son características de los Telephorales (Raidl & Müller, 1996). Las exudaciones que aparecen en las paredes externas de las hifas es una singularidad no compartida con ninguno de los morfotipos de éste grupo descritos hasta el momento.
Los Morfotipos VALONSADERO-MYCORRHIZA046, VALONSADERO-MYCORRHIZA048 y VALONSADERO-MYCORRHIZA049 comparten estos caracteres comunes al género *Tomentella*. El Morfotipo VALONSADERO-MYCORRHIZA048 comparte caracteres con varios de los tipos descritos por De Román (2003) en los encinares navarros, todos ellos pertenecientes al género *Tomentella* o muy próximos a él.
El Morfotipo VALONSADERO-MYCORRHIZA047 corresponde a un hongo del género *Cortinarius*, ya que presenta rizomorfos muy poco evolucionados, hialinos, manto plectenquimatoso laxo e hifas que emanan con fíbulas, lo que es una característica típica del género. Agerer (1999) describe las micorrizas del género *Cortinarius* como ligeramente curvadas, incluso tortuosas, con abundantes rizomorfos.
Del total de micorrizas competidoras caracterizadas, destaca el hecho de que dos son especies del género *Tuber*. También aparece *Cenococcum geophilum*, especie cosmopolita, ampliamente citada y bien adaptada por sus estrategias (hifas y esclerocios) a los ecosistemas mediterráneos.
Es remarcable la presencia de micorrizas de tipo AD, más habitual en plantaciones que en masas naturales productoras de trufa negra. Algunos autores apuntaron su posible correspondencia

a un morfotipo Telephoral (De Román, 2003). Recientemente ha sido descrito y denominado *Quercirhiza quadratum* por Águeda *et al.,* 2008, trabajo que ha revelado su identidad de Ascomycete Pezizal Pyronemataceae del género *Trichophaea.*
Destaca también la abundancia de micorrizas competidoras de tipos teleforoides. No parecen restar capacidad productora a las truferas. En el presente trabajo aparecen descritos 12 tipos pertenecientes a dicho orden, algunos de éstos presentan un alto grado de micorrización, hecho que está de acuerdo con la abundancia de estas micorrizas tanto en formaciones naturales de encina en Navarra (De Román & De Miguel, 2005, Clavería Y De Miguel, 2005), como en plantaciones truferas de toda España (De Miguel *et al.,* 2006).
En la provincia de Soria solamente se han recolectado 10 especies de carpóforos pertenecientes a los Telephorales (Pando, 2000), ya que son un grupo cuya fructificación es bastante difícil de observar. Sin embargo, las características morfológicas de sus ectomicorrizas son lo suficientemente específicas, representativas y diversas para ser uno de los grupos de más sencilla identificación anatómico-morfológica.
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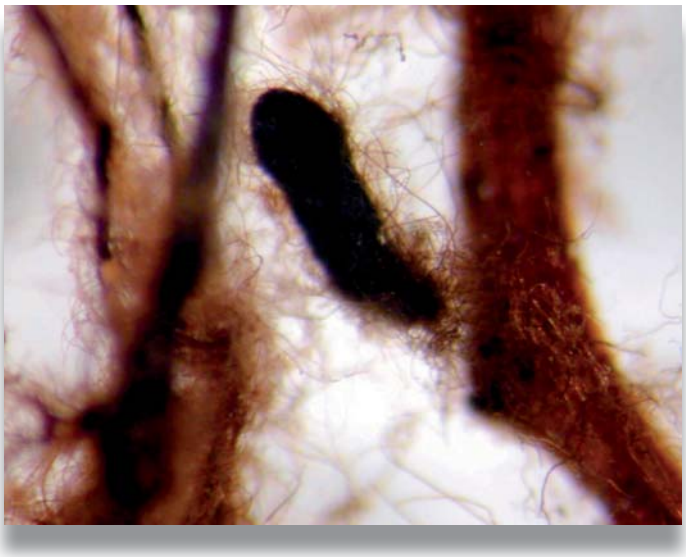
Figura 1. *a) Morfotipo VALONSADERO-MYCORRHIZA045, micorriza piramidal (20X); b) Morfotipo VALONSADERO-MYCORRHIZA045, hifa con gotas de exudados (1000X); c) Morfotipo VALONSADERO-MYCORRHIZA046, micorriza con hifas que emanan y rizomorfos (20X); d) Morfotipo VALONSADERO-MYCORRHIZA046, cistidio con la base engrosada (1000X); e) Morfotipo VALONSADERO-MYCORRHIZA047, micorriza lanosa (20X); f) Morfotipo VALONSADERO-MYCORRHIZA048, rizomorfos (1000X); g) Morfotipo VALONSADERO-MYCORRHIZA049, micorriza granulosa (20X); h) Morfotipo VALONSADERO-MYCORRHIZA049, manto con montones de células (1000X).*

a



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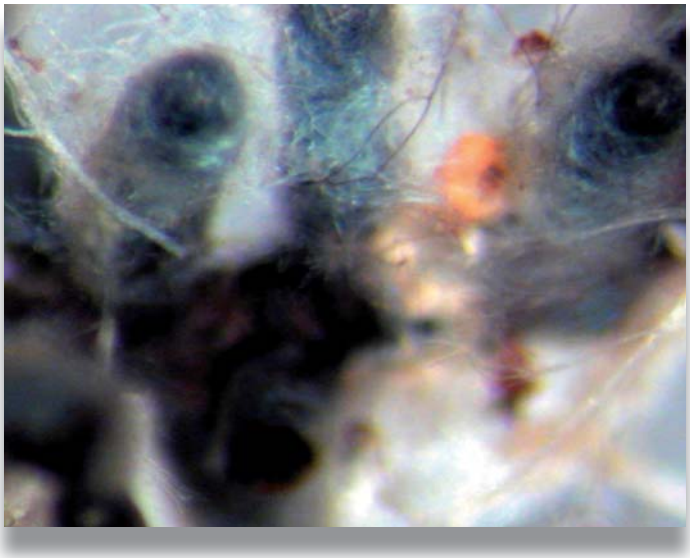

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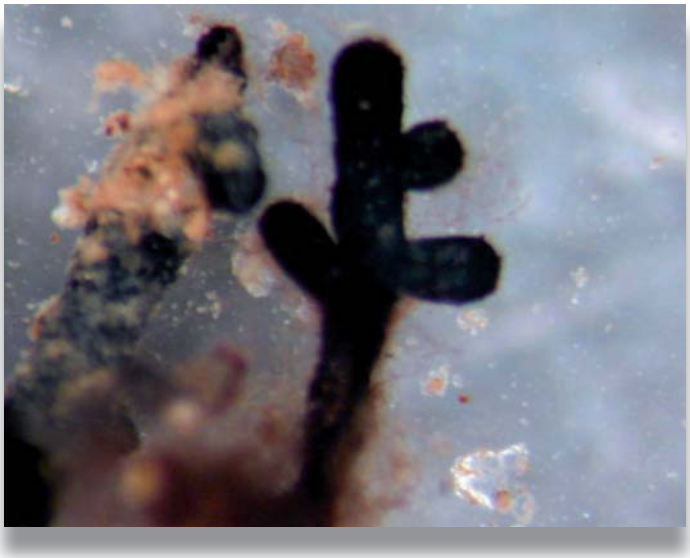

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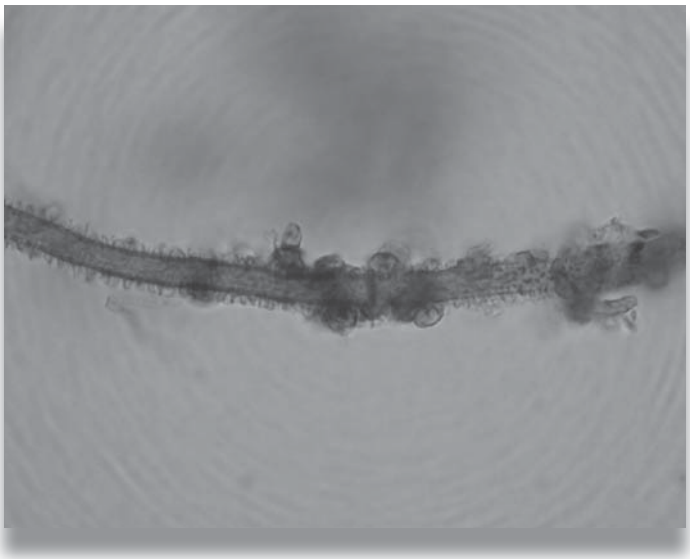

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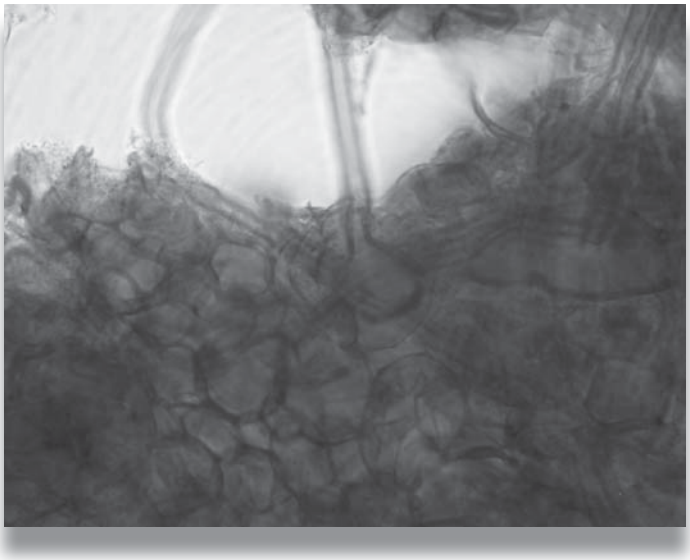

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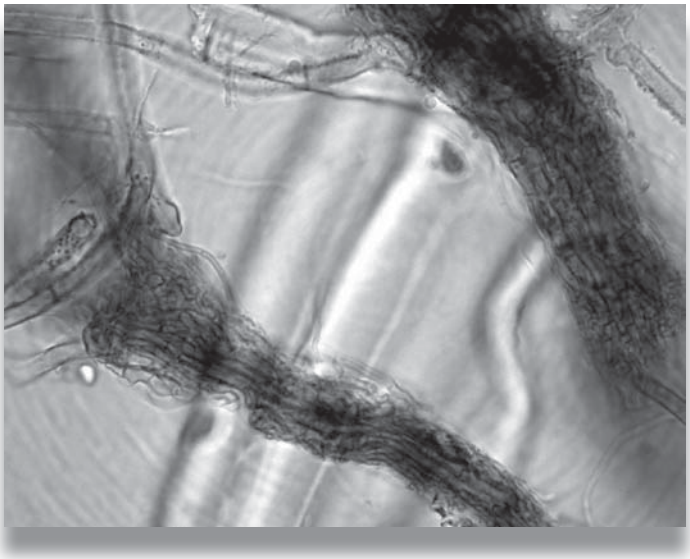

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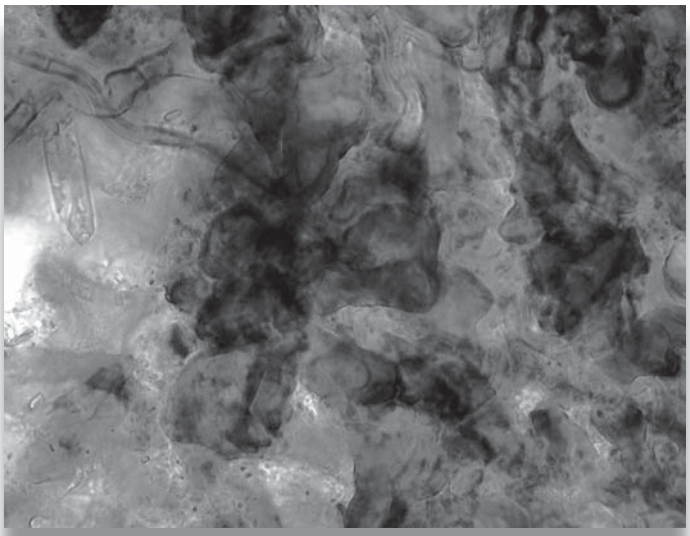

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TUBER MELANOSPORUM GROWTH-STIMULATING EFFECTS FROM VARIOUS *CISTACEAE* SPECIES

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Abstract
In vitro culture of slow-growing ectomycorrhizal fungi can be fastened through dual (monoxenic) culture with its own natural hosts, as it has been already demonstrated by using *Agrobacterium rhizogenes*-transformed root organ cultures to stimulate growth of *Tuber melanosporum* (Wenkart *et al*., 2001; Coughlan *et al*., 2001) or *Terfezia boudieri* (Kagan-Zur *et al*., 2003) mycelia. Most studies are currently limited to a single host species: *Cistus incanus*. By transforming seedlings of *Cistus* and *Helianthemum* with *Agrobacterium rhizogenes* strain A4, we demonstrated they also stimulate *in vitro* mycelium growth of *Tuber melanosporum*, although this effect was lowered by sugar concentration. *Helianthemum* transformed roots served as a control, indicating the stimulation is due to taxa-specific compounds more likely to be present in common truffle-mycorrhizal species.
Key words: *Cistus*, transformed roots, *in vitro.*

Introduction

In vitro culture of slow-growing ectomycorrhizal fungi can be fastened through dual (monoxenic) culture with their own natural hosts, as it has been already demonstrated by using *Agrobacterium rhizogenes*-transformed root organ cultures to stimulate growth of *Tuber melanosporum* mycelia (Wenkart *et al*., 2001; Coughlan *et al*., 2001) and *Terfezia boudieri* (Kagan-Zur *et al*., 2003). Most studies are currently limited to a single host species: *Cistus incanus*. This work aims to test *in vitro* culture effects of distinct species in the genus *Cistus* and *Helianthemum* on *Tuber melanosporum* mycelium growth.

Material and methods

Cistus albidus, *Cistus laurifolius*, *Cistus salvifolius* and *Helianthemum* spp. seedlings were germinated by heat shock in boiling water. Explants cut from 3-week-old seedlings were inoculated with *Agrobacterium rhizogenes* liquid cultures either by direct inmersion in 25ml of the mixture or previously wounding the explant with a syringe needle all over the surface and vascular system. Following an incubation period of 2-5 days, explants were properly washed in an 800 mg L-1 solution of the antibiotic cefatoxime and transferred to a 400 mg L-1 cefatoxime-supplemented MS agar culture medium. This washing protocol was repeated twice before roots were routinely grown in MS liquid medium.

Tuber melanosporum Vittad. strains AHTM08A, AHTM08B and AHTM0726 were grown in 90 mm Petri plates in presence and absence of *Cistus laurifolius*, on WM culture medium (Bécard & Fortin, 1988) amended with 0, 0.3, 3 or 30 g L-1 sucrose. WM salts were also employed as culture medium to test the other *Cistus* and *Helianthemum* species as *Tuber melanosporum* growth-stimulators.

Results & Discussion

Root organ cultures of all species were successfully established, while *Helianthemum* and *Cistus salvifolius* grew faster than *Cistus laurifolius* and *Cistus albidus*. No mycorrhizal association could be synthesized between *Tuber melanosporum* and its potential hosts in either modified WM medium or M medium. However, a conspicuous effect of root organ cultures on *Tuber melanosporum* mycelium growth rate was recorded, and sugar concentration couldn't mimick this effect (Fig.1). 30 g L-1 sucrose-amended culture media seemed to reduce growth

stimulation, may be due to root active growth and modification of the culture medium pH or a change in the production of stimulating compounds.

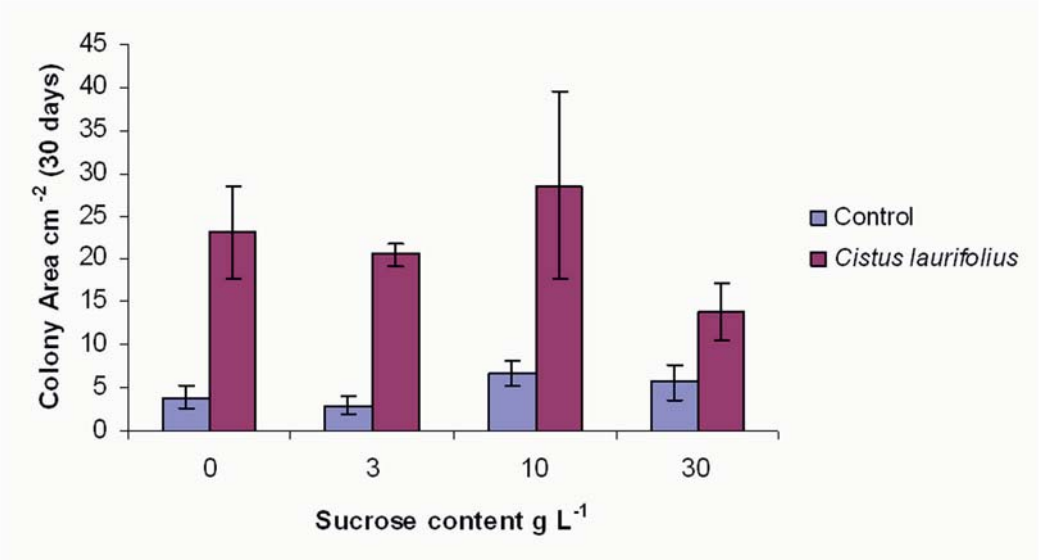

Fig. 1 Average *Tuber melanosporum* mycelium-colonized area in axenic (Control) and monoxenic (*Cistus laurifolius*) culture in relation with sucrose content.

Every *Cistus* species tested showed stimulating effects on black truffle mycelium growth, while *Helianthemum* increased *Tuber melanosporum* growth to a lesser degree, but still more than controls where no roots nor sugar was added (Fig.2, Fig.3). This means that either a small quantity of sucrose is introduced with root inoculation or that *Helianthemum* transformed roots have a lower but still present stimulatory effect on black truffle growth. Average growth rates of control and *Cistus*-stimulated *Tuber melanosporum* were comparable to those of Coughlan & Piché (2005) using *Cistus incanus*, so we conclude there's a common black truffle-stimulating biochemistry in the genus.

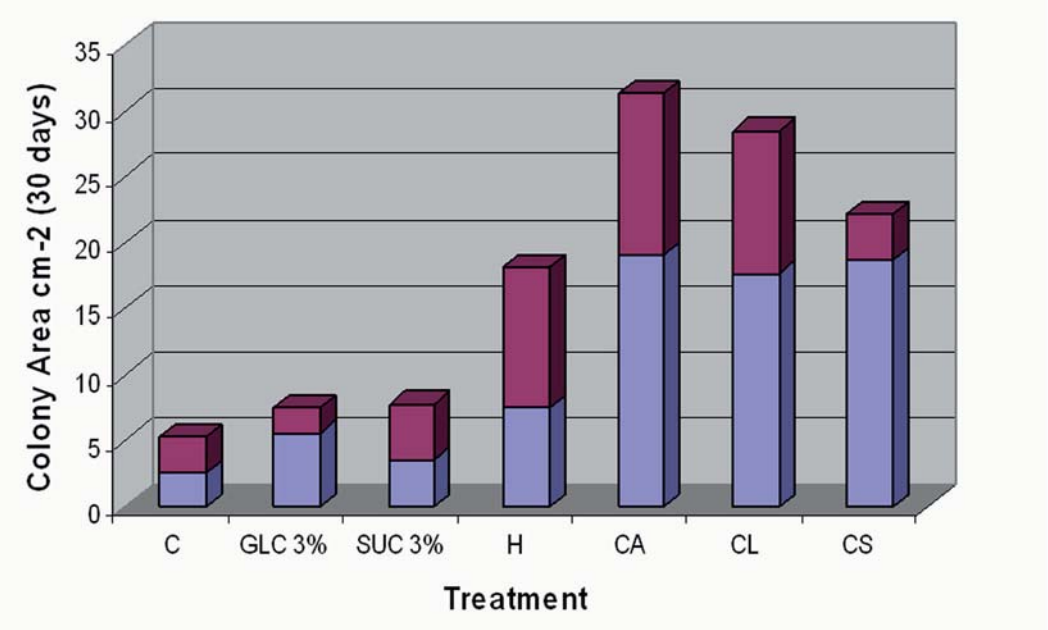

Fig. 2 Average *Tuber melanosporum* mycelium-colonized area in presence of *Helianthemum* sp. (H), *Cistus albidus* (CA), *Cistus laurifolius* (CL) and *Cistus salvifolius* (CS). Controls included: WM with no sugars (C), WM with 30 g L-1 glucose (GLC 3%) and WM with 30 g L-1 sucrose (SUC 3%). Upper volumes represent two-fold standard deviation.

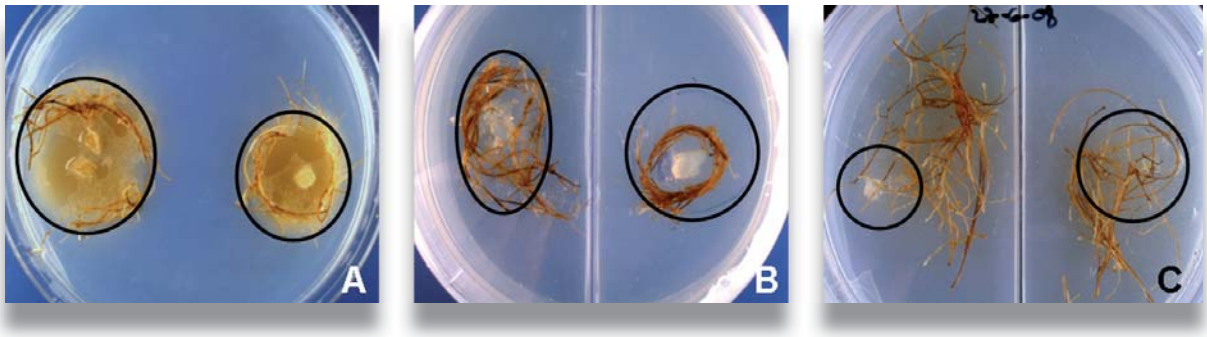

Fig. 3 Petri-dish cocultures of *Cistus albidus* (A), *Cistus laurifolius* (B) and *Helianthemum* sp. (C), with *Tuber melanosporum* (30 days). Mycelium colonized area is highlighted.

No effects of *Cistus laurifolius* transformed roots could be observed on *Tuber melanosporum* spores grown on WM-salts or water-agar medium after 3 months. Other fungi as *Amanita caesarea* or *Amanita ponderosa* behaved differently, colonizing root surface easily, but lacking enhanced growth rates on agar surface (Fig.4), suggesting there's some kind of specificity in the stimulation. The enhanced root surface colonization could suggest that a non-diffusible compound was causing local spread, but this resembled more a saprophytic relationship than a proper molecular signaling, as the effect was common on decaying roots. No effect at all could be detected in *Boletus edulis*-*Cistus laurifolius* transformed roots coculture.

Future perspectives

Transformed root-organ cultivation seems to be a useful model system to study biochemical signaling between mycorrhizal partners such as *Cistus* and *Tuber melanosporum*, irrespective from the particular host species employed. Isolation of active compounds from either roots or culture media should lead to a better understanding of root-fungus signaling and could be employed as commercial hormones.

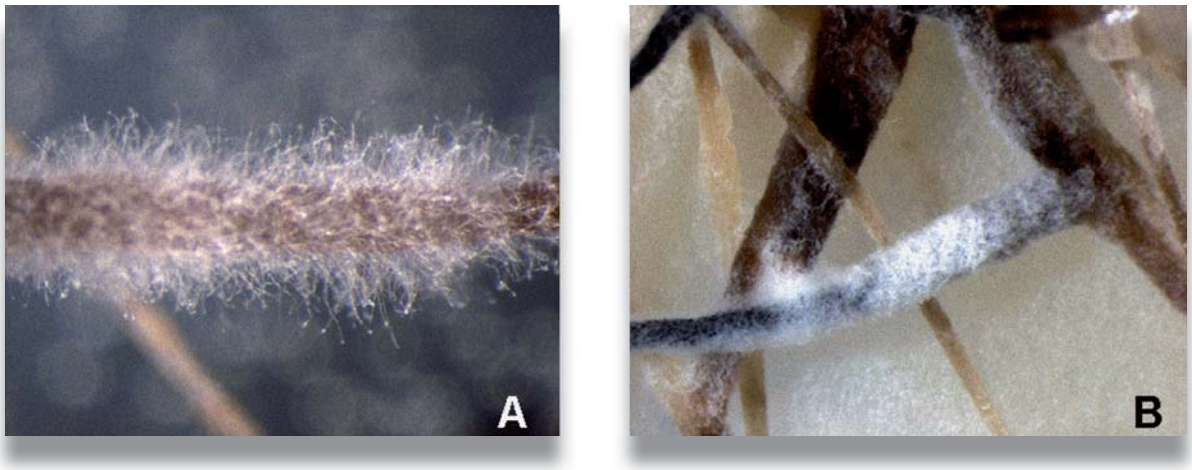

Fig. 4 *Amanita ponderosa* (A) and *Amanita caesarea* (B) mycelia growing on *Cistus laurifolius* transformed roots. Old and decaying roots were preferentially colonized by both fungi. No growth improvement was detected over the agar surface.

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UNIVERSITÀ DI PERUGIA: 20 ANNI DI CONTROLLO E CERTIFICAZIONE DI PIANTE TARTUFIGENE

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Abstract: University of Perugia: 20 years checking and certifying truffle plants
Since the 1980's the Department of Applied Biology at the University of Perugia has dealt with checking and certifying single mycorrhized plants and large quantities of plants from several nurseries in Italy and France. In the beginning, the evaluation was carried out using the morphological method by various researchers on behalf of the Ministry of Agriculture and Forestry and the Forestry Corp in 1987. Then, in 1995, a group of researchers was requested by the local governments of 10 Italian regions to elaborate a new method, easier than the previous, to evaluate truffle plants. Since October 2007, the Department has checked and certified all plants, following the regional law n.8 of 16/07/2007, using a morphological and molecular method of identification. The Department has drawn up agreements with many nurseries, checking approximately 80000 plants per year. Furthermore, the agreements with the nurseries also include the Department giving scientific and technical advice to improve the process of mycorrhization of truffle plants.
Other activities carried out by the Department are:
- checking and certifying root samples from truffle orchards to evaluate the situation, and/or carrying out expert analyses of these upon official request
- checking and certifying the fresh and preserved truffles and processed products, and again analyses upon official request.
Finally, the knowledge acquired in more than 30 years of studies and research has shaped the development of modern truffle cultivation.
Key words: mycorrhization, *Tuber*, truffle cultivation.

Gli studi sulla coltivazione del tartufo e le esperienze tecniche degli ultimi decenni considerano di fondamentale importanza la messa a dimora di piante tartufigene ben micorrizate con la specie di tartufo coltivata. Infatti, dopo la messa a punto di un metodo di produzione di piante tartufigene (Bencivenga, 1982), nacquero molti vivai specializzati per la produzione di piante micorrizate inoculate con specie di tartufi pregiati. Considerando che la produzione di queste piantine è abbastanza complessa e delicata, di fondamentale importanza è il controllo e la certificazione della micorrizazione prima della vendita e della messa a dimora.
Le analisi sulla micorrizazione prodotta dalle diverse specie di tartufo si concretizzarono nel 1987, con la stesura di un metodo di controllo e di certificazione delle piante micorrizate basato su caratteri morfologici. Questo primo metodo fu redatto da una commissione di esperti incaricata dal Ministero dell'Agricoltura e Foreste e dal Corpo Forestale dello Stato (Bencivenga *et al.*,1987). Da quel periodo, anche in base alla legge quadro nazionale n. 752/1985 che indica gli Istituti Universitari quali referenti scientifici per il controllo e la certificazione delle piante micorrizate, il nostro Dipartimento (oggi Dipartimento di Biologia Applicata) svolge attività di controllo e certificazione delle piante tartufigene prodotte dalla maggior parte dei vivai italiani ed alcuni francesi.
Il crescente interesse per la tartuficoltura, negli anni '90, ha portato ad un miglioramento del metodo di controllo e certificazione delle piante tartufigene; infatti, nel 1995, 10 Regioni italiane incaricarono alcuni esperti del settore per redigere un nuovo metodo (Govi *et al*., 1995), in seguito alle numerose esperienze scientifiche acquisite sulla morfologia delle micorrize delle specie di tartufo (Bencivenga e Granetti, 1990; Fontana *et al*., 1992; Granetti, 1995; Rauscher

et al., 1995; Zambonelli e Govi, 1990; Zambonelli *et al.,* 1995) e di alcuni funghi inquinanti (Agerer (ed.) 1995/96; Bencivenga *et al*., 1995; Donnini e Bencivenga, 1995).
Ad oggi, il Dipartimento fornisce ai vivaisti convenzionati supporto scientifico e tecnico per produrre piante micorrizate sempre migliori.
Questa attività si è ulteriormente evoluta nel tempo introducendo:

a) il controllo dei tartufi utilizzati per inoculare le piante;

b) l'analisi biomolecolare delle micorrize prelevate durante il controllo morfologico, dall'ottobre 2007 in adempimento al Regolamento della Regione Umbria n. 8/2007 (Amicucci *et al*., 1998; Rubini *et al*., 1998; Mello *et al*., 2002).

L'intenso lavoro di ricerca e di certificazione ha consentito un evidente miglioramento della tartuficoltura: dalla qualità della pianta tartufigena alla produzione del tartufo (Bencivenga *et al*., 1997; Comandini e Pacioni, 1997; Donnini *et al*., 2007). Negli ultimi anni vengono controllate e certificate dal nostro Dipartimento, circa 80.000 piante tartufigene ogni anno.
Altre attività svolte sono:

- controllo della micorrizazione di campioni di radici di tartufaie coltivate;
- controllo e certificazione di tartufi freschi, conservati e trasformati.

Il Dipartimento di Biologia Applicata dell'Università degli Studi di Perugia con la pluridecennale esperienza maturata nel settore tartufo e tartuficoltura continuerà ad impegnarsi allo scopo di fornire ulteriori validi contributi alla coltivazione delle specie pregiate di tartufo.

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LIBYAN TRUFFLES

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Abstract
Libyans have been traditionally appreciated truffles as delicious food. Libyan truffles are normally collected by men, who look for soil cracks made by expansion of the truffles. Two types of wild desert truffles were purchased from the local market and were lately identified as *Terfezia* (known as red or black truffle) and *Tirmania (*known as white truffle). The protein content and fat content of both truffles were investigated. The results showed that *Tirmania* and *Terfezia* contained 20% and 35% protein respectively.
Key words: ecology, desert truffle, *Terfezia, Tirmania, Helianthemum spp*.

Introduction

Desert truffles are a complex family of mycorrhizal hypogeous fungi mainly containing species of the genera *Tirmania*, *Terfezia*, *Picoa* and *Balsamia*. Desert truffles (*Tirmania* and *Terfezia*) are mostly endemic to arid and semi-arid areas, where they are associated with *Helianthemum* species (Dìez *et al*., 2002). Desert truffles are not ectomycorrhizal with trees, because their habitat area contains of lichen, moss and perennial sedges. Truffle fruiting depends on several parameters such as climate conditions, soil type and properties and quantity of the rainfall (Bokhary & Parvez, 1988). In the region of Murcia, Spain, after a year with a rainfall of between 350-400 mm, the estimated desert truffle production in natural areas varied between 50-170 kg/ha (Honrubia *et al.,* 2003). Libyan Desert truffles grow naturally in many parts of the country especially in Hamada Al-Hamra region where truffles are highly appreciated as delicious food. The price of the Libyan truffles varies from season to another whereas the price varies between 12-55 Euros/kg. The purpose of this study was to obtain knowledge about the chemical composition of the Libyan truffles.

Materials and methods

Truffles

Truffle ascocarps were purchased from the open market (Tripoli, Libya). The truffles were washed free of adhering soil. Several representative tubers from each of the two truffle variety were cut into small pieces and then blended separately by a Brabender chopper. Samples of the fresh both truffles variety (in triplicates) were used for the determination of fat content by a 16 hours soxhlet extraction using petroleum ether (30-40 B.P.), water was measured by heating the truffle samples at 75 C in a vacuum oven (45-50 mm Hg) to a constant weight and ash and total crude protein (N x 6.25) by AOAC methods (1980).

Results and discussion

Truffles

Truffles used in this study were morphologically identified as *Tirmania* and *Terfezia*. The chemical compositions of the Libyan truffles studied in this study are listed in Table 1.

Table 1 Proximate composition of Libyan truffles

Truffle type	Moisture %	Ash %	Total Fat %	Total Protein %
Tirmania	75.3	3.6	1.7	19.9
Terfezia	74.5	3.8	1.3	34.9

Our results showed that *Tirmania* contained lower level of protein (20%) than *Terfezia* (35%). Sawaya *et al*., (1985) showed that the brown species (Gibaah) of Saudi truffles (*Terfezia*) contained 25% protein (lower than Libyan *Terfezia*) while the white *Tirmania* species (Zubaidi) contained 27% protein, which is much lower than the value of Libyan *Tirmania*. Dabbour and Takruri (2002) showed that Jordanian mushrooms (*Agaricus macrospores*, *Tricholoma terreum* and *Pleurotus ostreatus*) having more or less same amount of protein as Jordanian *T. claveryi* (11%). The water content of Libyan truffles was 75% of fresh weight that is close to values (78%) reported for Saudi truffles (Bokhary *et al*., 1987; Bokhary and Parvez, 1993).

Acknowledgements
We are grateful to the Regional Council of Southern Savo, Finland for their financial support.

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FLAVOUR PROFILE OF THE *TUBER MELANOSPORUM* VITTAD.

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Abstract
The Mondo Tartufo Association was born in 2007 with the purpose to promote, plan, coordinate and develop study activities, research, documentation and realization of projects finalized to the spreading of the knowledge of truffle.
Among the various developed activities, Mondo Tartufo has created a Panel Test finalized to the recognition of the main describers that characterize every single typology of truffle: from the *Tuber magnatum* Pico to the *Tuber melanosporum* Vittad., considering also the less known species, that despite of that have a good gastronomic and sensorial quality. In order to make this, the Association has used some the Sensorial Analysis.
Key words: Sensorial Analysis, Truffle, *Tuber melanosporum* Vittad.

The Association Mondo Tartufo
The Mondo Tartufo Association was born in 2007 with the purpose to spread the knowledge of truffle, in Italy and abroad.
Belong to the Association: biologists, chemists, geologists, food and wine scientists, sommelier, technicians of truffle cultivation, traders of fresh and processed truffles, breeders of truffle dogs, truffle experts, nurserymen, truffle-growers and simple truffle lovers.
Among the various developed activities, Mondo Tartufo has created a Panel Test finalized to the recognition of the main describers that characterize every single typology of truffle, as the *Tuber melanosporum* Vittad.. In order to make this, the Association has used some the Sensorial Analysis.

Sensorial Analysis
The “Sensorial Analysis” is a technique allowing an evaluation – through our sensory organs – of the products intrinsic value. The links created between the brain and the external receptors let us taste the different kinds of food, analysing them and describing their features: it is then possible to tell what the senses let us explore. The ability to detect smells and tastes belongs to the concentration of the element that defines such smells and tastes, but also to the experience: a product being analysed for a long time let us train our sensory memory, which keeps its knowledge.
Today many techniques are available, able to define to chemical level the presence of determined substances in the truffles, but only through training and passion for truffle, we become expert tasters, able to appreciate all the sensory interactions that no other professional instrument is able to give us.
Studies and searches have been effected in order to define the sensory profile of every typology of truffle. To this intention Mondo Tartufo has elaborated a card of evaluation that takes into consideration all the aspects involved in the evaluation of this appreciated tuber, describing some for every typology the sensory profile.
For example the *Tuber melanosporum* is characterized for its aromas that mainly remember dark chocolate, black olives, glutammato, aromas of brushwood and liquorice, spread by an alcoholic note.

Description of the card
The evaluation cards take into consideration the three basic aspects for the sensorial analysis of the truffles: visual examination, tactile examination and olfactory examination.

The evaluation of the gustatory examination is not considered in this phase, because it would be destructive and therefore the truffles, after such evaluation, could not be marketed anymore.
The most representative describers have been identified. They concur to determine the sensory profile of the *Tuber melanosporum* Vittad. (listed in the image below) and based on their presence and interaction with the other components the quality of the analysed truffle is determined.
Some of the parameters we considered are: the intensity of the aromas, the fineness or elegance, the complexity and the description of the single aromas that participate in the formation of the bouquet.
The *Tuber melanosporum* Vittad. has an agreeability and a complexity olfactory that have made it one of the more appreciated truffle on the tables of the whole world. His sensorial profile is characterized by aromas that mainly remember dark chocolate, black olives, glutammato, aromas of brushwood and liquorice, spread by an alcoholic note; then depending on the stadium of maturation and/or on the place in which are picked up they can underline signs more delicate or more definite that further differentiate them.

Gastronomic suggestions
The evaluation of the gustatory examination, that is not considered in the sensory analysis, has however a fundamental role to appreciate it fully and to make truffle successful on the tables of the best gourmets all over the world, both in the past and at present.
During the course of the year Mondo Tartufo organizes suppers and guided tastings to find out the best recipes and all the possible matches.
These meetings are offered by famous chef or technical staff of the Association that, every time, will suggest innovative or traditional suggestions to enjoy which will allow to appear well on special occasions or everyday.

THE GENUS *TUBER* IN SPAIN: NEW TECHNIQUES FOR YOUR INVESTIGATION

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Abstract

Several methodologies have been employed during decades for the identification and classification of Ascomycetous: the study of the ecosystem were they are isolated and their symbiont, light microscopic and magnifying glass observations for the study of their morphological characters, organoleptic recognition, fungi reaction front diverse reactives, etc. The continuous appearance of new equipment and their application to the fungi investigation are allowing us to a better knowledge of this fungus kingdom no described so far. The success of the application of cryogenic scanning electron microscopy (cryo-sem) to the study of microscopic fungi in previous investigations done in our laboratory allow us to discover new morphological characters no described by other researchers yet. This technique has not been applied to the study of these hipogeus fungi. Early studies say that genus *Tuber* in Spain it's composed by twenty species, six edibles nor the rest. The purpose of this investigation was the study of diverse autochthonous species of this genus by means of classical techniques and cryo-sem observations.

Key words: *Tuber*, cryogenic scanning electron microscopy, morphologic characters.

Introducción

El género *Tuber* en España está comprendido según estudios recientes por veinte especies de las cuales 6 son comestibles y el resto no. El éxito de la aplicación de la microscopía electrónica de barrido de bajas temperaturas (cryo-sem) en investigaciones realizadas previamente en el laboratorio del Fondo de Diversidad Fúngica de la Comunidad Valenciana con hongos microscópicos ha permitido descubrir nuevos caracteres morfológicos previamente no descritos por otros investigadores. Para el estudio de hongos hipógeos esta técnica no se ha aplicado hasta el momento.

El objetivo de este trabajo fue investigar y caracterizar distintas especies autóctonas del género *Tuber* utilizando las técnicas tradicionales junto a la microscopía electrónica de barrido de bajas temperaturas.

Materiales y Métodos

Aislados

Los carpofóros de las especies utilizadas en la presente investigación: *Tuber aestivum, Tuber brumale, Tuber melanosporum* y *Tuber brumale* fueron obtenidas de distintas zonas productoras de España. Posteriormente se hizo un muestreo aleatorio de los ejemplares recogidos.

Examen morfológico

Antes de su examen utilizando las distintas técnicas, los ascocarpos fueron lavados con agua.

La observación de las esporas al microscopio óptico se realizó utilizando agua o colorante azul algodón de lactofenol. Previamente se realizaron cortes utilizando un microtomo de congelación.

Otras veces se utilizó el microscopio óptico de epifluorescencia para poder diferenciar las diferentes estructuras.

Cuando se utilizó la lupa los carpóforos fueron seccionados utilizando un bisturí y depositados sobre la platina para realizar las distintas observaciones.
Para examinar las muestras al microscopio electrónico de barrido de bajas temperaturas, los distintos cortes de carpóforo realizados con un bisturí fueron adheridos al portaobjetos y congelados con nitrógeno líquido. Ya en el microscopio se sublimaron a 90ºC durante 15 minutos, cubiertos con oro durante 30 segundos y visualizados a 10 kV.

Resultados y Discusión

Tuber aestivum Vittadini

Comestible pero de menor valor comercial que *Tuber melanosporum*.
Ascocarpo: de tamaño variable y forma irregular. Según Granetti *et al*., (1990), Astier (1998) y De Miguel and Reyna (2007) globoso y de tamaño que va desde una pelota de golf al de una naranja.

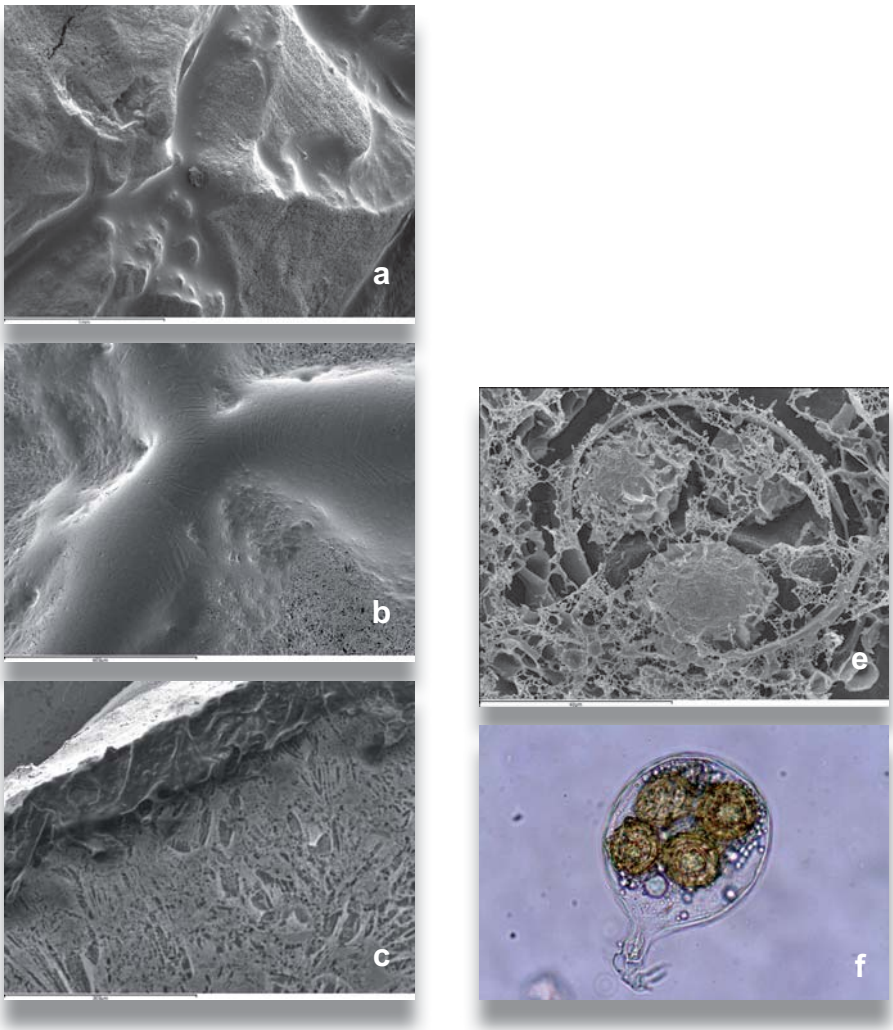

Figura 1. **a**, **b**, **c**, **d**: Fotografías de cryo-sem de *T. aestivum*. **a**: Verruga con fisuras (x50). **b**: Detalle de la pared que separa las verrugas (x150). **c**: Corte transversal del carpóforo. **e**, **f**: Ascas con esporas (**f**: microscopia óptica). **d**: Gleba (lupa x12.5).

Peridio: De color rojizo en su inmadurez que oscurece progresivamente hasta alcanzar una tonalidad marrón-negruzca. Formado por grandes verrugas piramidales recorridas algunas veces longitudinalmente por fisuras. Con el ápice deprimido y con una base poligonal mayoritariamente irregular, de 5 a 7 lados (Fig. 1a). Abundantes poros o hifas truncadas en las paredes de las celdas que componen las verrugas, mientras que la textura de la pared de las fisuras como la que las separa es completamente lisa y sin poros (Fig. 1a y 1b).

En un corte transversal del ascoma hipógeo se distingue claramente el peridio de grosor variable y con un entramado hifal compacto que lo diferencia de la gleba (Fig. 1c).
Gleba: Blanca en los ascocarpos inmaduros. A medida que transcurre el tiempo, se va volviendo de color pardo en el que se observan a la lupa unos puntos de color marrón: las ascas. Internamente recorrida por venas blancas, sinuosas más finas en los extremos y más gruesas en la parte central (Fig. 1d).

Ascas: De forma variable según el estado de madurez. Cuando son inmaduras su forma es globosa. Normalmente suele albergar de 1 a 4 esporas y sus dimensiones depende del número de éstas. Pedunculadas (Fig. 1e y 1f).
Esporas: Globosas y elípticas, con una coloración marrón clara. Tanto internamente como externamente de pared rugosa. Además la pared externa esta recubierta por unas celdas reticuladas que recuerdan los panales de abeja (Fig. 1e y 1f).
Época de maduración: Fundamentalmente en verano.
Simbiosis: Principalmente con *Quercus ilex* L. subsp. *ballota* y subsp. *ilex* y *Quercus fagínea* Lamk.

***Tuber brumale* Vittadini**
Comestible. Valor comercial superior al ascoma hipógeo *Tuber aestivum*.
Ascocarpo: Más irregular que el de *T. aestivum*, también de tamaño y forma variable. Subgloboso según Montecchi and Sarasini (2000) y con un tamaño medio de 4,5 cm (Granetti *et al*., 1998).
Peridio: Frágil, se desprende con facilidad. Verrugas piramidales con una base poligonal irregular pentagonal o hexagonal, con el ápice excavado, de color negro en la madurez y con fisuras longitudinales. De menor tamaño que las verrugas de *T. aestivum* (Fig. 2a).
Las verrugas son más porosas que las de *T. aestivum* con mayor número de hifas emergentes y truncadas, siendo su textura muy irregular. Muy similar a la de *T. melanosporum* (Fig. 2b).
Entre las distintas verrugas no existe una separación física. El grosor del peridio es menor que el de *T. aestivum* y el de *T. melanosporum* (Fig. 2c).

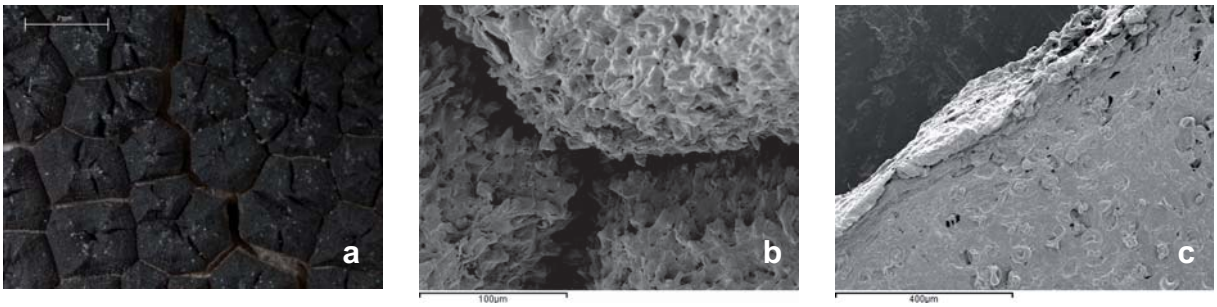

Fig. 2 a: Observación a través de la lupa del peridio (x8). b, c: Fotografías de cryo-sem de *T. brumale.* b: Detalle de las verrugas (x500). c: Corte transversal del ascocarpo en el que se distingue la gleba con las ascas del peridio (x150).

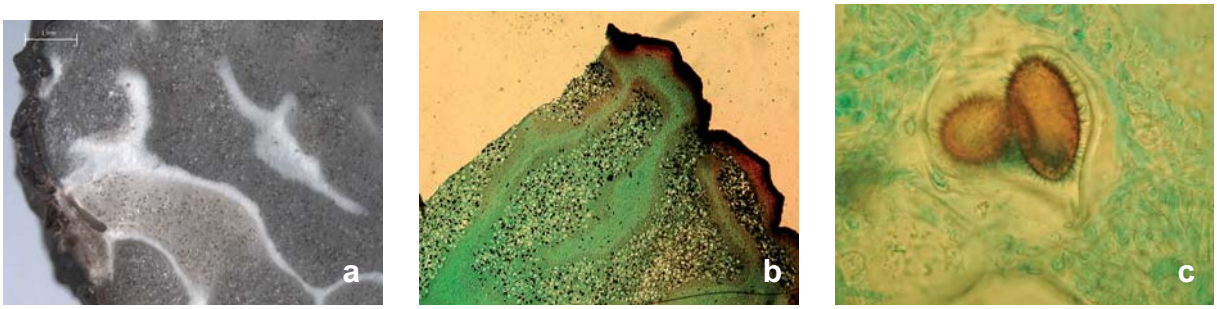

Fig. 3 a, b: Corte transversal del carpoforo (a: lupa x8) (b: microscopio óptico x40) c: Observación al microscopio óptico de un asca en la gleba (x400).

Gleba: de gris a marrón que va oscureciendo con la madurez. Venas blancas (Figg. 3a y 3b).
Ascas: Globosas cuando son inmaduras, en cuyo interior se encuentran de 1-7 ascosporas. Con pedúnculo muy corto (Figg. 3b y 3c).
Esporas: Tamaño ligeramente superior a las de *T. aestivum*. De color marrón claro, translúcidas, elípticas. Muy ornamentada con estructuras similares a aguijones de tamaño variable en la madurez que se van desarrollando a medida que la espora madura (Fig. 3c).
Época de maduración: Noviembre-Febrero.
Simbiosis: Los mismos simbiontes que *T. melanosporum*.

***Tuber melanosporum* Vittadini**

Es la trufa de mayor valor comercial y gastronómico que se recolecta en España.
Ascocarpo: Subgloboso aunque se pueden encontrar formas muy variables. Según De Miguel and Reyna (2007) su tamaño puede oscilar generalmente desde una pelota de ping-pong a una de tenis. Rojo en su inmadurez pero cuando madura se vuelve negro.
Peridio: Formado por verrugas, a veces con hendiduras de un tono anaranjado. La diferencia respecto a *T. brumale* es que su peridio es adherente y no se desprende fácilmente. Otro aspecto que los diferencia, es que en *T. melanosporum* hay una clara distinción entre el peridio y la gleba siendo el grosor del peridio mayor (Fig. 4b).
Al igual que *T. brumale* no existe una separación física entre las verrugas (Fig. 4a).
Gleba: Se asemeja a la de *Tuber brumale*. En *Tuber melanosporum* sin embargo, las ascas aparecen sobre el himenio como puntos blancos cuando se visualiza a la lupa.
Recorrida por venas blancas que cuando el cuerpo fructífero madura se minimizan.
Ascas: En su interior alberga diferentes números de esporas, existiendo discrepancia entre los distintos autores. Globosas inicialmente, con un pedúnculo corto (Fig.ura 4c y 4d).
Esporas: Marrones que a medida que maduran se van ornamentando de acúleos rectos y lisos. (Fig. 4c y 4d).

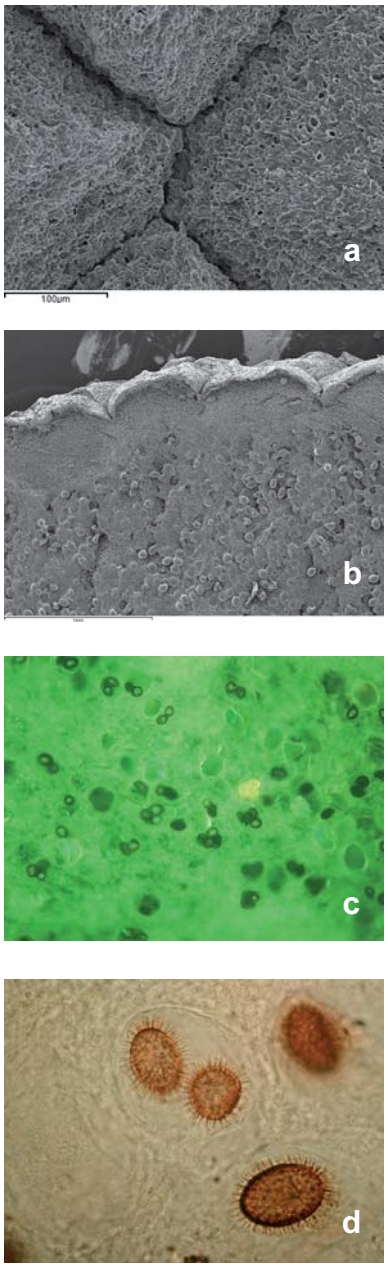

Fig. 4 **a**, **b**: Fotografías de cryo-sem de *T. melanosporum*. **a**: Detalle de la textura verrugas y su separación (x350). **b**: Corte transversal del carpóforo (x50). **c**: Gleba (Observación al microscopio óptico por epifluorescencia x100). **d**: ascas con ascosporas (x400).

Época de maduración: De noviembre a marzo.
Simbiosis: De forma natural las hifas establecen una simbiosis ectomicorrícica con las raíces del *Quercus ilex* L. subs. *ballota*, *Quercus faginea* Lamk., *Quercus coccifera* L. *Tilia platyphillos* Scop, *Quercus humilis* Millar y *Corylus avellana*.

***Tuber rufum* Pico**
No comestible. Carece de valor comercial.
Ascocarpo: de pequeño tamaño. Según De Miguel and Reyna (2007) no suelen sobrepasar los 2-3 cm. Globoso a subgloso su forma al igual que las otras especies, se ve condicionada por distintos factores.
Peridio: verrugas ligeramente marcadas, planas con una base poligonal muy variable, y con una textura muy irregular y marcada debido al entramado hifal (Figg. 5a y 5b).

Peridio de grosor más ancho que *T. melanosporum*, que se diferencia claramente por su estructura compacta (Fig. 5c).

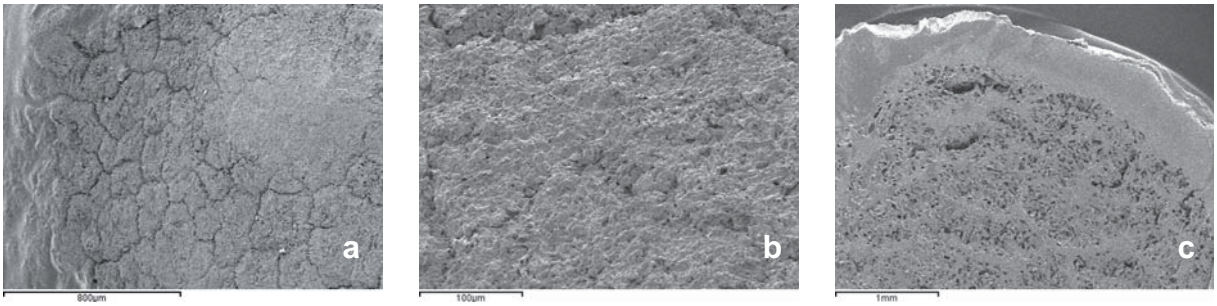

Fig. 5 a, b: Fotografías de cryo-sem de *T. rufum*. a: Peridio (x75). b: Detalle de la textura del peridio (x350). c: Corte transversal del carpóforo (x35).

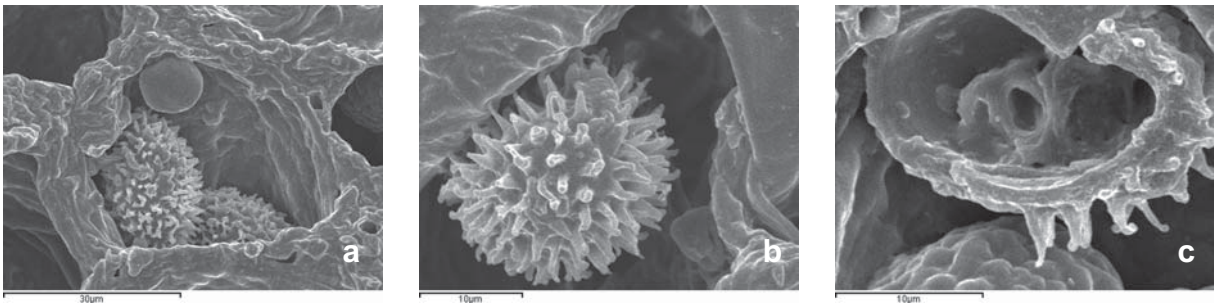

Fig. 6 Fotografías de cryo-sem de *T. rufum*. a: Esporas con distinto grado de desarrollo en el interior de un asca (x3500). b: Ascospora ya madura (x2000). c: Corte transversal de una espora (x5000).

Gleba: Inicialmente blanquecina.
Ascas: En la madurez, pared interna y externa rugosa (Fig. 6a). Ascas inmaduras globosas. Pedunculadas.
Esporas: Se encuentran de 3 a 4 en el interior de las ascas (Fig. 6a). Elipsoidales y redondeadas en los extremos, translúcidas, de color amarillento. Con una ornamentación a modo de espículas que recubren toda la espora (Figg. 6b y 6c).
Simbiosis: Principalmente con las raíces de *Corylus avellana*.

Conclusiones

La microscopía electrónica de barrido de bajas temperaturas constituye una buena herramienta para el estudio de los caracteres morfológicos de la trufa.
La textura del peridio fue distinta para las distintas especies que se investigaron pudiendo llegar a ser un carácter de diferenciación.
El estado de maduración de las ascas en un mismo carpóforo es distinto, al igual que el de las esporas contenidas en ellas.

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CURRENT CHALLENGES IN TRUFFLE RESEARCH IN KOREA

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Abstract
Growing the edible ectomycorrhizal mushrooms (EEMM) has proved to be a greater challenge than first anticipated due to the complex interdependencies in which fungi play a critical role. The challenge we face is to tilt the balance so that species of our choice can take up residence in such a complex natural setting-to design habitats in which it can grow. Many EEMM growers hope for huge profits when they try to grow European truffles, that sell at very high prices. Perigord truffle cultivation is also encouraged by governments in several countries as an alternative source of income to conventional agriculture. There are significant problems with the cultivation of EEMM on their host plants, and there are difficulties delivering quality products to the consumer. This paper will outline science and industry to this challenges in Korea, their successes in first steps of truffle fungi isolation and identification, production of *Tuber* sp. infected plants and some failures, the current state of our knowledge and will suggest a vision for the future.
Key words: Edible ectomycorrhizal mushrooms, truffle, *Tuber* spp. isolation, *Tuber* infected plants production.

I FUNGHI IPOGEI RACCOLTI INSIEME A *TUBER AESTIVUM*

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Abstract
The hypogeous fungi mixed with the commercialised *Tuber aestivum* Vittad. were studied between May and October 2008.
Macroscopic and microscopic observation led to the identification of twenty-five species of hypogeous fungi belonging to *Ascomycetes* and *Basidiomycetes*. Some rare truffles such as *Tuber panniferum* Tul. and *Tuber malenconii* Donadini, Riousset, G. Riousset & G. Chev. were identified together with *Fischerula macrospora* Mattir. and other species belonging to the genera *Genea, Melanogaster, Gautieria*, *Hymenogaster*, *Hydnotrya* and *Stephensia*.
According to this study two important considerations arise: 1) there are many other different of hypogeous fungi in the same site where *T. aestivum* grows, probably sharing similar ecological requirements; 2) it is evident that collectors are often unable to recognize the hypogeous fungi harvested together with *T. aestivum.* Therefore the identification by the Company's personnel is very important before the commercialization, in particular as concern truffles used as inoculum to produce truffle-infected plants, in order to avoid undesirable truffle contamination in the nurseries.
Key words: taxonomy, hypogeous fungi, harvesters, truffles, identification.

Introduzione

L'Italia, insieme a Francia e Spagna è uno dei principali produttori mondiali di tartufi. In questi paesi, che presentano caratteristiche pedo-climatiche ideali per la crescita spontanea dei tartufi più pregiati, da sempre, tradizionalmente, questi vengono raccolti e consumati (Ciani *et al.*, 1992).
Dagli anni '70, alla produzione naturale dei tartufi, si è aggiunta quella derivata dalla coltivazione che, grazie allo sviluppo di sempre migliori tecniche colturali e di micorrizzazione è in progressivo aumento (Ciani *et al.*, 1992).
Di pari passo nel nostro Paese si sono sviluppati anche i settori della lavorazione e della commercializzazione dei tartufi, a tal punto che, negli anni '80-'90, si è registrato un costante incremento del flusso di importazione sia del prodotto fresco che conservato, da altri paesi produttori quali: Francia, Spagna, Repubblica di Andorra, ex Jugoslavia, Tunisia, Stati Uniti (Ciani *et al*., 1992). Sempre negli stessi anni, l'esportazione del prodotto fresco verso i mercati tradizionali quali Francia, Germania, Austria, Stati Uniti, Canada e Gran Bretagna ha subito oscillazioni, mentre la costante domanda di paesi quali Australia, Giappone, Cina, fa sperare in un incremento (Ciani *et al*., 1992). Oggi i maggiori paesi importatori sono la Spagna, la Slovenia, l'Ungheria e la Romania (Gogan, Urbani, comunicazione personale).
Insieme ai pregiati *Tuber magnatum* Pico e *Tuber melanosporum* Vittad., trovano notevole e crescente interesse commerciale anche *Tuber borchi* Vittad. e *Tuber aestivum* Vittad.. Quest'ultima specie (*T. aestivum*), oggetto del presente lavoro, risulta largamente più diffusa rispetto alle altre specie citate e il suo areale di crescita si estende dalla Spagna fino all'Est Europa e dalla Svezia fino al Nord Africa; infatti si sviluppa in ambienti diversi ed è associato a varie essenze arboree come *Fagus sylvatica*, *Carpinus betulus*, *Ostrya carpinifolia*, *Corylus avellana*, *Quercus robur* e *Quercus ilex* (Hall *et al*., 2007). Sul nostro territorio *T. aestivum* è

presente in molte regioni: Piemonte, Lombardia, Liguria, Veneto, Toscana, Lazio, Umbria e Campania (Granetti, 1982; Mannozzi Torini, 1984; Zambonelli e Morara, 1985; Ceruti *et al.*, 2003; Venturella *et al.*, 2004, 2006).
I dati statistici a nostra disposizione, relativi alla produzione italiana dei tartufi pregiati a partire dagli anni cinquanta al 2002, sono insufficienti e non ci permettono di distinguere, se non in maniera approssimativa, la quantità percentuale delle diverse specie. Conosciamo in ogni caso la stima riferita alla quantità prodotta nel nostro Paese dagli anni 2003-2006 espressa in tonnellate, sia dei tartufi bianchi (*Tuber magnatum* e *Tuber borchii*) sia di quelli neri (*Tuber melanosporum*, *Tuber aestivum* e probabilmente *T. brumale*) (Tab. 1) (Pettenella et al. 2004; Bollettino mensile di statistica, ISTAT).

Tab. 1: Produzione italiana di tartufi, 1950-2005.

Anno	Totale (tonnellate)
1950	30,4
1960	76,4
1970	83,8
1980	71,8
1990	107,4
1991	77,2
1992	846,7
1993	138,5
1994	74
1995	195,5
1996	84,2
1997	85,7
1998	65,3
1999	86,5
2000	97,9
2001	69,3
2002	118
2003*	84,9/13,1
2004*	65,6/16,2
2005*	81,6/18,4

*Tartufi neri (Prev. *T. melanosporum* e *T. aestivum*) / Tartufi bianchi (*T. magnatum* e *T. borchii*).

Pettenella et al. 2004; Bollettino mensile di statistica, ISTAT, 1990-2006.

La produzione di tartufi, solo in parte destinata alla commercializzazione locale, è massimamente assorbita dall'industria di conservazione, che in Italia si aggiudica circa il 60-80% della produzione totale annuale dei tartufi reperibili sul mercato (Ciani, 1990). Le aziende che si occupano della trasformazione e della lavorazione del tartufo, rappresentano quindi una realtà economica in espansione, anche per l'apertura di nuovi mercati interni ed esteri e per il sempre maggior interesse che questo prodotto riscuote nel mondo (Ciani, 1990). *T. aestivum*, grazie alle sue eccellenti qualità organolettiche, è sempre più apprezzato e richiesto anche dal mercato internazionale, pertanto è nata l'esigenza di conoscere meglio le sue dinamiche di produzione, raccolta e commercializzazione.
Una fase essenziale, dalla raccolta alla lavorazione del prodotto, è l'accertamento dell'identità della specie per poter eliminare quelle estranee e soprattutto quelle non commerciabili. A tale scopo il presente lavoro si è rivolto alla ricerca e allo studio di tutte quelle specie, commerciabili e non, di funghi ipogei occasionalmente raccolti e erroneamente immessi all'interno delle partite di *T. aestivum*.

Materiali e metodi

Lo studio è stato condotto nell'anno 2008, presso l'Azienda Urbani Tartufi s.r.l (Santa Anatolia di Narco, Perugia), in occasione dello stage previsto dal Master in Micologia Agroalimentare organizzato dall'Università degli Studi di Bologna. Il periodo di svolgimento dello stage ha coinciso con quello di massima raccolta di *T. aestivum*, ovvero dall'inizio del mese di maggio fino alla fine di ottobre anche se questo, secondo le attuali norme legislative **(**Legge 16 dicembre 1985, n. 752 e Legge 17 maggio 1991, n. 162) si prolunga fino al 31 dicembre. Tali norme, infatti, consentono la raccolta di *T. aestivum* Vittad. dal 1 maggio al 30 novembre e di *Tuber uncinatum* Chatin dal 1 ottobre al 31 dicembre, distinguendo le due specie, nonostante che gli attuali orientamenti scientifici, basati su recenti ricerche molecolari, le considerino solo due ecotipi (Paolocci *et al.*, 2004).

In laboratorio si è provveduto all'identificazione dei tartufi scartati dalle partite di *T. aestivum* destinate alla commercializzazione, in seguito ad una accurata cernita effettuata dal personale specializzato dell'Azienda.

Si può ipotizzare che le partite analizzate siano pervenute da diverse regioni italiane e presumibilmente anche dall'estero, anche se si è riscontrata un'oggettiva difficoltà nell'ottenere informazioni sulla loro reale provenienza, legata alla riservatezza dei cavatori, dei commercianti e dell'Azienda stessa. Questa condizione di incertezza ha pertanto reso impossibile la raccolta dei dati per un'elaborazione statistica delle quantità del prodotto e delle sue zone di raccolta.

Da una prima valutazione macroscopica degli sporomi, si è passati ad una osservazione microscopica che ci ha permesso l'identificazione di numerose specie di funghi ipogei con caratteristiche morfologiche non riferibili a *T. aestivum*, ma erroneamente raccolte dai "cavatori".

L'indagine microscopica è stata effettuata mediante l'utilizzo di un microscopio ottico munito di fotocamera digitale (ECIPSE TE 2000-E microscope (Nikon) (200X e 400X).

Per alcune specie sia di Basidiomiceti che di Ascomiceti sono stati eseguiti disegni delle spore, degli aschi e dei basidi e sono stati rilevati alcuni caratteri micromorfologici: numero delle spore per asco o basidio, tipo di ornamentazione e misurazioni delle spore. Per ogni specie sono state effettuate almeno 30 misurazioni sporali e in particolare quelle sugli Ascomiceti si riferiscono ad aschi contenenti un numero variabile di spore.

I campioni studiati, una volta essiccati, sono stati depositati presso l'erbario del Centro di Micologia di Bologna (CMI-UNIBO).

Come riferimenti bibliografici per l'identificazione e la descrizione delle specie, sono state utilizzate le informazioni anatomiche e morfologiche macro e microscopiche riportate da: Pegler *et al.* (1993), Montecchi e Sarasini (2000) e Granetti *et al.* (2005).

Le specie più rare ed interessanti individuate, sono state oggetto di ulteriore indagine bibliografica, con il fine di formulare alcune ipotesi sulla possibilità di condivisione di condizioni ecologico-ambientali con *T. aestivum*.

Risultati

Lo studio effettuato ha portato all'identificazione di 25 specie di funghi ipogei tra Ascomiceti e Basidiomiceti. Talora le specie sono state riconosciute con facilità, poiché gli sporomi, ancora sostanzialmente integri, mantenevano riconoscibili le caratteristiche macroscopiche, confermate poi dall'indagine microscopica che in certi casi, si è resa determinante a causa della vetustà e del degrado degli ascomi e dei basidiomi, soprattutto ai fini dell'identificazione di alcuni Basidiomiceti.

Le specie di Ascomiceti risultate più comuni e facilmente riconoscibili macroscopicamente, sono state per quanto riguarda il genere *Tuber*: *Tuber aestivum* Vittad., *T. borchi* Vittad., *T. brumale* Vittad., *T. dryophilum* Tul., *T. excavatum* Vittad., *T. macrosporum* Vittad., *T. maculatum* Vittad., *T. melanosporum* Vittad., *T. mesentericum* Vittad. e *T. rufum* Pico; per il genere *Genea*: *Genea fragrans* (Wallr.) Sacc., *G. sphaerica* Tul. e C. Tul. e *G. verrucosa* Vittad.. Un altro esempio di specie appartenenti agli Ascomiceti ci è stato offerto dalla sporadica presenza di ascomi relativi a *Stephensia bombycina* (Vittad.) Tull. e *Hydnotrya cerebriformis* Harkn..

Interessante, e oggetto di ulteriori approfondimenti, è stata l'osservazione di un discreto

numero di ascomi appartenenti a due specie del genere *Tuber* apparentemente legate ai climi caldi, ovvero: *Tuber malenconii* Donadini, Riousset, G. Riousset e G. Chev., che risulta essere piuttosto rara in Italia e *T. panniferum* Tul. (Montecchi e Sarasini, 2000).
Suggestivo per la sua rarità è stato il ritrovamento di due esemplari di *Fischerula macrospora* Mattir. poiché, secondo quanto riportato in letteratura, questa specie è stata segnalata soltanto per l'Italia e la Spagna (Montecchi e Sarasini, 2000; Moreno *et al.*, 2005, Venturella *et al.,* 2006).
Le specie di Basidiomiceti talora presenti all'interno delle partite di *T. aestivum*, crescono comunemente sia nelle tartufaie naturali che negli impianti artificiali e pertanto, vengono facilmente ritrovate dai cani utilizzati nella ricerca e nella raccolta dei tartufi (Montecchi e Sarasini, 2000).
Queste appartengono ai generi *Hymenogaster*, *Melanogaster* e *Gautieria* e in particolare sono: *Hymenogaster bulliardii* Vittad., *H. citrinus* Vittad., *H. hessei* Soehner, *Melanogaster ambiguus* (Vittad.) Tul. & C. Tul., *M. broomeianus* Berk., *M. variegatus* (Vittad.) Tul. & Tul. e *Gautieria graveolens* Vittad.
In tabella 2 sono riportate le misurazioni dei caratteri sporali di alcune specie di Ascomiceti e Basidiomiceti.

Tab. 2: Misurazioni sporali.

Lista delle specie	N° di erbario	N° di spore[1]	Ornam.[2]	Diam. Mag. (µm)[3]	Diam. Min. (µm)[3]	Q*
Ascomiceti						
Tuber aestivum Vittad.	3564	(1) 2-4 (5,6)	RA	(23) 24,4-30,6 (38)	(20) 20,7-24,5 (26)	1,21
Tuber borchi Vittad.	3565	(1) 2-3 (4)	RA	(26) 27,6-36.6 (41)	(25) 26,4-33 (38)	1,07
Tuber excavatum Vittad.	3568	(2) 3-4	RA	(34) 35,8-41,8 (45)	(22) 23,5-28,3 (35)	1,5
Tuber macrosporum Vittad.	3569	1-3 (4)	A	(46) 49.3-64,9 (72)	(27) 29,6-37,4 (43)	1,7
Tuber maculatum Vittad.	3570	(1) 2-3 (4)	RA	(28) 30,2-48,2 (64)	(21) 22,9-31,9 (36)	1,43
Tuber malenconii Donadini, Rious., G. Rious. & G. Chev.	3571	(6) 7-8	RAP	(19) 19,9-21,9 (23)	(13) 14,1-15,5 (16)	1,41
Tuber melanosporum Vittad.	3572	(1) 2-4 (5)	A	(25) 26,6-36,2 (44)	(17) 18,4-23,8 (29)	1,48
Tuber mesentericum Vittad.	3573	(1) 2-4 (5,6)	RA	(27) 29,4-33,8 (35)	(20) 21,5-25,7 (28)	1,34
Tuber panniferum Tul.	3574	(3-5) 6-8	A	(23) 24,1-29,5 (32)	(17) 17,5-22,3 (26)	1,34
Tuber rufum Pico	3575	(1,2) 3-4	A	(24) 26-36 (46)	(16) 16,8-22 (26)	1,6
Fischerula macrospora Mattir.	3576	(1) 2-5 (6)	V	(45) 46,3-58,3 (67)	(35) 37,6-46,6 (53)	1,24
Genea fragrans (Wallr.) Sacc.	3577	8	V	(26) 28,4-34,4 (40)	(22) 23,9-28,9 (32)	1,19
Hydnotrya cerebriformis Harkn.	3580	8	AF	(20) 21,1-24,7 (29)	(20) 21,1-24,7 (29)	1
Basidiomiceti						
Hymenogaster bulliardii Vittad.	3584	4	L	(16) 20,1-24,7 (28)	(8) 11-14,2 (16)	1,8
Hymenogaster citrinus Vittad.	3585	1-3 (4)	R	(22) 25,1-30,3 (33)	(12) 13,5-17 (18)	1,83
Melanogaster ambiguus (Vittad.) Tul. & C. Tul.	3587	4	L	(14) 15,1-17,5 (20)	(8) 8,8-10,2 (11)	1,71
Melanogaster broomeanus Berk.	3588	4	L	(6,5) 7,1-8,7 (10)	4-5 (5,5)	1,76
Melanogaster variegatus (Vittad.) Tul. & C. Tul.	3589	4	L	(5,5) 6,2-8 (10)	(4) 4,2-5 (5,5)	1,53

[1]Numero spore per asco (Ascomiceti), per basidio (Basidiomiceti).
[2]Tipo ornamentezione spora (Sigla): A = Aculeata, AF = Aculeata finemente, RA = Reticolata-alveolata, RAP = Reticolata-alveolata con maglie piccole, V = Verrucosa, L = Liscia, R = Rugolosa.
[3](Valore minimo) Valore medio ± deviazione standard (Valore massimo).
*Valore medio.

Fig. 1 Caratteristiche macro e microscopiche di funghi ipogei non commerciabili presenti all'interno delle partite di *T. aestivum*: *Tuber panniferum* (1a ascomi, 1b fotografia di spore al microscopio ottico, 1c disegno di asco e spore), *Tuber malenconii* (2a ascomi, 2b fotografia di spore al microscopio ottico, 2c disegno di asco e spore), *Fischerula macrospora* (3a ascomi, 3b fotografia di spore al microscopio ottico, 3c disegno di asco e spore), *Tuber excavatum* (4a ascomi e 4b fotografia di spore al microscopio ottico), *Tuber maculatum* (5a ascomi e 5b fotografia di spore al microscopio ottico), *Tuber rufum* (6a ascomi e 6b fotografia di spore al microscopio ottico).

Discussione

La maggior parte dei campioni di funghi ipogei esaminati, appartengono a specie del genere *Tuber* considerate comuni e abbondanti in Italia e la loro presenza all'interno delle partite di *T. aestivum* è riconducibile alla condivisione degli habitat di crescita di questa specie che come è noto si sviluppa in condizioni ambientali e climatiche assai diverse tra loro (Chevalier e Frochot, 1997). Presumibilmente è questo un motivo per il quale i "cavatori" spesso trovano e raccolgono specie diverse da *T. aestivum* che non vengono da loro distinte con una prima grossolana e superficiale valutazione.

La presenza delle due specie *T. malenconii* e *T. panniferum*, fa supporre che il luogo di

provenienza di alcune partite di *T. aestivum* sia riconducibile all'ambiente mediterraneo. *T. malenconii* infatti, descritto per la prima volta in Francia in Provenza (Donadini *et al.*, 1978), è stato successivamente segnalato in Italia nell'isola della Sardegna, in Spagna nel Barcellonese (Montecchi e Sarasini, 2000) e in Andalusia in provincia di Cordoba (Moreno *et al*., 2005); *T. panniferum* è stato ritrovato sia in Francia e Spagna che in Italia, dove è presente nelle regioni centro-meridionali (Montecchi e Sarasini, 2000): Molise, Lazio e Campania (Mattirolo, 1933), della Sardegna (Bencivenga, 1994; Montecchi e Sarasini, 2000) e della Sicilia (Venturella *et al*., 2006); nel sud della Francia si trova in Provenza (Montecchi e Sarasini, 2000) e nel sud della Spagna in Andalusia (Moreno *et al*., 2005).
Fischerula macrospora indubbiamente assai rara, è stata ritrovata e descritta per la prima volta nel 1887, in Lombardia sui monti del Lago di Como dal Mattirolo; successivamente è stata segnalata in Toscana a Vallombrosa (Fi) nel 1928 e a Sora al confine fra il Lazio e la Campania nel 1933 (Mattirolo, 1933). Recenti segnalazioni in Emilia-Romagna (Montecchi e Sarasini, 2000), in Basilicata (Rana *et al.*, 2006) e in Sicilia (Venturella *et al.,* 2006, 2008), fanno pensare ad una finora inaspettata diffusione di questa specie.
Il reperimento di due ascomi nel materiale di studio, è significativo e fa ipotizzare che la sua ecologia, considerata finora legata solo alle latifoglie di ambiente montano o comunque fresco quali *Corylus avellana* e *Fagus sylvatica*, possa estendersi anche ad altri habitat tipici del *T. aestivum.,* come ad esempio boschi più termofili a prevalenza di leccio, come segnalato per la Sicilia da Venturella *et al.* (2008) e per l'Andalusia in Spagna da Moreno *et al.* (2005).
Nelle medesime partite, il rinvenimento anche di Basidiomiceti ipogei e semiipogei, è da ricondurre, come già affermato, ad una grossolana somiglianza con *T. aestivum*, legata in alcuni casi alla colorazione brunastra dei corpi fruttiferi, tipica di alcune specie ed in altri conseguente al loro invecchiamento.

Conclusioni

Da questi studi emerge che differenti specie di funghi ipogei anche piuttosto rare, possono crescere insieme a *Tuber aestivum* condividendone, a seconda della specie, ambienti e condizioni ecologiche diversi. Queste specie vengono mescolate agli ascomi di *T. aestivum* dai "cavatori", in fase di raccolta, in quanto non sempre sono in grado di riconoscerle. L'identificazione dei corpi fruttiferi di *T. aestivum* è fondamentale per la loro commercializzazione e in particolare per quella dei tartufi utilizzati come inoculo per la produzione di piante micorrizate, al fine di scongiurare indesiderate contaminazioni all'interno dei vivai.

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STUDIO DELLA STRUTTURA E DELLA QUALITÀ DEI TARTUFI TRAMITE RISONANZA MAGNETICA DI IMMAGINI

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Abstract: Internal structure and quality assessment of fresh truffle by means of Magnetic Resonance Imaging spectroscopy

Magnetic Resonance Imaging spectroscopy (MRI), being a non destructive and non invasiveness analytical technique, offers a very powerful tool for the investigation of the internal morphology of foodstuff and of modifications induced by "stress agents", e.g. fungal invasion, diseases, contaminants, etc. In the present paper MRI was applied, to the best of our knowledge for the first time, to study the internal structure of fresh black truffle; decaying process and fungal invasion were observed and quality evaluation, which up to now has not yet yielded an accepted technical standard being based on smell and consistency on handling, was exploited by means of MRI.

Key words: MRI, *Tuber melanosporum*, freshness, fungicolous fungus, Conservation, commerce and valorization.

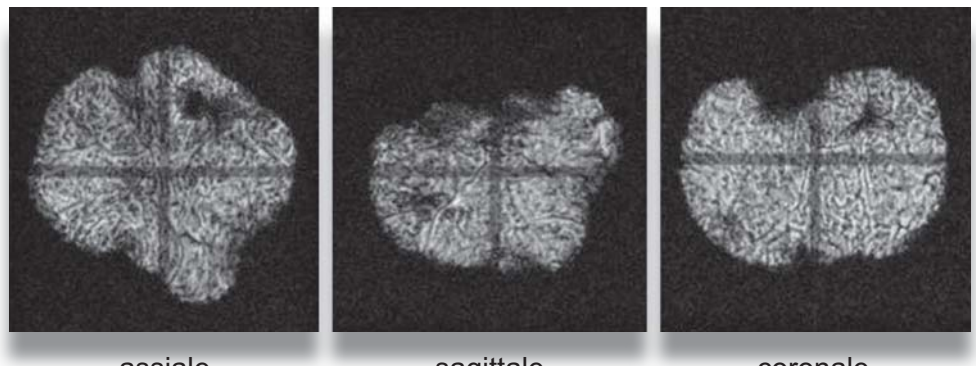
Introduzione

La Risonanza Magnetica di Immagini (MRI), tecnica analitica avanzata che permette di ottenere immagini ad alta risoluzione in modo non invasivo e non distruttivo, negli ultimi anni ha trovato una nuova applicazione nel campo agro-alimentare: il controllo della qualità dei prodotti (Clark *et al.,* 1997; Sequi & Valentini, in press). Questo approccio è particolarmente rilevante per tutti quegli alimenti che hanno un elevato valore economico, come ad esempio i tartufi. Per essi non esiste un metodo analitico per valutarne la qualità, la freschezza e l' eventuale presenza di malattie. Tali valutazioni si basano ancora su determinazioni tramite odore e consistenza al tatto (Hall *et al*., 2003).

La MRI è stata utilizzata per studiare la struttura di tartufi neri freschi (*Tuber melanosporum*) e per valutarne conservabilità e presenza di agenti fungini.

Determinazione della struttura dei tartufi

Nell'**esperimento Gradient-Echo (GEFI)** il segnale NMR è direttamente proporzionale alla densità protonica ovvero al contenuto d'acqua. Esso ha permesso di evidenziare in dettaglio la morfologia interna del tartufo: gleba, peridio e i micro canali delle vene esterne adibiti al rilascio di H_2O e dei composti organici volatili. (Fig.1)



assiale sagittale coronale

Fig. 1 Immagini esperimento GEFI su un esemplare di *Tuber melanosporum*.

Nell'**esperimento Multi-Slice-Multi-Echo (MSME)** il segnale NMR è direttamente proporzionale al tempo di rilassamento trasversale T_2 dell'H_2O, che in linea generale rifletta la capacità delle molecole di H_2O di muoversi all'interno dei tessuti cellulari. Immagini più chiara indicano molecole di H_2O con maggiore mobilità, legame con il substrato cellulare più debole, mentre zone più scure sono caratterizzate da T_2 più brevi, avente H_2O legata più intensamente e con minore capacità di muoversi. Il risultato di una analisi MSME è mostrato nelle Figura 2.

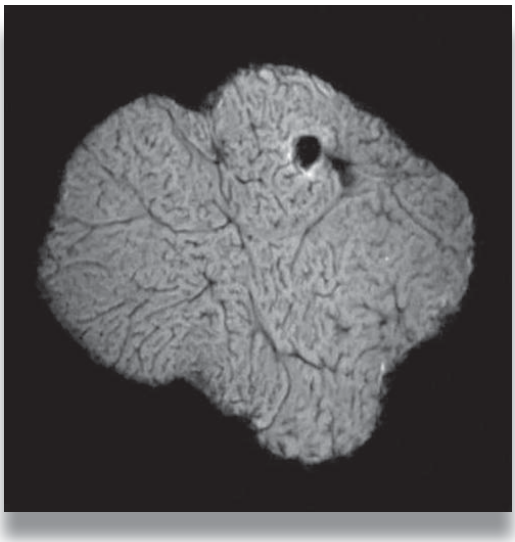


Fig. 2 Immagine ottenuta con esperimento MSME su un esemplare di *Tuber melanosporum*.

L'intensità del segnale è piuttosto omogenea all'interno del tartufo, ciò suggerisce che:

- i tessuti hanno simile consistenza;
- le molecole di H_2O hanno interazioni comparabili con i componenti cellulari in tutto il tartufo.

<u>Determinazione di miceli fungini ospiti</u>

Recentemente (Pacioni *et al*., 2007) è stata dimostrata la presenza di miceli estranei all'interno della gleba dei tartufi. La presenza di questi miceli estranei non è sempre legata ad alterazioni patologiche della gleba, come ad esempio un "marciume". Un caso di una particolare sintomatogia prodotta da un massiccio sviluppo di un ifomicete fungicolo bruno, non ancora identificato, è stata osservata mediante immagini pesate in T_2 (Fig. 3).

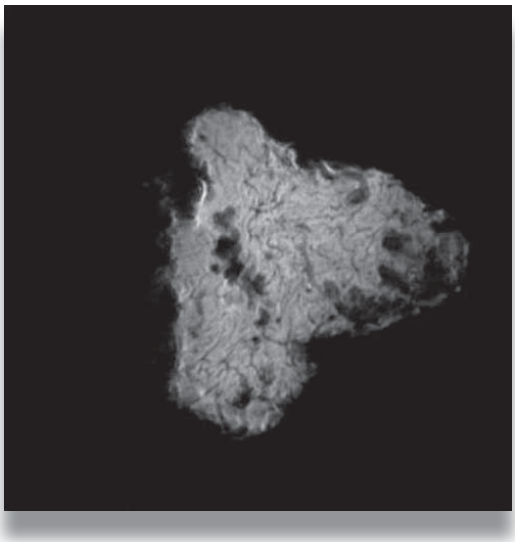
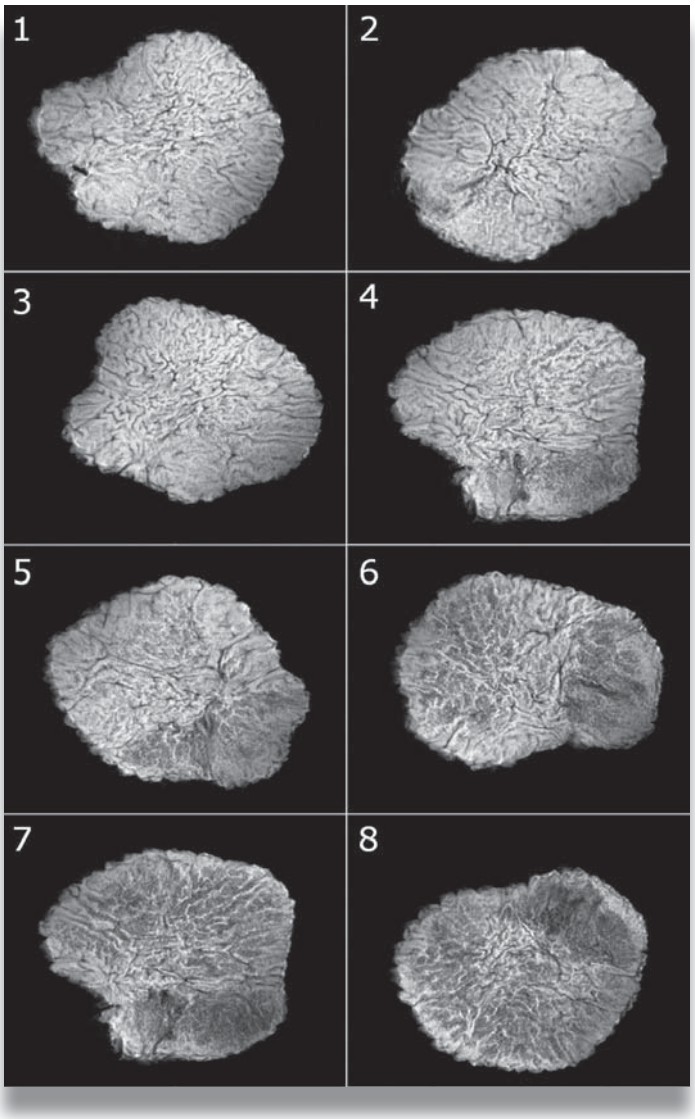


Fig. 3 Immagine MRI pesata in T_2 di un tartufo fresco invaso da un micelio fungicolo.

Questo micelio lascia inalterato odore e consistenza del tartufo cambiando però profondamente in peggio le proprietà organolettiche e rendendo praticamente immangiabile il tartufo stesso. Sino ad oggi è stato possibile valutare la presenza di questo problema solo sezionando i tartufi, tramite immagini MRI pesate in T_2 invece è stato possibile visualizzarlo in modo non distruttivo.
Le macchie scure, presenti soprattutto nella parte destra del campione, rilevano la presenza di un micelio fungicolo che, oltre ad abbassarne la qualità del tartufo dal punto di vista organolettico, porta ad un indurimento consistente delle zone interessate. In queste zone il contenuto di H_2O è molto basso, e la poca presente è fortemente legata al substrato cellulare, tanto da non poter essere osservata neanche con misure MRI di tipo Singol Point Imaging, che permette di misurare $T_2 < 0.5$ ms. Nelle altre zone la struttura delle vene e del peridio rimane inalterata. Questa è probabilmente la ragione per cui odore e consistenza rimangono pressoché invariate.

Studio dello stato di conservazione

Il tempo di rilassamento trasversale T_2 è stato usato come indicatore della conservabilità. Le immagini pesate in T_2 (Fig. 4) registrate a ritardi crescenti dal momento della raccolta mostrano come all'aumentare del tempo di conservazione vi sia una diminuzione del segnale NMR; le immagini MRI diventano più scure e i valori di T_2 si accorciano.



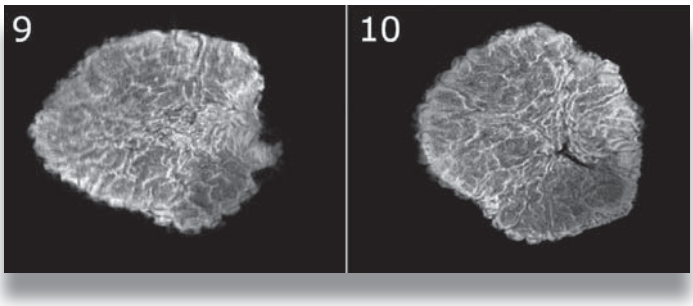


Fig. 4 Immagini MRI pesate in T_2 di un *Tuber melanosporum* acquisite nel corso del periodo di conservazione: 1) tartufo fresco; 2) dopo 2 giorni di conservazione; 3) dopo 4 giorni; 4) dopo 8 giorni; 5) dopo 11 giorni; 6) dopo 15 giorni; 7) dopo 18 giorni; 8) dopo 22 giorni; 9) dopo 25 giorni; 10) dopo 32 giorni.

I tempi di rilassamento T_2 sono stati ottenuti interpolando i dati con una funzione di tipo esponenziale decrescente di ordine 1

$$y = y_0 + W \exp^{(-t/T_2)}$$

dove:
T_2 è il tempo di rilassamento trasversale espresso in ms;
y_0 è l'intensità a t=∞
W è il fattore pre-esponenziale
I risultati dei dati rilevati in corrispondenza di un'area centrale di 0,25 cm² all'interno dell'ascoma, sono illustrati nella Fig. 5.

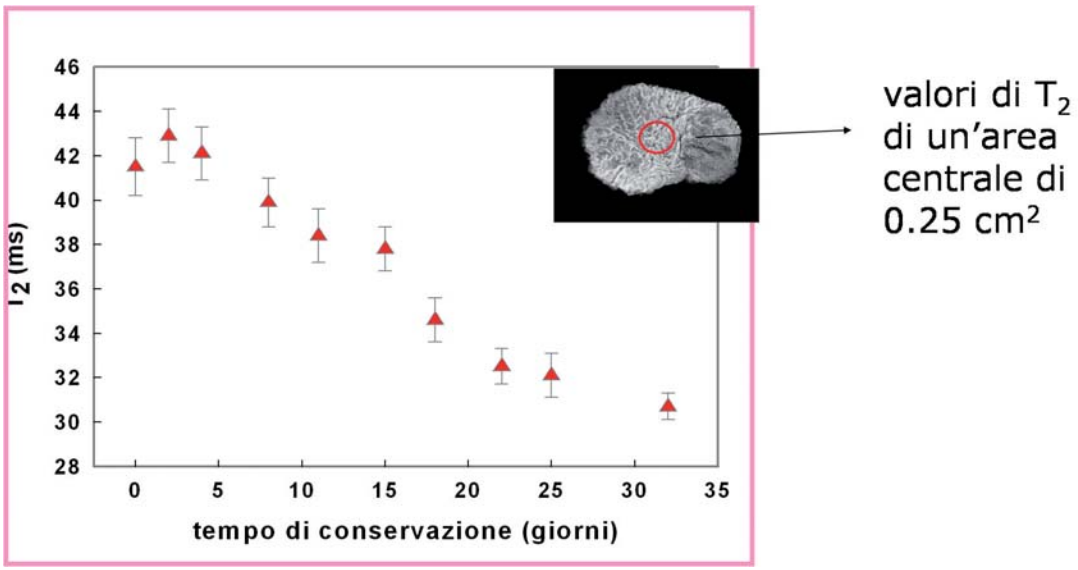


Fig. 5 variazione di T_2 in funzione del tempo di conservazione. T_2 è stato calcolato come la media nella zona evidenziata in rosso.

Il valore di T_2 rimane costante e pari a circa 42 ms, nei primi 4 giorni e poi inizia a diminuire quasi linearmente sino al giorno 25, dove diviene pari a ca. 32 ms. Questo valore viene poi mantenuto per un'ulteriore settimana.
Questo andamento può essere dovuto al processo naturale di:

1. *evapo-traspirazione*; particolarmente significativo per le molecole di acqua con elevata capacità di muoversi
2. *traslocazione* delle molecole H_2O progressivamente compartimentata in specifici tessuti cellulari.

Conclusioni

L'MRI ha permesso di studiare in dettaglio la struttura interna di tartufi neri, evidenziando particolari morfologici relazionabili con la qualità generale del prodotto. La non invasività tipica della tecnica adoperata ha permesso di evidenziare miceli che deteriorano le proprietà organolettiche del tartufo non rilevabili dall'esterno, ed il tempo di rilassamento trasversale è stato utilizzato come indicatore nel monitoraggio delle modificazioni strutturali durante il periodo di conservazione. La Risonanza Magnetica di Immagini potrà essere quindi un importante tecnica per una diagnostica analitica delle condizioni qualitative dei tartufi e per validare le metodologie di conservazione del prodotto fresco.

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CONTENUTO DI METALLI PESANTI NEI TARTUFI ABRUZZESI

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Abstract: **Heavy metal content of Abruzzi truffles**
Many kinds of food have been analyzed for their content of heavy metals over the past few years. In some cases, it has turned out that foods of vegetal origin, including fungi, exhibit a strong tendency towards "bioaccumulation", defined as the capability to concentrate particular toxic substances inside somatic structures. Since truffles have been analyzed only in terms of the nutritionally relevant salt content, we deemed it necessary to undertake this kind of research on a representative sample of the diverse species and of the various soil types of the Abruzzi Region. The analyses have been conducted by using Atomic Absorption Spectrometry (AAS), to determine the presence of barium, cadmium, chromium, nickel, lead, copper, zinc and manganese, and by using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). In contrast to what has been found out about some fungi considered excellent edibles - and until now allowed to be traded by the food law in force - which, in point of fact, have been proved to be bio-accumulators of heavy metals, Hg and Cd, in particular, or of radioisotopes (Cs and Sr), at the moment, truffles appear to be a perfectly safe food from this point of view. In fact, the results obtained by two different analytic methods match up about the selectivity in ion absorption from the solutions present in the soil by truffles. It has repeatedly been emphasized that truffles strongly accumulate useful ions such as K and S, while very little attention has been devoted to harmful bioaccumulations, which, luckily however, do not seem to occur in truffles. The data gathered in this research is consistent with what emphasized by the only work on the subject which focused on Abruzzi truffles. It remains to be clarified, therefore, whether the absence of heavy metal bioaccumulation is a general feature of truffles or rather it is related with the features of the truffle-producing soils. Abruzzi soils favourable to the fruiting of truffles are, in fact, characterized by a low content of heavy metals and an alkaline pH which limits metal gain. Even though it is necessary to carry on further analyses in order to obtain statistically significant data, Abruzzi truffles, both from natural truffle-grounds and farming productions, have been proved to be safe and of top quality and the soils of production may be expected to be the same.
Key words: Truffle, Heavy metals, Bioaccumulation, AAS, ICP-OES, Conservation, commerce and valorization.

Introduzione

Da qualche anno in molti alimenti è stato analizzato il contenuto di metalli pesanti, ed, in alcuni casi, alimenti di origine vegetale, inclusi i funghi, hanno evidenziato una spiccata attitudine al "*bioaccumulo*", definito come la capacità di concentrare determinate sostanze tossiche all'interno delle strutture somatiche. Scopo della presente indagine è stato quello di definire i livelli di accumulo dei tartufi nei confronti dei metalli pesanti nella Regione Abruzzo, dal momento che risulta un argomento di ricerca decisamente trascurato.

Materiali e metodi

Le analisi sul contenuto di metalli pesanti sono state condotte con l'impiego della Spettrometria ad Assorbimento Atomico (AAS) (Fig. 1) e Spettrometria Ottica in Emissione con Plasma Accoppiato Induttivamente (ICP-OES) (Fig. 2) prendendo in considerazione Bario, Cadmio, Cromo, Nichel, Piombo, Rame, Zinco e Manganese.

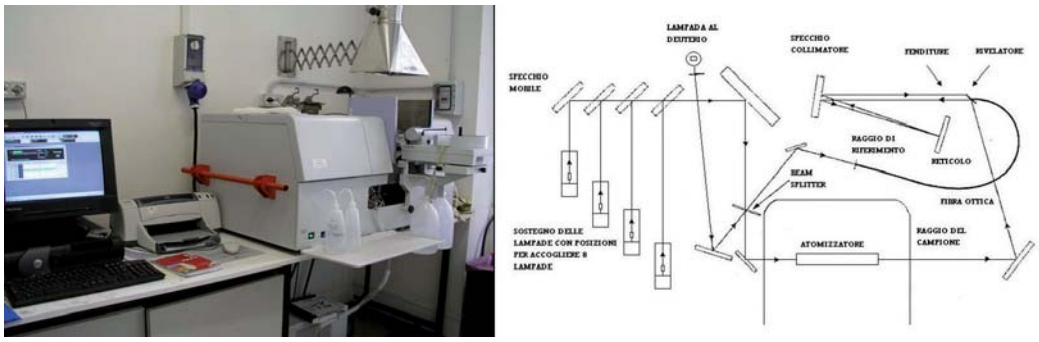

Fig. 1 Spettrofotometro ad Assorbimento Atomico AA Analist 800 *Perkin Elmer*

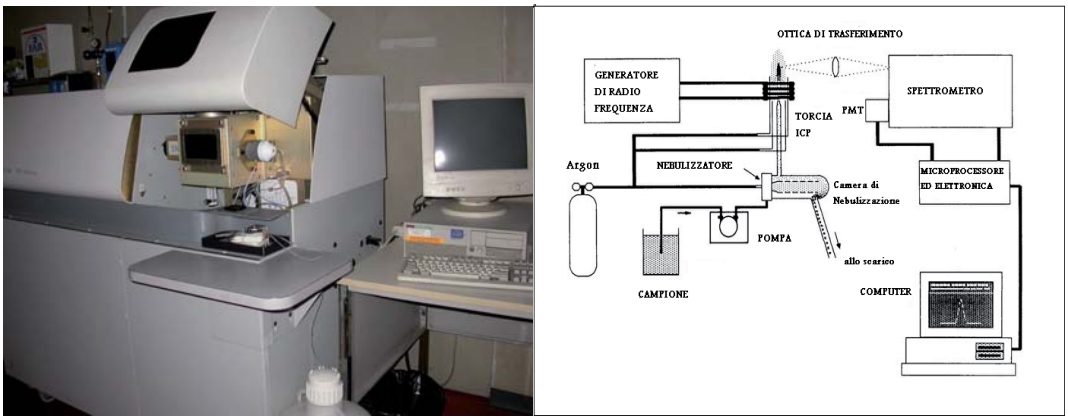

Fig. 2 Sistema ottico dell'ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry) *Perkin Elmer*

La analisi sono state condotte seguendo le seguenti metodologie ufficiali:
Tartufi
ISTISAN 96/34 per tutti i metalli
EPA 7470 A (CV-AAS) per il Hg
Terreni
D.M.13/09/1999 per tutti i metalli
EPA 7470 A (CV-AAS) per il Hg

Si è ritenuto perciò necessario procedere a questo tipo di indagine su campioni rappresentativi delle diverse specie e dei diversi terreni della Regione Abruzzo come individuati dalla specifica cartografia regionale (De Laurentiis *et al.,* 2005).

Tab. 1Tartufi e suoli analizzati con le rispettive aree di raccolta

TARTUFO	***ZONA***
Tuber melanosporum	Menzano-Forcella (AQ)
Tuber aestivum	Menzano-Forcella (AQ)
Tuber aestivum	Menzano-Forcella (AQ)
Tuber borchii	Pedicciano-Fagnano A (AQ)
Tuber borchii	Pedicciano-Fagnano A (AQ)
Tuber aestivum	Scoppito (AQ)
Tuber macrosporum (zona magnatum)	Tagliacozzo (AQ)
Tuber aestivum	Pietransieri-Roccaraso (AQ)
Tuber aestivum	Pietransieri-Roccaraso (AQ)
Tuber aestivum	Pietransieri-Roccaraso (AQ)
Tuber melanosporum	S.Giorgio- Castel Raimondo (TE)
Tuber magnatum	Bisenti (TE)
Tuber magnatum	Bisenti (TE)
Tuber rufum	Bisenti (TE)
Tuber macrosporum	Bisenti (TE)
Tuber melanosporum	Montefino (TE)
Tuber melanosporum	Montefino (TE)
Tuber magnatum	Cellino Attanasio (TE)
Tuber mesentericum	Santo Stefano di Sessanio (AQ)
Tuber magnatum	Pietransieri-Roccaraso (AQ)
Tuber magnatum	Pietransieri-Roccaraso (AQ)
Tuber macrosporum	Corbellino Fagnano (AQ)
Tuber macrosporum	Castello, Montefino (TE)

Risultati

A differenza di quanto è emerso per alcuni funghi epigei, considerati ottimi commestibili e sinora ammessi al commercio dalla vigente normativa sugli alimenti, che si sono in realtà rivelati preoccupanti bioaccumulatori di metalli pesanti, in particolare Hg e Cd, o radioisotopi (Cs e Sr) (Cocchi & Vescovi, 1997; Cocchi *et al.,* 2005), i tartufi sembrano, al momento, essere un alimento perfettamente sicuro da questo punto di vista (Fig. 3). I risultati ottenuti con i due diversi metodi analitici, infatti, evidenziano una ridotta assunzione di ioni dalle soluzioni circolanti nel suolo da parte degli stessi come in parte era stato evidenziato in precedenza in uno studio (Giaccio *et al*., 1992) che aveva esaminato la concentrazioni dei metalli pesanti nei soli corpi fruttiferi dei tartufi, senza verificare i corrispondenti valori nel terreno di crescita (Tab. 2).

Tab. 2 Concentrazioni medie di metalli pesanti nelle sei specie di tartufi, raccolti in diverse aree della regione Abruzzo (valori in mg/kg ppm).

tartufo	Fe	Cd	Al	As	Be	Co	Cr	Cu	Hg	Ni	Pb	Se	V	Zn
T. melanosporum	131,78	0,41	85,70	0,05	0,01	0,01	0,21	4,56	0,05	0,38	0,12	0,04	0,20	74,00
T. aestivum	133,01	0,11	111,01	0,04	0,01	0,03	0,22	1,88	0,02	0,25	0,26	0,01	0,24	29,27
T. borchii	340,16	0,31	692,81	0,16	0,04	0,00	0,98	3,08	0,01	0,37	0,30	0,00	1,35	41,93
T. macrosporum	105,83	0,03	12,75	0,01	0,00	0,00	0,05	4,46	0,01	0,04	0,13	0,01	0,05	25,27
T. mesentericum	99,44	0,06	68,74	0,03	0,00	0,00	0,19	6,69	0,01	0,09	0,32	0,01	0,21	156,40
T. magnatum	130,75	0,16	150,52	0,03	0,01	0,01	0,22	3,56	0,06	0,16	0,18	0,09	0,27	70,98

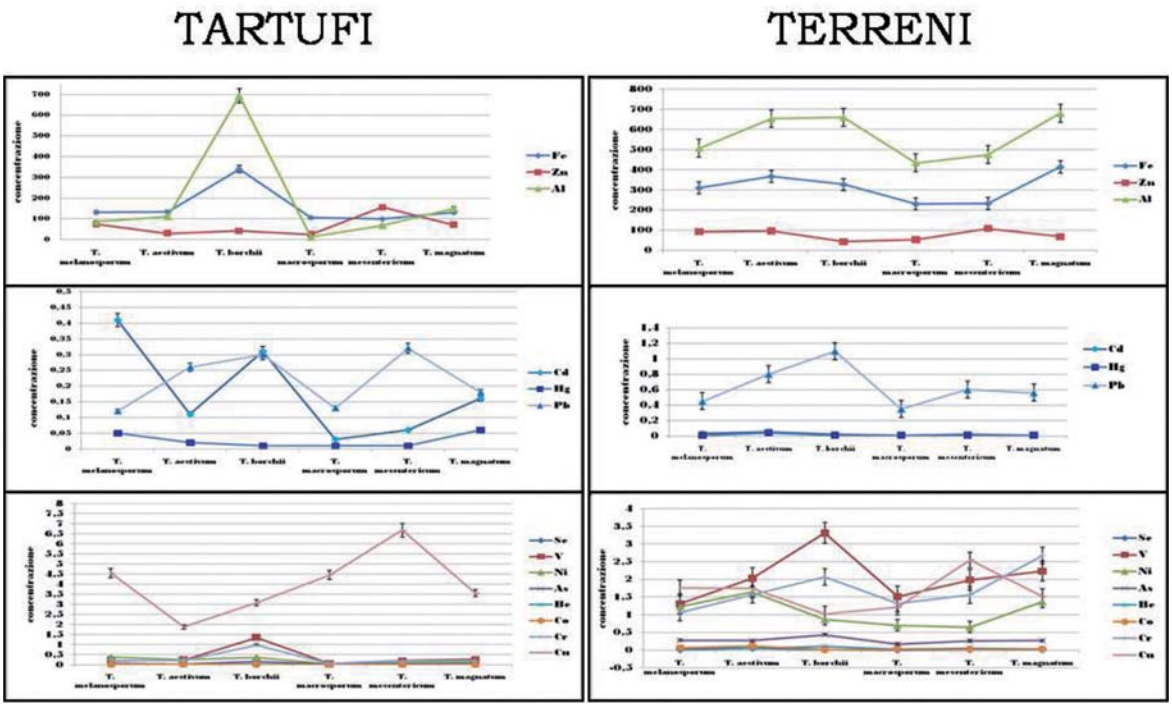

Fig. 3 Concentrazione dei metalli pesanti nelle diverse specie di tartufo confrontata con il loro contenuto nel suolo di crescita.

Conclusioni

Questo lavoro, che rappresenta il primo studio sul bioaccumulo dei metalli pesanti da parte dei tartufi, ha evidenziato la loro assoluta qualità e sicurezza. Già in precedenza, l'unico lavoro esistente sul contenuto dei metalli pesanti nei tartufi, anch'esso realizzato in Abruzzo (Giaccio *et al.,* 1992), aveva mostrato che tali valori risultavano ampiamente sotto la soglia di sicurezza. Mancando dati di ricerche analoghe effettuati in altre aree, rimane da chiarire se la mancanza di bioaccumulo di metalli pesanti sia una caratteristica generale dei tartufi o sia, in realtà, connessa con le caratteristiche dei suoli tartuficoli.

I terreni tartuficoli abruzzesi sono caratterizzati da un basso contenuto di metalli pesanti e da un pH alcalino (che ne limita l'assunzione), e per questo, a differenza dei funghi, i tartufi sembrano essere dei buoni indicatori di ambienti tellurici salubri (Geoffrey & Gadd, 2007).

Ringraziamenti

Siamo grati a Valentina Ferrari ed alla dott.ssa Rosita Abbate dell'ARTA per il prezioso aiuto tecnico nell'uso della strumentazione, e, per la raccolte del materiale studiato, al dr. Gabriele De Laurentiis e Domenico Spinelli dell'ARSSA, a Carmine Visca Università dell'Aquila, ed ai signori Laurentino Oddis e Silvio Guardiani.

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TRUFFLE IN BHUTAN

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Abstract
In 1991 thirty oaks inoculated with *Tuber melanosporum* Vittad., *Tuber magnatum* Pico and *Tuber aestivum* Vittad. obtained from the Research Centre in San Angelo in Vado (Italy) were sent to the National Mushroom Centre, under the Ministry of Agriculture in Bhutan through the then Director of the Institute. These plants were planted in the area around the Centre. Only later on starting from 1995 periodical management (removal of the lower branches to allow light) and adding of lime was carried out. In 2001 upon digging around one of the trees some truffles were discovered. They were very small 1-2 cm in diameters, soft and without any aroma. It has been concluded that they were not the main truffles inoculated on the plants but contaminations which were encouraged to take over because of the nature of the soil and pH. The pH of the soil is found to be below 6 also after liming.
There is only one report of natural truffles in Bhutan in 1998 while eight truffles of around 200 g each were discovered collecting pine saplings from a forest nursery near Thimphu, Western Bhutan. These truffles were similar to *Tuber borchii* Vittad. (bianchetto) for the smooth yellowish red peridium and the reticulated spores. The host plant was *Pinus wallichiana* (2 years old) and the soil pH 6.2. Molecular analysis will be necessary to identify these whitish truffles.
The spores of these truffle might have come along with the forest litter collected from the near by forest where lime stone and dolomite are found.
The presence of *T. himalayensis* and *T. indicum* has not been reported in Bhutan but has been suspected since they were found in the other parts of the Himalayas.
Key words: ecology, whitish truffles, Himalayas.

UP-TO-DATE ACQUIREMENTS ON TRUFFLES AND FALSE TRUFFLES OF BASILICATA AND APULIA (SOUTHERN ITALY)

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Abstract
Authors report results of a research carried out in the last tree years on hypogeous fungi of Basilicata (Matera and Potenza provinces) and Apulia (Brindisi, Lecce and Taranto provinces) regions (southern Italy). Seven and 22 taxonomic entities (including also two semi-hypogeous species) were discovered for the first time in the first and second region, respectively. So the number of such fungi until now found in the above Italian regions grew up to 56 and 47 units, respectively. The presence of *Pachyphloeus prieguensis* is reported for the first time in Salentum and in Italy.
Key words: Truffle biodiversity, False truffles, Basilicata, Apulia, Southern Italy.

Studies on hypogeous fungi of Basilicata and Apulia regions conducted from 1996 until the beginning of spring 2006 showed that 49 and 22 taxonomic entities were present in their respective territories (see, among others, Rana *et al.*, 2006, 2007 and 2008). Further investigations carried out in the last three years allowed some new knowledge advances that are hereafter summarized. The numerous localities explored during this research are included in provinces of Matera and Potenza, in Basilicata, and Brindisi, Lecce and Taranto, in Apulia. Dogs used for finding the hypogeous fungi mostly belonged to breeds Lagotto Romagnolo and Epaniel Breton but were also small hybrids well trained to truffle search. For identification and correct classification of fungi found during this study, treatises of Montecchi & Sarasini (2000), Riousset *et al*. (2001), Granetti *et al*. (2005), Gori (2005) and papers by Pacioni & El-Kholy (1994) and Hibbet *et al*. (2007) were referred to.
The new species, varieties and *formae* of hypogeous and semi-hypogeous fungi found in the two regions [the relative *exsiccata* were deposited in the mycological collection of *Herbarium Lucanum* (HLUC) at University of Basilicata - Potenza] are hereafter listed (see Rana *et al*., 2008, 2008 a, b, and Signore *et al*., 2008) for details, i. e., locations and dates of findings, *exsiccata* number/s of each mycete, macro- and microscopic features, colour pictures):

BASILICATA

Ascomycota - Pezizomycotina - Pezizomycetes - Pezizomycetidae - Pezizales**:** *Incertae sedis*: *Picoa lefebvrei* (Pat.) Maire (1906); *Pezizaceae*: *Terfezia olbiensis* Tul. & C. Tul. (1851); *Pyronemataceae*: *Stephensia bomycina* (Vittad.) Tul. (1851); *Tuberaceae*: *Reddellomyces donkii* Malençon (1973); *Tuber magnatum* Pico var. *vittadinii* Daprati; *T. rufum* Pico: Fr. fo. *lucidum* (Bonnet) Montecchi & Lazzari (1993) (the last two, although here mentioned, must be not considered true taxonomic entities because, probably, genetically identical to respective species);
Basidiomycota - Agaricomycotina - Agaricomycetes - Phallomycetidae - ***Gomphales*** - *Gomphaceae*: *Gautieria graveolens* var. *otthii* (Trog) Zeller & Dodge. (1934); ***Hysterangiales*** - *Hysterangiaceae*: *Hysterangium inflatum* Rodway (1918).

APULIA

Ascomycota - Pezizomycotina - Pezizomycetes - Pezizomycetidae - Pezizales**:** *Incertae sedis*: *Picoa juniperi* Vittad. (1831); *Pezizaceae*: *Pachyphloeus citrinus* Berk. & Broome (1846); *P. ligericus* Tul. & C. Tul.: Berk. (1851); *P. prieguensis* Mor.-Arr., J. Gomez & Calonge (1996); *Terfezia arenaria* (Moris) Trappe (1971), *T. boudieri* Chatin (1891) and *T. olbiensis*; *Pyronemataceae*:

Genea fragrans (Wallr.) Sacc. (1889); *G. sphaerica* Tul. & C. Tul. (1851); *G. verrucosa* Vittad. (1831) and its var. *badia* Mattir. (1900); *Geopora arenosa* (Fuckel) S. Ahmad (1978); *Stephensia bombycina*; *Tuberaceae*: *Reddellomyces donkii*; *Tuber puberulum* Berk. & Broome (1846).
Basidiomycota - Agaricomycotina - Agaricomycetes - Agaricomycetidae - Agaricales - *Strophariaceae*: *Hymenogaster bulliardii* Vittad. (1831); *Incertae sedis*: *Setchelliogaster tenuipes* (Setch.) Pouzar (1958); ***Boletales*** - *Paxillaceae*: *Melanogaster ambiguus* (Vittad.) Tul. & C. Tul. (1843); *Rhizopogonaceae*: *Rhizopogon roseolus* (Corda) Th. Fr. (1909); ***Phallomycetidae - Geastrales*** - *Geastraceae*: *Schenella pityophila* (Malençon & Riousset) Estrada & Lado (2005) (see Signore *et al*., 2008); ***Hysterangiales*** - *Hysterangiaceae*: *H. crassum* (Tul. & C. Tul.) E. Fischer (1938), *Hysterangium inflatum*; *H. stoloniferum* Tul. & C. Tul. (1843).
These recent findings temporarily bring up the number of species and varieties/formae of hypo- and semi-hypogeous fungi so far reported in Basilicata and Apulia to 56 and 47 units, respectively (including among those found in the second region, although rare, *T. melanosporum* Vittad. and *T. magnatum* Pico). Finally, it seems worthwhile to underline the first finding, in Salentum and in Italy, of *Pachyphloeus prieguensis* in symbiosis with *Quercus ilex* L. This rare hypogeous fungus, so far reported as present in Spain and in Hungary (Montecchi & Sarasini, 2000), has been recently found also in Greece (Signore *et al*., unpublished results).

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MONITORING THE DIVERSITY OF ECTOMYCHORRIZAL AND SOIL FUNGI UNDERLYING NATURAL AND MAN-MADE *TUBER MELANOSPORUM* PLANTATIONS

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Abstract

The production of truffles is the offspring of several intermingled factors that mediate the transition from the vegetative to the reproductive phase. Among these determinants, the fungal and microbial diversity of the soil likely stands out as a key player. Thus, monitoring microflora dynamics next to and on apical root-tips within truffle grounds is of paramount importance. Below ground, ectomycorrhizal communities are often species-rich. Yet, our knowledge on fungal biodiversity underpinning truffle plantations is still scant. Now, characterization of the different ectomycorrhizas (ECM) at the species level can be achieved by combining detailed morphological and anatomical descriptions with molecular approaches. Further to this, reliable procedures are available to isolate and analyse microbial and fungal DNA from soil samples. Lastly, the ongoing acquisition on public databases of sequence information relative to phylogenetically important genomic traits such as the small subunit (SSU) and the internal transcribed spacers (ITS) of the rDNA region for an increasing number of fungi has greatly improved the chances of giving species name to mycorrhizal structures and unculturable soil fungi. To investigate the fungal biodiversity underlying both productive and unproductive *T. melanosporum* natural and/or man made truffle plantations, we have sampled ECMs from truffle grounds located in central Italy (Latium and Umbria) and analysed them morphologically and molecularly via ITS rDNA region amplification. From these collection sites, by isolating and amplifying the fungal ITS region from soil samples, we generated a dataset of the soil fungal community. Both ectomycorrhizal and soil samplings were performed during the years 2007-2008. Until today, we were able to cluster ECMs into different morphotypes while approximately 50 ECM taxa on the basis of ITS analysis have been monitored. Interestingly, most of these ECMs have been also sampled into soil, likely as free living mycelia.

The ongoing analysis will help up tracking the fungal biodiversity on truffle fields over time and under different ecological situations. It will also enable us to test whether the presence or absence of specific ECM taxa is associated with truffle production.

Key words: fungal biodiversity, ectomycorrhizas, soil fungi, ITS, truffle plantations, ecology.

TAXONOMIC STUDY OF DESERT TRUFFLES IN TUNISIA

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Abstract
Desert truffles are met in the arid and semi arid areas with warm climate around the Mediterranean basin, associated with roots of some perennial and annual species of *Helianthemum* and *Cistus* of the *Cistaceae* family. Their fruitbodies are very appreciated by local people for their organoleptic qualities and their therapeutic virtues.
Based on morphological criteria (form of spores and asci, spores ornamentation, structure of the peridium and the gleba), a mycological study has been done in the Range Ecology Laboratory of the Arid Lands Institute of Médenine, in order to identify desert truffles species which are present in southern Tunisia. Six species of truffles have been identified; *Terfezia boudieri* Chatin, *Terfezia claveryi* Chatin., *Tirmania nivea* Trappe, *Tirmania pinoyi* (Maire) Malençon, *Picoa carthusiana* Tul. & C. Tul. and *Picoa juniperi* Vittadini. The last two species have been recently found in Tunisia.
Key words: Taxonomic study, desert truffle, morphological criteria, *Terfezia*, *Tirmania*, *Picoa.*

LA GERMINAZIONE DELLE SPORE DI *TUBER MAGNATUM* PICO

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Abstract
The aim of this work was to elucidate and to describe the processes that favour *Tuber magnatum* Pico spore germination. All the phases that follow spore release from the *ascus* up to the primary mycelium formation are described. Spore behaviour in the presence of the symbiotic plant and in its absence is also analysed.
Key words: biology, ascus, mycelium.

Premessa
E' noto che la coltivazione del tartufo bianco (*Tuber magnatum* Pico), al contrario di *Tuber melanosporum* Vittad. sta offrendo grosse difficoltà soprattutto nei riguardi della produzione delle piante micorrizate. Assume, pertanto, molta importanza la conoscenza dei fattori che favoriscono la germinazione delle spore di questa specie.
La conoscenza di tali fattori, oltre ad una notevole importanza scientifica, ha una grossa importanza pratica perché può consentire di dar vita alla coltivazione di *Tuber magnatum* e alla salvaguardia delle tartufaie naturali sottoposte ad una raccolta troppo intensa: distribuire spore sulla superficie delle tartufaie quando sono presenti le condizioni ambientali idonee alla loro germinazione può consentire il mantenimento di un sufficiente grado di micorrizazione delle piante simbionti responsabili della produzione di corpi fruttiferi.

Materiali e metodi
Questo lavoro è stato eseguito seguendo le modalità di inoculo sporale usate generalmente nella micorrizazione delle piante in vivaio. Allo scopo di verificare l'influenza esercitata dalla pianta simbionte sulla germinazione delle spore sono stati eseguiti inoculi con pianta simbionte e senza pianta simbionte.
Nel mese di novembre 2006 corpi fruttiferi di *Tuber magnatum* raccolti nel Bresciano, dopo essere stati individuati, sono stati lasciati a maturare in campo nel punto di fruttificazione per altri 15 giorni. Dopo la raccolta sono stati sterilizzati in superficie con alcool etilico al 95%, omogeneizzati e messi in cella frigorifera a 5° C.

Inoculo senza pianta simbionte
Nel mese di Marzo 2007 dieci fitocelle dalle dimensioni di cm 7 di diametro x cm 24 di altezza sono state riempite per due-terzi di terreno sterilizzato prelevato da una tartufaia di *T. magnatum* in produzione. Al centro è stato disposto l'inoculo coperto da uno strato di sabbia calcarea setacciata, lavata, sterilizzata, del diametro di 2 - 4 mm. e coperta da un ulteriore strato di terra.
I campioni sono stati depositati su una griglia metallica in tunnel non riscaldato.

Inoculo con presenza della pianta simbionte.
Nel mese di marzo 2007, semenzali di *Quercus pubescens* Willd. fatti germinare in cassoni contenenti una miscela al 50% di perlite e vermiculite sterile sono stati sistemati in fitocelle riempite con terreno sterilizzato della stessa natura geologica sopra descritta. L'inoculo veniva depositato principalmente nella parte superiore delle radici in ragione di gr. 10 per prova, successivamente è stato ricoperto con uno strato di sabbia sterile; la fitocella è stata poi riempita di terreno. I preparati sono stati trasferiti in tunnel protetto non riscaldato e posti sopra una griglia metallica.

Dopo 6 mesi, a cadenza mensile, una piccola parte dell'inoculo veniva prelevato e sottoposto a controllo microscopico per valutare lo stato delle spore e l'eventuale modificazione dell'episporio.
All'ottavo mese il 70% delle spore presentava l'episporio degradato, mentre nei rimanenti 30% il degrado è avvenuto nei due mesi successivi. In seguito, parte della sospensione sporale veniva prelevata e sistemata in micro piastre Petri, che venivano mantenute ad una temperatura di 20 gradi centigradi. Successivamente parte del prelievo veniva sistemato su vetrini con aggiunta di acqua sterile e sottoposto periodicamente a controllo con microscopio ottico.

Risultati

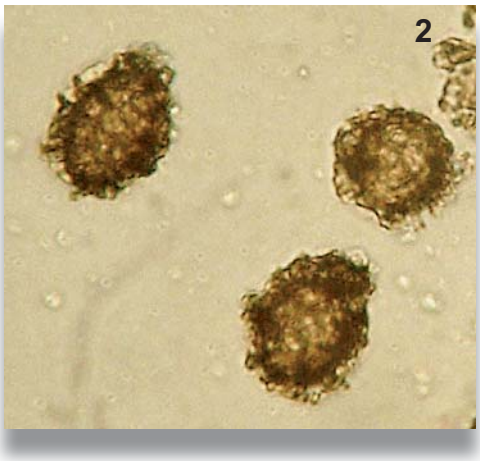
Campioni senza pianta simbionte
La totalità dei campioni inoculati nel terreno senza la presenza della pianta simbionte ha dato origine solo ad abbozzi di germinazione senza un ulteriore sviluppo del micelio.

Inoculo con presenza della pianta simbionte
La maggior parte dei campioni inoculati (sette su dieci) ha dato origine ad una germinazione molto incostante ed aleatoria dove la maggior parte delle spore non ha proseguito oltre l'apertura del poro germinativo, solo alcune di esse hanno completato la germinazione. I tre campioni rimasti hanno dato risultati interessanti con oltre il trenta per cento di spore germinate.
Tutte le fasi di questo lavoro sono state ampiamente documentate con fotocamera elettronica, in parte anche con diapositive.

Fasi della germinazione spore di *Tuber magnatum* Pico
Vorrei premettere che, in questo lavoro, riporto quanto ho notato in anni di osservazioni convincendomi che si tratta di germinazione delle spore e non di eventuali miceli inquinanti. Ovviamente l'analisi molecolare degli elementi osservati avrebbe tolto ogni dubbio.

1 Uscita delle spore dall'asco
L'uscita delle spore dall'asco è determinata principalmente dall'azione corrosiva dei batteri presenti nel corpo fruttifero, essa può prolungarsi anche per la durata di alcuni mesi (Foto n° 1).

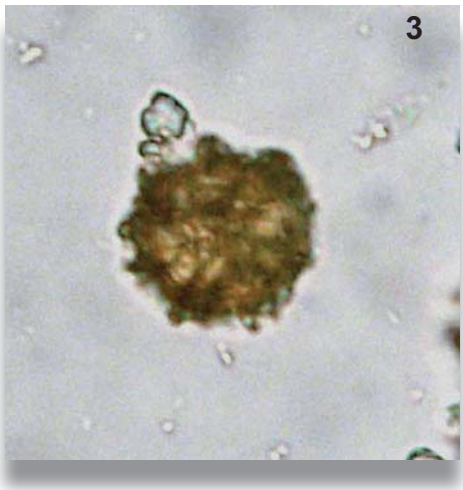
<u>Deterioramento dell'episporio</u>
In *Tuber magnatum* la resistenza dell'episporio può ostacolare la germinazione. Sono stati osservati eventi in cui l'integrità dell'episporio ha creato grosse difficoltà all'uscita del micelio primario.
Il degrado avviene in tempi abbastanza lunghi (alcuni mesi, Foto n° 2).

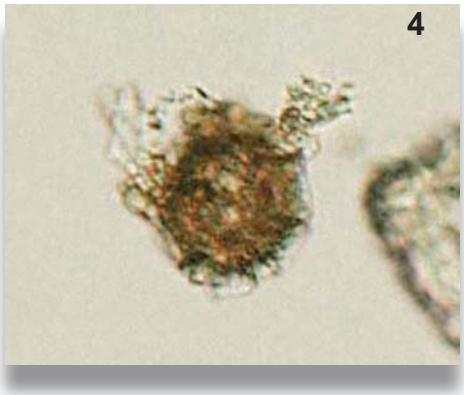


Esso si presume avvenga per l'azione congiunta di vari elementi tra cui: secrezioni radicali, componenti del terreno e batteri. Questi ultimi possono rivestire un ruolo importante nel processo metabolico sia della maturazione del corpo fruttifero che del deterioramento dell'episporio, in funzione anche delle temperature relativamente basse del periodo di maturazione degli sporocarpi.

Apertura del poro germinativo

Durante le varie osservazioni, e la numerosis-sima documentazione fotografica realizzata, in *Tuber magnatum* l'uscita dell'ifa è stata osservata avvenire da un solo poro germinativo (Melendez – Howell, 1967). All'inizio si evidenzia con una minuscola cavità seguita dalla formazione di una protuberanza disposta tra due creste dell' episporio (Foto N° 3 - 4).



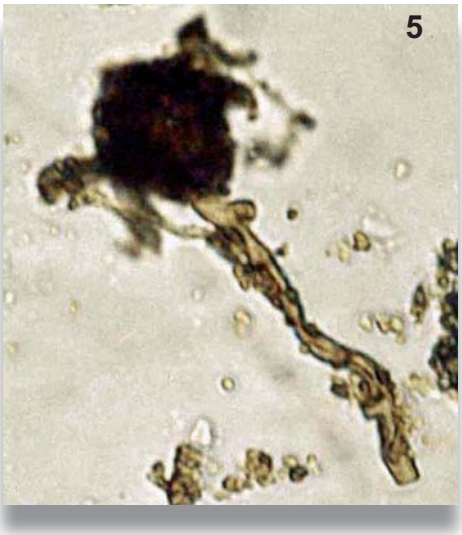


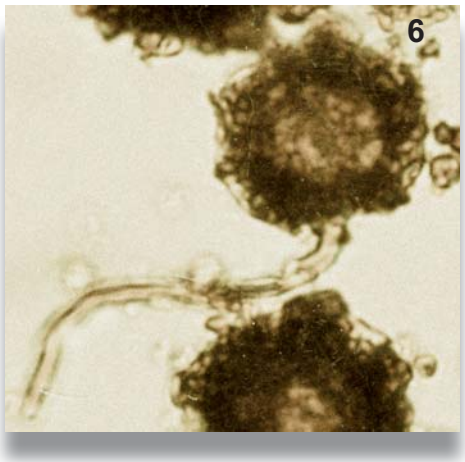
Ma può apparire anche come una minuscola lacerazione con espulsione di frammenti di episporio; probabilmente ciò è dovuto alla pressione che si forma all'interno della spora durante lo sviluppo del micelio primario. Essa può presentarsi anche come una autentica spaccatura nei casi in cui alla germinazione sono interessate contemporaneamente più ife.

Inizio della germinazione

L'inizio della germinazione avviene generalmente dopo alcuni giorni dall'apertura del poro germinativo. Il micelio primario, in uscita, è anticipato o accompagnato dall'espulsione e o trascinamento di alcuni frammenti di episporio, o altri componenti della spora. Come detto in precedenza, la maggioranza delle spore generano una sola ifa, ma si sono avuti esempi anche di uscite di più ife. Di frequente, dopo la prima fase iniziale della germinazione, non segue alcun progresso e la spora rimane in stato di quiescenza per lungo tempo.

Sviluppo del micelio
Lo sviluppo del micelio primario segue cicli periodici, dopo alcune fasi di crescita seguono periodi di riposo, è stato osservato giungere ad una lunghezza massima di 120 -150 micron, con una crescita di 13 micron ora. Successivamente, forse per mancanza di nutrimento, lo sviluppo si arresta (Foto N° 5-6).



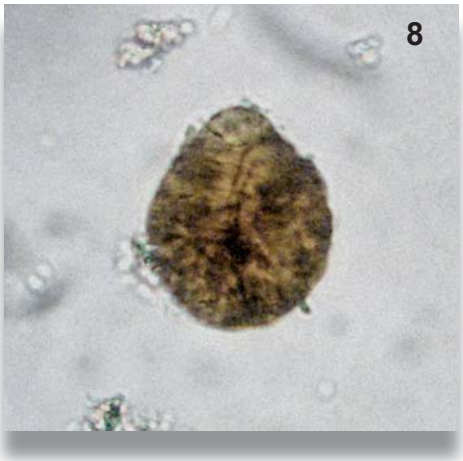


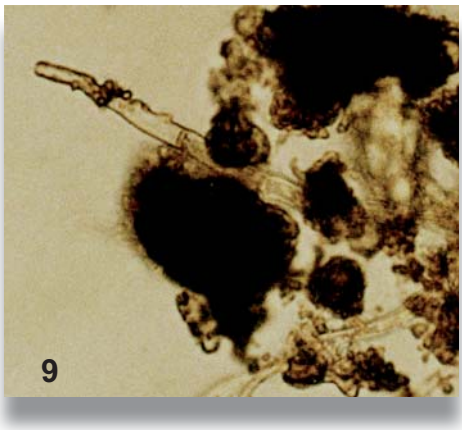
Caratteri del micelio
Il micelio generato dalle spore è settato, di colore ialino, con poche granulazioni ed un diametro medio di 4,5 micron. Raramente è stato osservato anche micelio dalle dimensioni di 2,3 micron. Spesso è presente un inizio di ramificazione a 90 gradi, (Foto N° 7) che non ha mai dato origine ad un ulteriore sviluppo. L'apice può essere arrotondato, ma spesso appare reciso (Foto N° 5-7).

In questo caso il micelio si sviluppa sfilandosi dall'interno (Foto N° 7). Questo particolare è stato notato anche nel micelio presente sulla superficie delle micorrize.

Collasso delle spore
Dopo dodici-tredici mesi dall'inoculo, parte delle spore non germinate (Ceruti, 1990) vanno incontro ad un normale disfacimento.
Ad alcune spore, prima della fase di collasso, si stacca il reticolo sporale lasciando l'episporio

psilato (Foto N° 8). Dopo la fase di collasso, sono state osservate porzioni o abbozzi di ife settate che si sviluppano e si accrescono come quelle osservate nella fase germinativa della spora. In altre, invece, si presenta come un abbozzo di ife singole o biforcate (Foto N° 9).





Unione di due spore

In due occasioni si è osservata l'unione dei miceli generati da spore germinate, mentre in un'altra si è vista l'unione di miceli generati da spore collassate (Foto N° 10-11). In tutti i casi, tuttavia, non è stato possibile documentare le fasi di avvicinamento.

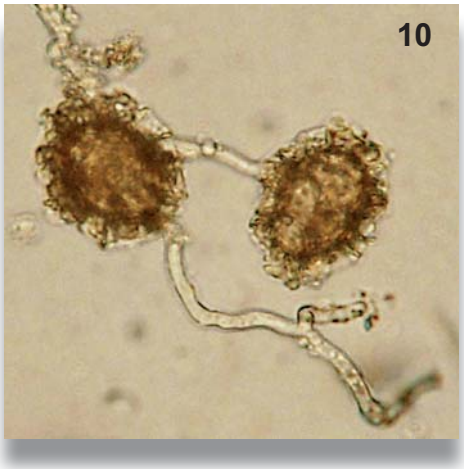


Foto n° 10 - Unione di miceli generati da due spore

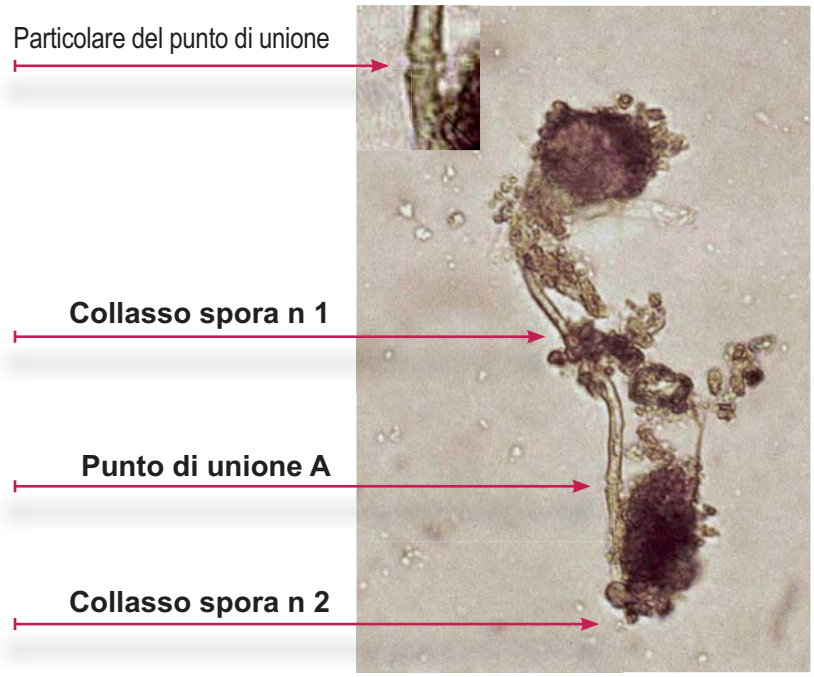


Foto n° 11 Unione di miceli da spore collassate

Conclusioni

La scarsa conoscenza degli elementi che favoriscono la germinazione delle spore, i tempi di dormienza, le temperature ideali di germinazione, le difficoltà a seguire il processo nelle varie fasi, i problemi derivanti dai prelievi, mi hanno in molti casi dissuaso a completare lo studio di questo evento.

Questo lavoro, iniziato ed in parte realizzato nel 1996 (Vezzola, 2004), è stato recentemente completato con la documentazione dell'ultima parte del percorso: l'unione di ife generate da due spore.

Tutte le fasi del processo sono state seguite realizzando una ampia documentazione fotografica dei singoli eventi, dalla uscita delle spore dall'asco fino all'unione dei miceli generati dalle spore. Tuttavia non è stato rilevato nessun elemento che evidenzi caratteri distintivi delle spore a cui attribuire differenze di polarità tra i due soggetti.

La germinazione delle spore di *Tuber magnatum* avviene in tempi più lunghi rispetto a quelli delle altre specie di *Tuber* testate, *T. melanosporum* Vittad., *T. macrosporum* Vittad. e *T. aestivum* Vittad..

Ciò può essere causato da vari elementi quali: il grado di maturazione del corpo fruttifero, la rottura dell'asco, la corrosione dell'episporio e componenti del terreno. Questi elementi, nel complesso, sono risultati importanti per favorire tale processo, ma singolarmente non sono stati identificati. E' stato evidenziato che la presenza della pianta simbionte è importante nello stimolare l'inizio del processo, e la conseguente formazione del micelio primario. Molte spore per motivi sconosciuti non riescono a germinare e dopo un periodo prolungato (dieci- dodici mesi) dall'inoculo collassano. Dopo la fase di collasso, in alcuni casi, si possono notare ife singole o biforcate unite alla base. Ritengo che questo lavoro debba essere ulteriormente approfondito per verificare i motivi della bassa percentuale di spore che hanno completato il ciclo evolutivo e verificare la realtà delle mie osservazioni effettuate con semplici mezzi da laboratorio.

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GERMINAZIONE DELLE SPORE DI *TUBER MELANOSPORUM* VITTAD.

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Abstract
The author describes the various phases occurring during *Tuber melanosporum* Vittad. spore germination, from the spore release from the *ascus* up the primary mycelium formation. Spore behaviour in the presence of the symbiotic plant and in its absence is also analysed.
Key words: spore, mycelium, symbiotic plants.

Scopo della ricerca

La germinazione delle spore di *Tuber melanosporum* Vittad. è un elemento fondamentale nelle applicazioni pratiche della tartuficoltura. Scopo della ricerca è stato quello di chiarire alcuni aspetti che concorrono alla germinazione delle spore (Fig. 1) alla formazione delle micorrize e documentare i vari eventi che si susseguono dall'apertura del poro germinativo allo sviluppo del micelio primario, fino all'unione dei miceli provenienti da spore diverse.

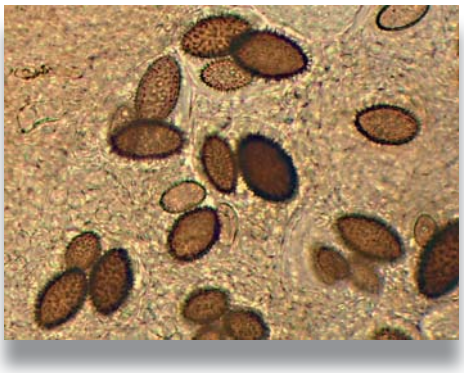

Fig. 1

Materiali e metodi

Corpi fruttiferi di *Tuber melanosporum* ben maturi, raccolti nel mese di gennaio 2007, sono stati sterilizzati in superficie con alcool etilico al 95%, stratificati in sabbia sterile e posti in cella frigorifera a 5° C. Nel mese di marzo 2007, i carpofori sono stati tagliati a pezzi, aggiunta acqua distillata sterile, omogeneizzati per due minuti a 2.500 r.p.m. ed usati come inoculo sporale in due tesi: una con la presenza della pianta simbionte, una senza la pianta simbionte. Iniziando dopo sei mesi, parte delle spore sono state prelevate, e si è osservato il grado di germinazione.

Tesi numero uno

Nel mese di marzo 2007, dieci fitocelle di centimetri dieci di diametro per ventiquattro di altezza, sono state riempite per due-terzi con terreno raccolto in una tartufaia naturale di *Tuber melanosporum* in produzione, precedentemente sterilizzato a vapore fluente per un'ora. Nel terreno, al centro della fitocella è stato depositato l'inoculo sporale in ragione di grammi dieci per prova. La sospensione è stata coperta con uno strato di sabbia calcarea sterile dello spessore di 3-5 millimetri e ricoperta da un ulteriore strato di terra.

Tesi numero due

Dieci semenzali di *Quercus pubescens* Willd., fatti germinare in cassoni contenenti una miscela di perlite e vermiculite al 50% sterile, sono stati depositati al centro di fitocelle riempite

di terreno come descritto nella prova precedente. Nella parte superiore delle radici sono stati depositati dieci grammi di sospensione sporale, coperta con uno strato di sabbia calcarea sterile dello spessore di 3-5 mm e riempiti di terra. Successivamente, le fitocelle usate nelle due prove sono state depositate su una griglia di ferro in tunnel non riscaldato.

Risultati

Inoculo senza pianta simbionte

Nella tesi numero uno (senza pianta simbionte), dai controlli seguiti sia dopo sei mesi dall'inoculo che in quelli successivi (otto, nove, dodici mesi), si è osservato solo un minuscolo accenno alla germinazione, determinato da una lieve protuberanza in prossimità del poro germinativo. Mentre la maggior parte delle spore sono rimaste integre.

Inoculo con la presenza della pianta simbionte

Nella tesi con pianta simbionte, le spore hanno presentato un grado di germinazione incostante, che si è prolungato per alcuni mesi. Dopo sei, sette mesi dall'inoculo (Fig. 2), si sono avuti i primi risultati, questi hanno riguardato principalmente le spore inoculate sulle piante meglio sviluppate, mentre in quelle con la crescita più stentata la germinazione è avvenuta nei mesi successivi.

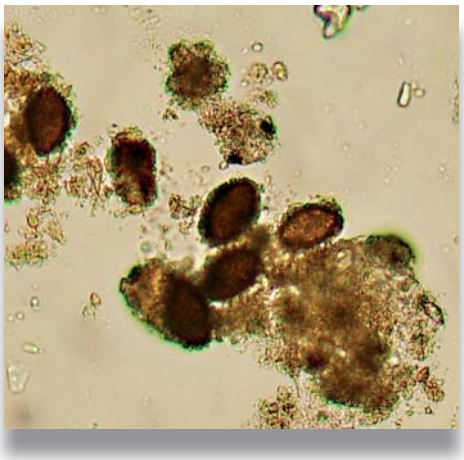

Fig. 2 Dopo sei mesi

Molte spore, durante la fase di germinazione, si sgretolano mettendo in evidenza porzioni o abbozzi di micelio come osservato in *Tuber magnatum* (Fig. 3), mentre alcune rimangono integre per lungo tempo.

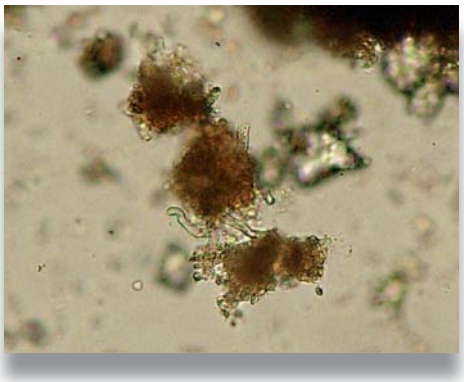

Fig. 3 dopo 12 mesi

Fasi della germinazione

L'apertura del poro germinativo si evidenzia con una minuscola protuberanza disposta sulla parete della spora. In base alla numerosa documentazione raccolta è stata osservata una sola

ifa per tubo germinativo. Il poro germinativo non ha una posizione ben definita, può presentarsi al centro della parete sporale, o spostato verso una delle due estremità, ciò fa supporre che esistano più pori germinativi (Fig. 4-5-6).

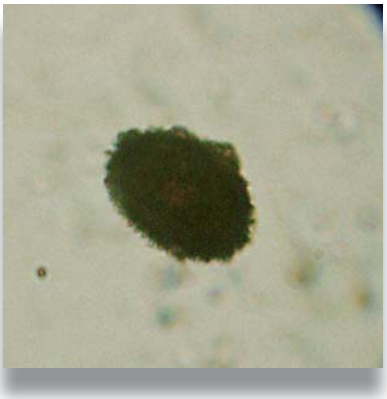

Fig. 4

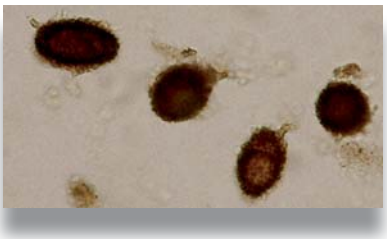

Fig. 5

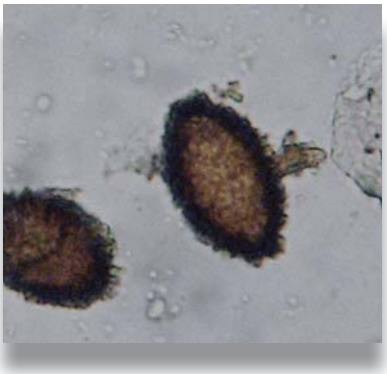

Fig. 6

Caratteri del micelio

Il micelio generato dalle spore è settato, con poche granulazioni ed un diametro medio di 4,6 micron. Dopo una crescita nella fase iniziale di otto - dieci micron, lo sviluppo si arresta (Fig. 6). Successivamente, riprende, fino a raggiungere unalunghezza massima osservata di micron duecento (Fig. 7).

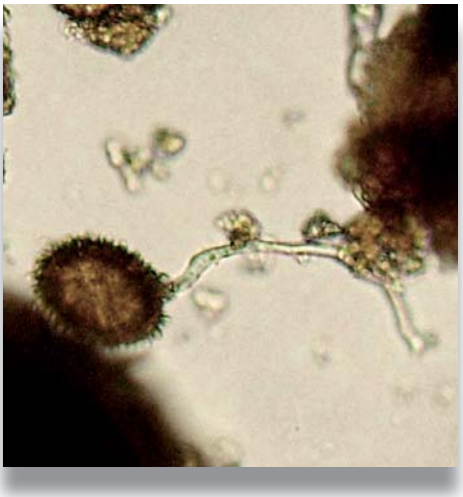

Fig. 7

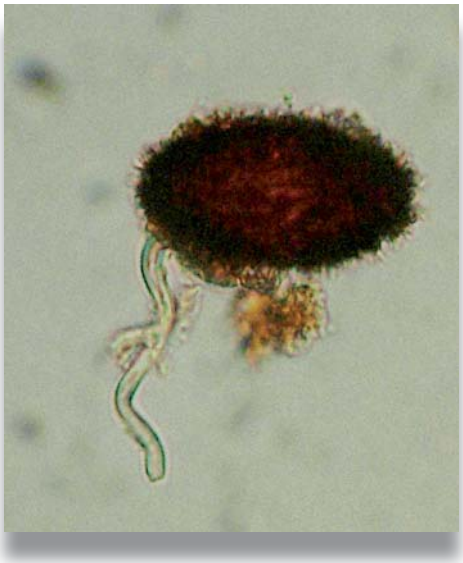

Fig. 8

Frequentemente è presente un inizio di ramificazione che non ha mai dato origine ad un ulteriore sviluppo (Fig. 8). La velocità di crescita riscontrata non è stata omogenea, nella fase iniziale è stata di circa otto micron ora, in altre osservazioni, di dodici.

Unione dei miceli provenienti da spore diverse

L'unione del micelio primario generato da due spore diverse è stata documentata in più occasioni. Tuttavia (come rilevato in *Tuber magnatum),* non è mai stato osservato nessun elemento distintivo a cui attribuire, alle spore, differenze di polarità (Fig.9).

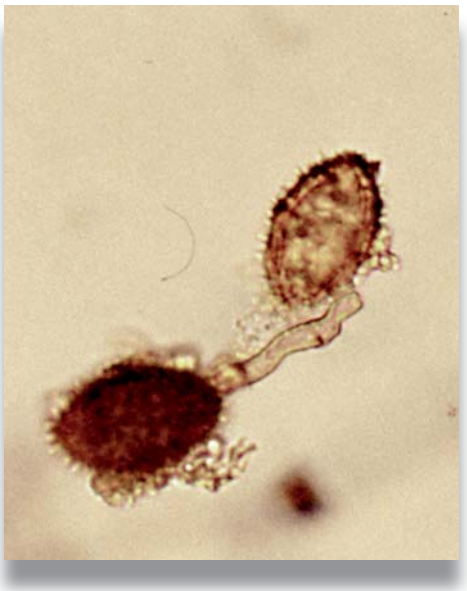

Fig. 9

Conclusioni

La germinazione delle spore di *Tuber melanosporum* è un evento molto aleatorio, ed incostante, essa avviene gradualmente, in tempi che oscillano tra i sei ed i quindici mesi. Molte spore nella fase germinativa vanno incontro ad un naturale disfacimento liberando nel terreno porzioni di micelio come osservato nella germinazione delle spore di *Tuber magnatum*. La presenza del simbionte è risultata determinante nel favorire l'inizio del processo di germinazione, come molto importante è risultato il grado di sviluppo dell'essenza arborea. E' stato interessante notare l'influenza che esercitano sulle spore le piante con un apparato radicale più sviluppato le quali stimolano la germinazione un numero maggiore di spore. E' stato osservato che l'apertura del poro germinativo non avviene in un punto ben definito, ma può avvenire in punti diversi, ciò fa supporre l'esistenza di più pori germinativi ed è evidenziata da una piccola protuberanza presente sulla parete sporale che, a volte, si manifesta molto tempo prima dell'uscita del tubo germinativo.

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Conservation, Commerce and Valorisation Session

COMMERCIO E CONSERVAZIONE DEI TARTUFI IN ITALIA

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Abstract: Conservation and commerce of truffles in Italy
L'Autore, prendendo in considerazione le attività svolte nel laboratorio di trasformazione e commercializzazione dei tartufi della "Urbani Tartufi", si sofferma sulle problematiche:

- legate alla salubrità/qualità dei tartufi commercializzati;
- legate alla regolamentazione dell'etichettatura;
- fiscali relative agli enormi impedimenti in materia di IVA ed altri similari in confronto alle normative vigenti nel resto d'Europa.

Conclude evidenziando le differenze legislative che esistono nei paesi europei produttori di tartufi ed auspicando una normativa europea che possa superare leggi nazionali equiparando, almeno per quanto riguarda la proprietà del tartufo e l'entità dell'IVA, l'Italia ai diversi paesi europei.
Key words: truffle conservation, truffle commerce.

Premessa
La Urbani Tartufi è l'azienda che da anni è presente nel settore della raccolta, trasformazione, produzione e commercializzazione di tartufo e prodotti a base di tartufo. L'Azienda opera sempre nel rispetto della Legge 16 dicembre 1985, n. 752, Normativa quadro in materia di raccolta, coltivazione e commercio dei tartufi freschi o conservati destinati al consumo. Tale legislazione è integrata con le normative dei paesi esteri dove ha rapporti commerciali.
La passione che da anni questa azienda ha per il tartufo porta la direzione non solo al rigoroso rispetto delle normative vigenti ma anche a seguire le norme volontarie, è così che si è certificata ISO 9001/ISO 14001/ISO 22000 e ha ottenuto con High level BRC and IFS, Certificazione Sillikert col massimo dei voti per il mercato USA, il tutto sempre per essere stati competenti, chiari, affidabili, trasparenti.
Vengono qui affrontate alcune attività e problematiche relative alla commercializzazione e trasformazione dei tartufi:

- legate alla salubrità/qualità dei tartufi commercializzati;
- legate alla regolamentazione dell'etichettatura;
- fiscali relative agli enormi impedimenti in materia di IVA ed altri similari in confronto alle normative vigenti nel resto d'Europa.

A**)** *salubrità/qualità dei tartufi commercializzati*
La Urbani Tartufi, per fornire ai consumatori, agli altri soggetti interessati e alle controparti commerciali, fiducia nell'azienda, ha da sempre preso in seria considerazione la messa in commercio di alimenti sicuri e sani, considerando questo aspetto fondamentale per il mercato interno ed esterno. Tutto ciò per contribuire in maniera significativa alla salute e al benessere dei consumatori nonché a tutelare gli interessi sociali ed economici. La politica della nostra azienda è garantire un livello elevato di tutela della vita e della salute umana in applicazione delle politiche comunitarie ed in particolare nel rispetto del REGOLAMENTO (CE) N. 178/2002 che stabilisce i principi e i requisiti generali della legislazione alimentare, e fissa le procedure nel campo della sicurezza alimentare. La rintracciabilità di un alimento prescinde, infatti, dal fatto che questo sia in commercio sul mercato interno o su quello internazionale.
Per affrontare il problema della sicurezza alimentare in maniera sufficientemente esauriente la Urbani Tartufi ha predisposto controlli e messo in atto disposizioni, tutte volte a garantire che alimenti a rischio non siano immessi sul mercato; ha inoltre implementato istruzioni per individuare i problemi di sicurezza degli alimenti e reagire ad essi.
E' logico che per garantire la sicurezza degli alimenti occorre considerare tutti gli aspetti della filiera produttiva iniziando dalla produzione primaria inclusa; per tale motivo l'Azienda prende

in considerazione il ricevimento delle materie prime, gli imballaggi, l'origine, le tecniche di produzione, la trasformazione, il magazzinaggio, il trasporto, e infine la vendita o l'erogazione al consumatore.
Al fine di avere un totale controllo sia della rintracciabilità (Reg CE 178/2002) che della tracciabilità, e affinché vi sia un clima di fiducia, le valutazioni del rischio vengono svolte in modo indipendente, obiettivo e trasparente e sono basate sulle informazioni e sui dati scientifici disponibili. Per questo oltre alle rigide regole interne, si procede ad un elevata selezione dei fornitori, i quali vengono considerati qualificati dalla Urbani Tartufi, solo dopo essere sottoposti ad un lungo iter che prevede controlli documentali, audit aziendali, controlli analitici della merce fornita (imballi o alimenti) attraverso laboratori accreditati (anch'essi sottoposti a verifiche). La Urbani Tartufi rispetta tutte le Norme internazionali sugli alimenti, nonché le norme sanitarie e fitosanitarie. La nostra azienda per determinare il rischio di un alimento ha preso in considerazione quanto segue:

- le condizioni d'uso normali dell'alimento da parte del consumatore in ciascuna fase della produzione, della trasformazione e della distribuzione;
- le informazioni messe a disposizione del consumatore, comprese le informazioni riportate sull'etichetta o altre informazioni generalmente accessibili al consumatore, al fine di evitare specifici effetti nocivi per la salute provocati da un alimento o categoria di alimenti**;**
- considera i probabili effetti immediati, a breve termine o a lungo termine dell'alimento sulla salute di una persona che lo consuma. Vengono cioè considerati i probabili effetti tossici cumulativi di un alimento;
- la particolare sensibilità, sotto il profilo della salute, di una specifica categoria di consumatori, nel caso in cui l'alimento sia destinato ad essa.
- per evitare che un alimento sia inadatto al consumo umano, si prendono in considerazione le possibili contaminazione dovute a materiale estraneo o fenomeni di deterioramento in seguito ad una non perfetta conservazione.

B) *Regolamentazione dell'etichettatura*
Le etichette della Urbani Tartufi sono state realizzate seguendo il decreto LEGISLATIVO 27 GENNAIO 1992, N. 109 in attuazione delle direttive 89/395/CEE e 89/396 CEE concernenti l'etichettatura, la presentazione e la pubblicità dei prodotti alimentari per il mercato nazionale, integrando tale decreto con le norme dei mercati esteri, dato che la nostra azienda è presente in tutto il mondo con i propri prodotti. Per etichettatura si intende l'insieme delle menzioni, delle indicazioni, dei marchi di fabbrica o di commercio, delle immagini o dei simboli che si riferiscono al prodotto alimentare e che figurano direttamente sull'imballaggio o su un'etichetta appostavi o sul dispositivo di chiusura o su cartelli, anelli o fascette legati al prodotto medesimo. La Urbani Tartufi dispone di un team di persone molto preparate che provvede alla realizzazione delle etichette, riportando tutte le informazioni necessarie, e altre, al fine di essere chiari e trasparenti verso i consumatori. I valori nutrizionali (non obbligatori) sono riportati nella maggior parte delle nostre etichette con il chiaro obiettivo di essere vicini alle esigenze del consumatore.

C) *Problematiche fiscali*
Innanzitutto vorrei fare delle considerazioni generali sul disegno di legge all'esame del senato relativo alle modifiche alla legge 16 dicembre 1985 n.752, in materia di raccolta, coltivazione e commercio dei tartufi freschi o conservati destinati al consumo.
Il previsto ripristino del diritto di proprietà del tartufo al proprietario del terreno, non potrà che portare vantaggio a tutti gli operatori del settore, sia esso l'agricoltore proprietario del fondo, sia al ricercatore e sia alle imprese che commercializzano e trasformano il prodotto. E' noto che l'Italia, da forte esportatore è divenuta un notevole importatore; la legge 752 del 1985 ed il conseguente trattamento fiscale della materia, ha avuto forti responsabilità nel creare tale situazione e ciò per i seguenti motivi:
1) L'aver privato l'agricoltore della proprietà del tartufo, l'aver addirittura disincentivato gli investimenti che poteva effettuare per incrementare la produzione ponendo degli assurdi limiti

all'estensione delle coltivazioni, l'aver di fatto autorizzato chiunque ed ovunque ad effettuarne la raccolta, ha avuto come risultato quello di ridurre la produzione nazionale ad un lento inesorabile declino.
Il trattamento fiscale, che contrariamente alle altre legislazioni dei paesi concorrenti della UE che trattano il tartufo un prodotto agricolo (in particolare Francia e Spagna), non ha fatto che contribuire a dare un forte impulso all'evasione fiscale del settore.
2) Oltre alle difficoltà sopra indicate che incontrano gli operatori, v'è da segnalare la concorrenza che debbono subire dagli altri paesi della UE in relazione al mancato adeguamento della legge 752 di modo che possa consentire la commercializzazione di altre specie di tartufo consentita in altri paesi; una modifica in tal senso non può pertanto che ristabilire il dovuto equilibrio dei fattori che portano ad una sana e leale concorrenza.
3) IVA e imposte indirette. Particolare rilevanza assumono le disposizioni recate dall'art.9 del DDL in discussione, che sono rivolte ad assoggettare i tartufi al regime speciale IVA di cui all'art.34 del DPR 633/72. La normativa attualmente in vigore che non inserisce il tartufo nella tabella A, parte I, allegata al DPR 633/72, costituisce una ulteriore penalizzazione per il settore impedendo lo sviluppo della tartuficoltura.
Ugualmente non risulta applicabile la disciplina contenuta nel successivo art.34 bis del richiamato DPR 633/72, riguardante l'importante settore delle attività connesse all'agricoltura, introdotto dall'art. 2 comma 7 della legge n° 350/03 (finanziaria per il 2004), in quanto anche questo regime appare subordinato all'esercizio di attività connesse che comportino la cessione dei prodotti elencati nella tabella A, parte I, allegata al DPR 633/72.
Difatti, su questo ultimo punto, una diversa interpretazione, tesa ad estendere questa disciplina attualmente in vigore a qualsiasi attività agricola, sebbene risulti compatibile con il dettato letterale del richiamato art.34-bis, appare in netto contrasto con la normativa comunitaria che, all'art.25 della VI direttiva, consente di attribuire un regime speciale ai soli beni specificatemente elencati negli allegati alla direttiva. In tal senso si è espressa anche l'amministrazione finanziaria con la circolare n.6/E del 16 febbraio 2005.
4) Imposte dirette. Pienamente apprezzabili sono le disposizioni che prevede il DDL in discussione per quanto attiene l'imposta diretta secondo il vigente TUIR n.917/1986. A questo riguardo si sarebbe utile apportare alcune modifiche negli articoli 28 e 34:

- All'art. 28 (determinazione del reddito dominicale Comma 4-bis: Il reddito dominicale delle superfici adibite alle colture prodotte in serra o alla funghicoltura, o alla tartuficoltura ivi comprese le superfici in cui avviene la raccolta dei frutti spontanei, in mancanza della corrispondente qualità del quadro di qualificazione catastale, è determinato mediante l'applicazione della tariffa d'estimo più alta in vigore nella provincia.
- All'art. 34 (determinazione del reddito agrario) Comma 4. Per la determinazione del reddito agrario delle superfici adibite alle colture prodotte in serra o alla funghicoltura o alla tartuficoltura ivi comprese le superfici in cui avvene la raccolta dei frutti spontanei si applica la disposizione del comma 4-bis dell'art. 28.

Le accennate modifiche dovrebbero perseguire l'importante obbiettivo di ricondurre a tassazione, secondo l'applicazione degli estimi catastali, l'attività della tartuficoltura, quella della raccolta (da parte del proprietario del fondo o da chi ne ha titolo) dei frutti spontanei (quindi anche i tartufi) e di ogni altra attività connessa così come previsto dall'art. 32 del citato DPR. 917/86.
In conclusione è auspicabile una legislazione europea in materia di raccolta, commercializzazione e trasformazione dei tartufi che superi le differenze nazionali e, nello stesso tempo, incentivi la tartuficoltura, attività agricola estremamente importante in Italia, nazione ricca di colline e montagne dove le colture tradizionali sono difficili e dove la tartuficoltura può consentire di fornire reddito nel rispetto assoluto dell'ambiente e di risolvere i problemi socio-economici delle popolazioni residenti. Si auspica, infine, che emerga in Italia il reddito annuo fornito dai tartufi raccolti nelle tartufaie naturali e coltivate, al fine di far emergere l'importanza economica del settore e di conseguenza richiamare una maggiore attenzione del legislatore verso il tartufo, la tartuficoltura e la ricerca scientifica ad essi collegata.

REGIONE UMBRIA: AZIONI PER LA TUTELA E LA VALORIZZAZIONE DEL PATRIMONIO TARTUFICOLO

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Abstract: Umbria Region: actions for the regional truffle resource conservation and development.
This presentation highlights the economical, social and environmental importance of truffles in the territory of Umbria, where all the truffle species admitted on the Italian market are well represented.
The effort of the Umbria Region to protect and develop the presence of spontaneous truffles, and to promote their cultivation, is reported.
The following aspects are shown, following also an historical analysis:

- available data, relative to this area;
- the Regional Truffle Plan;
- funds for truffle cultivation;
- the compulsory certification for mycorrhized plants produced and marketed in Umbria;
- the cartographic identification of the most suitable areas for truffle cultivation, and the following identification of truffle plantations exclusively within these areas;
- the funded research programs and projects;
- promotional and divulgative activities.

The talk ends with the presentation of a document mapping the most suitable local sites for truffle cultivation, carried out by the “Comunità Montana Alto Chiascio”.
Key words: experimentation, certification of mycorrhized plants, research, promotion and divulgation.

Il tartufo in Umbria

In Umbria sono presenti tutte le specie di tartufi commestibili riconosciute dalla legge italiana. La raccolta delle varie specie viene praticata nel corso di tutto l’anno, tranne nei mesi di maggio e settembre.
Nell’anno 2007 i raccoglitori di tartufi autorizzati ed in regola con il pagamento della tassa regionale sono stati 5.719. Negli ultimi anni la media è stata di circa 5.400 raccoglitori/anno.
La raccolta dei tartufi in Umbria, come in tutta Italia, è libera nei boschi e nei terreni non coltivati, fatta eccezione nelle tartufaie controllate ((tartufaie naturali migliorate) e coltivate (tartufaie artificiali realizzate con piantine micorrizate) dove la raccolta è riservata ai proprietari o conduttori dei terreni.
Secondo dati forniti dalle Comunità montane, in Umbria – dove quasi il 40% del territorio è coperto da boschi – sono presenti:

- n. 704 tartufaie controllate, per una superficie oltre 2.450 ettari
- n. 215 tartufaie coltivate legalmente riconosciute, per una superficie di 283 ettari.

La produzione regionale ufficiale di tartufi rilevata da ISTAT nel decennio 1998-2007 è stata in media di 25.000 KG/anno.
Il dato è sottostimato perché è praticamente impossibile rilevare tutto il prodotto raccolto.
Nel mese di agosto 2004, alle Camere di Commercio della Regione risultavano iscritte n. 65 Ditte dedicate alla commercializzazione del tartufo e n. 17 Ditte dedicate alla sua coltivazione.

Azioni della Regione Umbria per potenziare e tutelare la produzione tartuficola

Ricerca

A partire dal 1984 la Regione Umbria ha finanziato programmi e progetti di ricerca per lo sviluppo della tartuficoltura, con i seguenti obiettivi specifici:

- aumento delle conoscenze relative alla ecologia e biologia dei tartufi
- miglioramento delle tecniche di micorrizazione e controllo della qualità delle piantine micorrizate
- tutela e valorizzazione dei tartufi dell'Umbria.

Si segnalano in particolare:

a) Programma Tartuficolo Regionale
   Nel periodo 1984 -1987, su indicazioni del Dipartimento di Biologia Vegetale dell'Università di Perugia – referente scientifico del programma – le Comunità Montane hanno realizzato su terreni pubblici n. 55 impianti sperimentali dimostrativi per una superficie di 115 ettari.
   Su n. 28 campi sperimentali sono state impiegate piante micorrizate con *Tuber melanosporum* Vittad.; in n. 17 campi sono state impiegate piante micorrizate con *Tuber magnatum* Pico; nei restanti 10 impianti sono state utilizzate, in diverse combinazioni, piante micorrizate con varie specie.
   Il programma, ha fornito e sta ancora fornendo ancora importanti informazioni utili per la realizzazione e la coltivazione di tartufaie artificiali.

b) N. 4 programmi di prove sperimentali condotte dal 1997 al 2008 sulle tartufaie del Programma Tartuficolo Regionale dall'attuale Dipartimento di Biologia Applicata dell'Università di Perugia.

c) Progetto ECO.T (Ecologia del Tartufo)
   Il progetto, attuato (1990 –1994) dal Parco Tecnologico Agro – Alimentare dell'Umbria ha riguardato lo studio dell'ecologia del *Tuber melanosporum* Vittad., del *Tuber magnatum* Pico e del *Tuber aestivum* Vittad. in 9 aree della Regione.
   Lo studio ha fornito informazioni sulla composizione floristica delle tartufaie naturali, sulla composizioni fisico chimica dei terreni tartufigeni ed in generale sulle condizioni ambientali migliori per l'impianto di tartufaie artificiali.
   E' stata redatta una mappa in scala 1:25.000 delle aree che presentano le caratteristiche pedologiche, floristiche ed ambientali favorevoli allo sviluppo dei tartufi.

d) N. 3 studi (1995-2002), finanziati insieme ad altre Regioni, condotti dal Consiglio Nazionale delle Ricerche ed altri Istituti di ricerca, concernenti la biotecnologia della micorrizazione ed il metodo di controllo delle piante micorrizate con funghi del genere *Tuber*.

e) N. 4 progetti (1996-2008) del Consiglio Nazionale delle Ricerche - Istituto di Genetica Vegetale (Perugia), che hanno coinvolto anche Comunità Montane ed Azienda Vivaistica regionale Umbraflor, concernenti
   - lo studio della variabilità genetica e selezione di marcatori molecolari in *Tuber melanosporum* Vittad. ed in *Tuber magnatum* Pico
   - lo studio di un protocollo per la micorrizazione, con specifiche procedure di controllo biomolecolare, di piantine con *Tuber magnatum* Pico
   - indagini ecologiche, genetiche e molecolari.

Aiuti alla tartuficoltura

Gli aiuti all'impianto di tartufaie sono iniziati nel 1984 con finanziamenti regionali e proseguiti nell'ambito di programmi attuativi di Regolamenti comunitari con finanziamento a carico di Regione, Stato e Fondi europei.
Nel periodo 1984 – 1994 con contributi regionali e comunitari sono stati realizzati impianti tartuficoli per 398 ettari.
Dal 1994 al 1998 nell'ambito degli aiuti all'imboschimento di terreni agricoli previsti dal Reg. CE 2080/92 è stata concessa la possibilità di mettere a dimora anche piantine micorrizate. Le

superfici imboschite con piante tartufigene non sono state rilevate. Attualmente gli aiuti alla tartuficoltura sono previsti dal Programma regionale di Sviluppo Rurale 2007-2013.
Nel corso del precedente Programma regionale di Sviluppo Rurale 2000-2006 è stato finanziato il progetto "Filiera del tartufo" con aiuti alla coltivazione, conservazione, trasformazione, promozione e commercializzazione dei tartufi e contributi per studi volti alla certificazione ed il controllo di qualità del prodotto.

Regolamentazione
Per il miglioramento e la salvaguardia della produzione tartuficola la Regione ha introdotto norme particolari, non previste dalla legge nazionale, che riguardano la certificazione delle piante micorrizate e la realizzazione della carta delle zone vocate alla tartuficoltura.
L'obbligo della certificazione è stabilito per tutte le piante micorrizate prodotte, commercializzate o comunque distribuite in Umbria.
La certificazione deve basarsi su metodi di riconoscimento morfologici e biomolecolari ed è valida solo se rilasciata da Istituti scientifici autorizzati dalla Regione.
L'attestazione di tartufaia controllata e di tartufaia coltivata è rilasciata dalle Comunità montane a condizione che per il miglioramento o la realizzazione di tali tartufaie siano state impiegate piante micorrizate certificate.
Le aree comprese nella carta delle zone particolarmente vocate alla diffusione della tartuficoltura possono essere classificate dai Comuni quali zone di particolare rispetto naturalistico.
Nelle zone vocate è prevista una disciplina speciale per il taglio di specie arboree ed erbacee e per interventi che interessano i corsi d'acqua.
Inoltre solo in tali zone possono essere realizzate nuove tartufaie coltivate legalmente riconosciute.
La realizzazione della carta delle zone vocate alla tartuficoltura è in corso di realizzazione da parte delle comunità montane.

La Comunità montana Alto Chiascio ha realizzato un progetto di carta delle zone vocate che parte dall'organizzazione di una banca dati georeferenziata derivante dalle cartografie del progetto ECO.T. (vedi punto II.1.c) integrate dalla carta forestale, dalla carta dei pedopaesaggi e da informazioni su elevazione, esposizione e pendenza dei terreni.

Ciò ha portato alla definizione cartografica su base informatica delle aree vocate alla coltivazione e raccolta del tartufo.

Per ciascuna delle tre specie principali (*Tuber magnatum, Tuber melanosporum, Tuber aestivum*) la carta evidenzia in particolare le aree vocate, quelle vocate ma non utilizzabili e quelle non vocate.

Il progetto consente l'aggiornamento continuo delle informazioni contenute nella banca dati georeferenziata e rappresenta uno strumento semplice e completo in grado di fornire indicazioni utili sia per introdurre la coltivazione del tartufo nelle aree più idonee che per consentire azioni volte alla conservazione delle aree tartufigene naturali.

Promozione e divulgazione
La Regione finanzia annualmente esposizioni, fiere e manifestazioni varie che hanno per oggetto il tartufo, anche insieme ad altri prodotti tipici dell'Umbria.
Per divulgare le conoscenze sui tartufi e sulla tartuficoltura nel 2005 la Regione ha pubblicato il libro, distribuito gratuitamente, "Umbria terra di tartufi" di B. Granetti, A. De Angelis, G. Materozzi.

DALLA RACCOLTA ALLA CONSERVAZIONE: L'IMPEGNO DELLA REGIONE PER LA FILIERA TARTUFICOLA - PATRIMONIO NATURALE ED ECONOMICO SU CUI IL PIEMONTE PUNTA PER CREARE OPPORTUNITÀ DI SVILUPPO SECONDO CRITERI DI EQUITÀ E SOSTENIBILITÀ

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Abstract: From collection to preservation: the commitment of piedmont region in the truffle chain – a natural and economical heritage Piedmont stacks to create an opportunity of development under equity and sustainability criteria.
In the last 10 years Regione Piemonte have been promoting and financing many scientific research projects with the aim of creating an opportunity of development under equity and sustainability criteria for the truffle chain. These activities were realized in collaboration with different regional companies both public and private.
In particular the projects had been leading at the creation of truffle suitable areas maps in Piemonte, the creation of experimental truffle plantations by bedding certified mycorrhized bedders, the recovery of many *Tuber magnatum* Pico natural plantations, and the analysis of the rhizosphere components.
In addition economical studies to assess investment profitability of truffle growing were realized.
All these activities had leaded to write a regional law (L.R. 16/08), passed in the June of 2008, with the aim of increasing the truffle production and protecting suitable areas.
Moreover Regione Piemonte had promoted an international contest for the design and creation of a package suitable for the preservation and the enhancement of the *T. magnatum* Pico.
Key words: scientific research, improvement and preservation of Piemonte truffle heritage, Piemonte truffle chain.

Nell'ultimo decennio l'Amministrazione Regionale, per poter dare una risposta alla crescente domanda di prodotti di qualità per il turismo enogastronomico, tra cui il tartufo, prodotto di nicchia, ma strategico nell'economia piemontese, che, fungendo da volano, fa conoscere i prodotti tipici, le bellezze paesaggistiche e le attrattive turistiche, ha realizzato molte attività nel settore della tartuficoltura.
Tra le principali, in ordine cronologico, possiamo ricordare le seguenti.
Il progetto Verchamp, concluso nel 2006, frutto di un'importante cooperazione tra partners transfrontalieri (Hautes Alpes Développement e Chambre d'Agriculture des Hautes Alpes), attraverso il quale è stato possibile realizzare, con la collaborazione tecnico scientifica dell'Istituto per le Piante da Legno e l'Ambiente, la cartografia delle attitudini tartufigene del territorio collinare e pedemontano piemontese; inoltre, sono state messe a dimora piantine micorrizate e certificate, nel rispetto delle provenienze autoctone dei tartufi e delle piante ospiti.

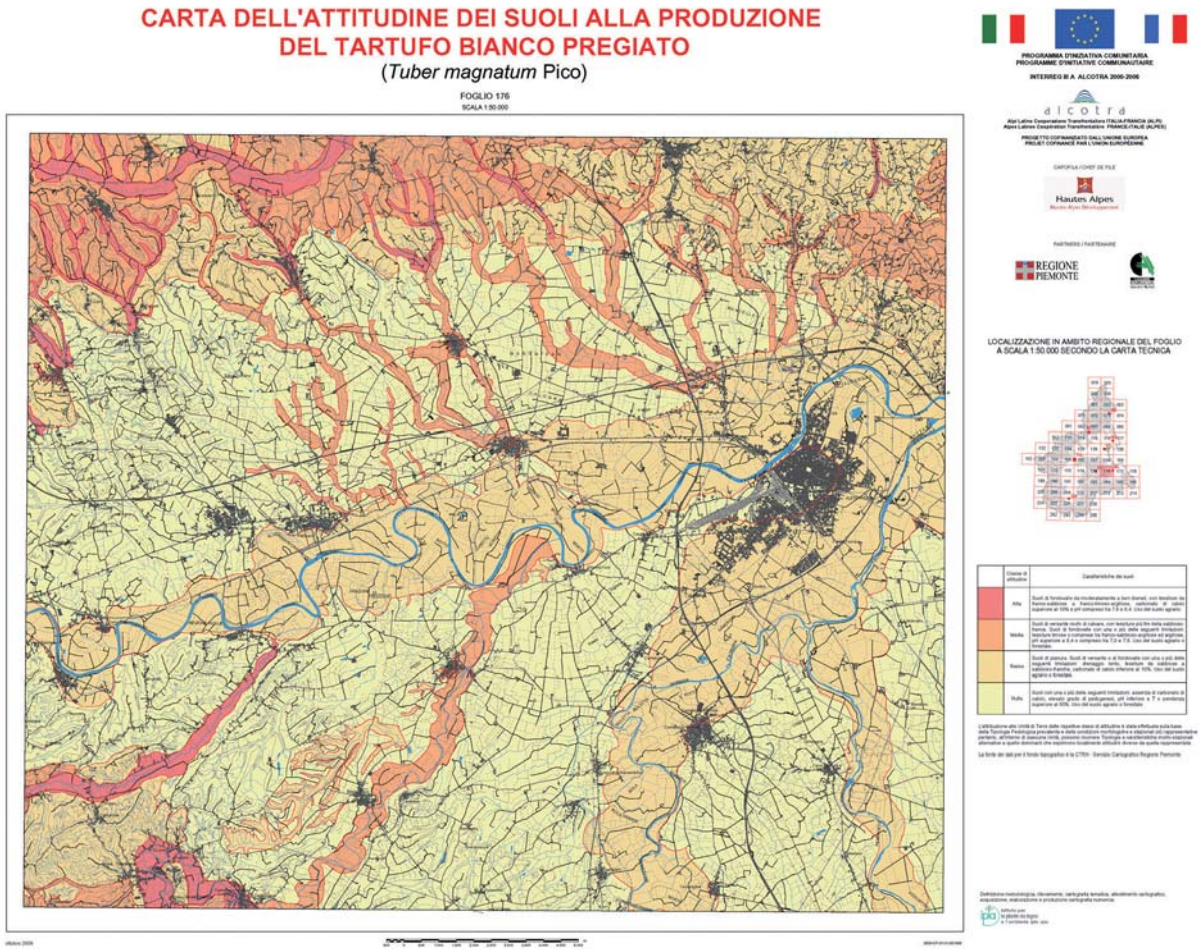

Fig. 1 Esempio di carta di attitudine alla produzione del *Tuber magnatum* Pico

L'incarico al Dipartimento di Economia ed Ingegneria Agraria, Forestale ed Ambientale dell'Università di Torino per valutare la redditività degli investimenti nell'ambito della tartuficoltura. Questi studi hanno confermato che il tartufo può essere, in molte zone considerate svantaggiate del territorio piemontese, la piattaforma sulla quale costruire un sistema economico-produttivo compatibile con l'ambiente.

Il recupero di numerose tartufaie nelle quattro province vocate (Alessandria, Asti, Cuneo e Torino), in collaborazione con il Centro Nazionale Studi Tartufo e con l'Unione delle Associazioni Trifulau Piemontesi; sotto la guida esperta dell'IPLA sono inoltre stati realizzati numerosi interventi tecnici sul territorio, al fine di incrementare la produzione di tartufo bianco pregiato, che negli ultimi anni ha accusato una flessione.

L'incarico al Consiglio Nazionale delle Ricerche – Istituto per la Protezione delle Piante di Torino – di effettuare una ricerca, finalizzata all'approfondimento delle conoscenze relative ai delicati equilibri che presiedono alla produzione del prezioso tartufo bianco pregiato, incentrata sull'analisi delle componenti presenti nella rizosfera, con tecniche biomolecolari.

L'incarico al Dipartimento di Biologia Vegetale dell'Università di Torino, in collaborazione con il Consiglio Nazionale delle Ricerche – Istituto per la Protezione delle Piante, per la realizzazione di una ricerca specifica per preservare le tartufaie piemontesi del pregiato *Tuber melanosporum* dalla presenza di *Tuber indicum*, con una attenta azione di monitoraggio.

Fig. 2 Esempio di tartufaia naturale in recupero

L'approvazione, nel giugno del 2008, della legge regionale 16 "Norme in materia di raccolta e coltivazione dei tartufi e di valorizzazione del patrimonio tartufigeno regionale". Tale legge punta alla valorizzazione del tartufo, allo snellimento delle procedure previste per la raccolta e la coltivazione ed alla salvaguardia ambientale dei territori. La norma riconosce agli ecosistemi tartufigeni il ruolo svolto nello sviluppo socio-economico delle popolazioni delle aree collinari e pedemontane del Piemonte e intende promuovere la conservazione e la diffusione delle provenienze autoctone dei tartufi e delle piante ospiti, il miglioramento e lo sviluppo della tartuficoltura ispirandosi a criteri di qualità, eccellenza e di tutela dei consumatori.

La Regione Piemonte, in occasione dell'evento importantissimo che ha visto Torino centro del design mondiale e, unendo le sinergie con Torino 2008 World Design Capital e con il Centro Nazionale Studi Tartufo di Alba, ha bandito il concorso "Packaging per il tartufo bianco pregiato" allo scopo di favorire l'ideazione e la creazione di una confezione capace di conservare e di valorizzare in modo adeguato il "*Tuber magnatum* Pico".
Tutti i prototipi dei progetti sono stati esposti nel mese di ottobre 2008 ad Alba, durante la "Fiera internazionale del Tartufo Bianco d'Alba"; nel mese di novembre a Roma, in occasione della "Borsa del Turismo Congressuale"e di alcune fiere piemontesi del Tartufo a carattere nazionale o regionale. Il vincitore del concorso è stato premiato in Alba durante la Fiera del Tartufo.

Gli interventi futuri che la Regione Piemonte intende realizzare e finanziare saranno improntati alla programmazione concertata e strategica, prevedendo il coinvolgimento di diversi attori (Enti, Associazioni di cercatori, Consorzi, Istituti scientifici e di ricerca) nella realizzazione di specifiche azioni di recupero e tutela della produzione del tartufo bianco pregiato e di sviluppo della produzione delle specie di tartufo nero coltivabili, nel rispetto dell'ecosistema, a conferma del ruolo della tartuficoltura nel sistema economico piemontese.

TARTUFI E TARTUFICOLTURA NELLA COMUNITA' MONTANA DEI MONTI MARTANI, SERANO E SUBASIO (GIÀ COMUNITÀ MONTANA DEI MONTI MARTANI E DEL SERANO)

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Abstract: Truffles and truffle cultivation in Comunita' Montana dei Monti Martani, Serano e Subasio (ex Comunità Montana dei Monti Martani e del Serano)
The Comunità Montana dei Monti Martani e del Serano is Local Agency founded in 1972 following up disposed law with final aim to promote the social and economical development of mountain territories. Since the beginning of its activity, it is interested at truffle problems, being a product economically very important inside the District.
Since its institution the Agency was also delegated to manage the administrative functions related to the truffle.
During 1976, it actuated, inside own green house, the production of mycorrized plants with *Tuber melanosporum* Vittad., with *Tuber aestivum* Vittad., with *Tuber brumale* Vittad. and with *Tuber uncinatum* Chatin. The green house production is still in operation towards a productive line which favours the local provenience of both symbiosis species, by recent technical and scientific knowledge.
During 1983, in co-operation with the actual Department of Applied Biology of University of Perugia, the Agency began the study and the research on the ecology of main black truffle species and the experimentation of agronomical techniques to use in cultivated truffle fields, realizing in a few years about 30 hectares of new truffle cultivations. This collaboration is still in progress.
In the town of Spoleto were organized two big International Conventions of Truffle: the first one, absolutely the first as international level, was organized in 1968 by Municipality of Spoleto in co-operation with Università degli Studi of Perugia and during 1988, after 20 years, was structured the second one promoted by Comunità Montana as well the one is going to be prepared during these days.
The Comunità Montana offers technical assistance to truffle seekers and truffle farmers and to ones are interested to truffles and to truffle cultivation.
Key words: experimentation, plant nursery, communication activity, administrative management.

La Comunità Montana dei Monti Martani e del Serano (oggi Comunità Montana dei Monti Martani, Serano e Subasio), istituita nel 1972 per favorire lo sviluppo socio-economico dei territori montani e svantaggiati, fin dall'inizio della sua attività si è interessata alle problematiche legate ai tartufi e alla tartuficoltura, essendo questi prodotti della terra molto importanti per l'economia del proprio Comprensorio di competenza.
L'Ente Locale è stato anche delegato dalla Regione Umbria a gestire le funzioni amministrative legate all'applicazione della specifica Legge Regionale che regolamenta la ricerca e la raccolta dei tartufi e la tartuficoltura.
Nel 1976, ha avviato nel proprio vivaio la produzione di piantine micorrizate con *Tuber melanosporum* Vittad., con *Tuber aestivum* Vittad., con *Tuber brumale* Vittad. e con *Tuber uncinatum* Chatin. La produzione vivaistica di piante micorrizate è ancora in atto e privilegia oggi l'utilizzazione di specie simbionti di provenienza locale, applicando le più recenti acquisizioni tecnico-scientifiche in materia.
Nel 1983, in collaborazione con l'attuale Dipartimento di Biologia Applicata dell'Università degli Studi di Perugia, la Comunità Montana ha dato inizio ad un interessante lavoro di studio e di ricerca sull'ecologia delle principali specie di tartufo nero e alla sperimentazione delle tecniche

agronomiche più adeguate da utilizzare in tartufaie coltivate. Con finalità sperimentali e di ricerca, nel corso degli anni sono stati impiantati circa 30 ettari di tartufaie nelle più disparate situazioni pedo-climatiche e utilizzando differenti specie arboree. Alcuni di questi impianti si sono rivelati molto importanti per i dati che dagli stessi è stato possibile rilevare nel tempo, altri, soprattutto impianti con *T. aestivum,* hanno dato risultati produttivi interessanti e sono oggi in produzione. La efficace collaborazione con l'Istituto di Ricerca Universitario è tuttora in corso.
Nella città di Spoleto si sono organizzati due grandi Congressi Internazionali sul Tartufo: il primo, in senso assoluto a livello internazionale, fu organizzato nel 1968 dal Comune di Spoleto in collaborazione con l'Università degli Studi di Perugia, il secondo, dopo venti anni, nel 1988, è stato promosso e organizzato dalla Comunità Montana così come questo terzo Congresso Internazionale.
La Comunità Montana, con il proprio personale tecnico-specialistico, fornisce anche un apprezzato supporto ai tartuficoltori del proprio comprensorio e a tutti coloro che dimostrino interesse nei confronti della tartuficoltura.

LA DISPONIBILITA' A PAGARE DEL CONSUMATORE PER PERCORSI DI QUALITA' CERTIFICATA NEL TARTUFO CON MODELLI A SCELTA DISCRETA

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Abstract: The willingness to pay of the consumer for food quality paths as the truffle with an application of a choice model
The willingness to pay is a very important information for the farmers because it is able to point out the ability of the consumer to compensate the economic costs stood by the farms in different actions to place in high-level markets the quality food; in this case there are a lot of costs to valorize the production and to certificate the rural productions as the truffle. The analysis on the willingness to pay have been carried out only for specific quality food put on consolidated agroindustrial systems, as extra virgin olive oil, wine, meats, producing some interesting results to define, in a short time, a lot of marketing strategies very helpful for the farms of rural areas.
The aim of this analysis is to describe the results obtained during a survey using questionnaire about the willingness to pay for a food quality certification of the truffle in an inner rural area, as the Rieti province very famous for significant production of truffle, and the economic outlook of quality certification for this area. The results of the questionnaire have treated with the application of statistical choice models, like Probit and Logit, to define the willingness of consumer to pay a premium price for the truffle, linked to a lot of different variable as sex of person interviewed, advertising campaigns for the promotion of the product and personal income. Moreover, during the consumer survey it has been estimated the role of the new information communication technologies could have both for the purchase behaviour of the consumers and for the farms, in particular for the truffle pickers and truffle firms, that operate on the rural territory of Rieti; even if, the statistical data has shown a significant results with regard to the role of Internet to advertise the truffle and the rural areas for the firms but not to increase the direct sale of the truffle. In fact the questionnaire has shown as the consumers prefer to buy the truffle directly in the farms using the traditional sales channels. The variable personal income and sex of person interviewed have had a fundamental role on the willingness to pay a premium price for the quality certification of the truffle; in fact the value of premium price and the percentage of price in addition of a base price are not be able to cover completely the costs for the quality certification.
Key words: willingness to pay, choice models, Rieti province, rural areas.

Introduzione

La disponibilità a pagare costituisce un'informazione molto importante per l'imprenditore in quanto indica la capacità del consumatore di compensare gli impegni economici messi in atto per consentire ad un prodotto di collocarsi in un mercato di alto livello, e che sappia valorizzare contemporaneamente sia i processi che gli impegni necessari alla certificazione del prodotto (Carbone e Sorrentino, 2004). Analisi sulla disponibilità a pagare sono state effettuate solo per prodotti che si collocano all'interno di circuiti Dop o Igp consolidati, quali olio extravergine, vino, salumi (Cicia e Perla, 2000). I risultati che sono emersi da tali indagini hanno evidenziato alcune indicazioni molto importanti per definire, nel breve periodo, delle strategie di marketing specifiche da applicare nei consorzi e utili indicazioni per gli imprenditori agricoli (Loseby e Mariano, 2004). In Italia le produzioni certificate rappresentano una quota di mercato molto interessante per le esportazioni agroalimentari; tuttavia, la maggior parte dei prodotti di qualità certificata trova delle difficoltà a collocarsi sul mercato poichè la maggior parte dello spazio

commerciale ed economico viene accaparrato da poche produzioni di eccellenza (Gay, 2005). Le aziende agricole, sopratutto quelle che si collocano nelle aree a ridosso della cimosa appenninica, sono quelle a maggior rischio di marginalizzazione e le produzioni certificate possono rappresentare una risposta alla multifunzionalità (Eboli, 2004) che il cittadino europeo richiede alle aziende agricole cui è ben disposto a fare erogare contributi specifici d parte del II pilastro della Politica agricola comunitaria. Le nuove tecnologie informatiche possono rappresentare una concreta possibilità per creare attorno al tartufo le condizioni necessarie per accorciare la filiera e far rimanere la ricchezza sul territorio riducendo il *buyer power* (Sodano, 2004) anche se permangono, come evidenziato per altri prodotti agroalimentari di qualità certificata, una serie di criticità legate al management aziendale e che potrebbero incrementare le opportunità di vendita (Galluzzo, 2006a; Galluzzo 2006b).

Obiettivi

La presente analisi ha voluto testare, mediante le risultanze emerse dalla somministrazione di apposito questionario, su un campione dell'area di studi a rischio marginalizzazione rappresentato dalla provincia di Rieti, le prospettive economiche che un percorso di certificazione della qualità potrà apportare al tartufo, il quale non ha ancora iniziato un proprio specifico percorso di tracciabilità e rintracciabilità commerciale e produttiva. L'obiettivo finale di tale tipologia di ricerca (Galluzzo, 2008) è stato quello di contribuire ad individuare e schematizzare un consumatore *target* evidenziando le proprie potenzialità e criticità al fine di indirizzare le linee strategiche future per quelle iniziative consortili che si vogliano costituire per valorizzare un prodotto, che si colloca, ancora, in ambiti di nicchia e che ha la necessità di valorizzarsi anche al di fuori del territorio sfruttando le nuove opportunità offerte dalle tecnologie informatiche.

Metodologia

Per poter procedere all'analisi della disponibilità a pagare è stata definita l'area di studio, rappresentata dal territorio della provincia di Rieti, la quale può identificarsi con una buona attendibilità alle altre aree interne dell'Appennino centrale a rischio marginalizzazione, in cui la produzione del tartufo rappresenta un elemento che caratterizza lo spazio rurale e un buon indicatore della multifunzionalità in agricoltura in senso ampio. Nell'area di studio si è proceduto, nel periodo gennaio-marzo 2008, a distribuire un questionario a risposta chiusa su un campione di 130 persone, anche se solo 116 hanno provveduto a compilare il questionario in maniera corretta e con dati utilizzabili per l'indagine sulla disponibilità a pagare.

La fase di *pre testing*, necessaria ad eliminare eventuali dubbi inerenti la chiarezza espositiva delle domande, non è stata effettuata perchè il questionario era stato già sottoposto ad un triplo processo di *screening* su altri campioni inerenti la disponibilità a pagare eseguiti su altri prodotti tipici dell'area di studio quali olio e castagne.

I dati del questionario, essendo di natura prevalentemente qualitativa, hanno consentito l'applicazione di modelli binari tra i quali possiamo annoverare i modelli a scelta discreta, Probit e Logit. In questi casi è stato possibile definire la disponibilità da parte del consumatore a pagare per prodotti di qualità certificata e per il processo di certificazione che potrà esser attuato nell'area di studio. Le variabili che sono state messe in relazione nel modello sono di due tipologie:

1. variabili dipendenti connesse alla risposta positiva o negativa fornita appartenenti alle variabili categoriali;
2. variabili indipendenti di tipo categoriale o non categoriale.

Un modello binario, come il modello Probit o Logit, ha come obiettivo la stima della probabilità che la variabile dipendente assuma valore 0 oppure valore 1 in funzione delle risposte contenute nelle variabili indipendenti e può essere formulato nel seguente modo (Verbeek, 2006):

Prob (Y_i=1) = F(βX_i)
i = 1, 2, 3, ……n

Y_i con valore binario 0 o 1
X_i vettore di variabili esplicative
β vettore dei parametri da stimare
F è una funzione di distribuzione della probabilità cumulata e che è rappresentata da una normale nel modello Probit o una logistica nel Logit
I valori stimati della probabilità al di sotto della soglia di 0,5 fanno si che la Y calcolata assuma valore 0 mentre per valori superiori a 0,5 la Y calcolata assume il valore di 1 (Sodano e Verneau, 2006).
Nel questionario è stato chiesto all'intervistato la propria disponibilità a pagare un premio ulteriore ad un prezzo soglia del tartufo e collegato ad una molteplicità di variabili indipendenti rappresentate dal sesso dell'intervistato, dal titolo di studio, dal numero di componenti la famiglia e, infine, dalla variabile reddito disponibile. I modelli empirici ottenuti con il metodo della massima verosimiglianza sono stati due Logit e Probit in maniera tale da fare un confronto della bontà dei due modelli, o meglio, delle funzioni di distribuzione della probabilità cumulata. Per avere un maggiore e migliore quadro interpretativo dei modelli da utilizzare nella presente ricerca, si è utilizzato il modello dei minimi quadrati ordinari (OLS) al fine di avere un quadro organico dell'analisi, dando comunque maggiore importanza ai modelli a scelta discreta rispetto al modello OLS.
Inoltre, nel corso dell'indagine quantitativa, mediante l'applicazione di tabelle di contingenza sono state verificate le ipotesi di dipendenza statistica tra le variabili indipendenti testate e inserite nel modello a scelta discreta. Alla parte quantitativa della ricerca si è affianca la parte qualitativa necessaria per valutare il ruolo che le nuove tecnologie informatiche potranno avere. sia per il comportamento di acquisto del consumatore sia per le imprese che operano sul territorio e per l'indotto, il quale in analogia con quanto esposto in precedenza, dovrà ancora avvenire direttamente nei canali di vendita tradizionali.

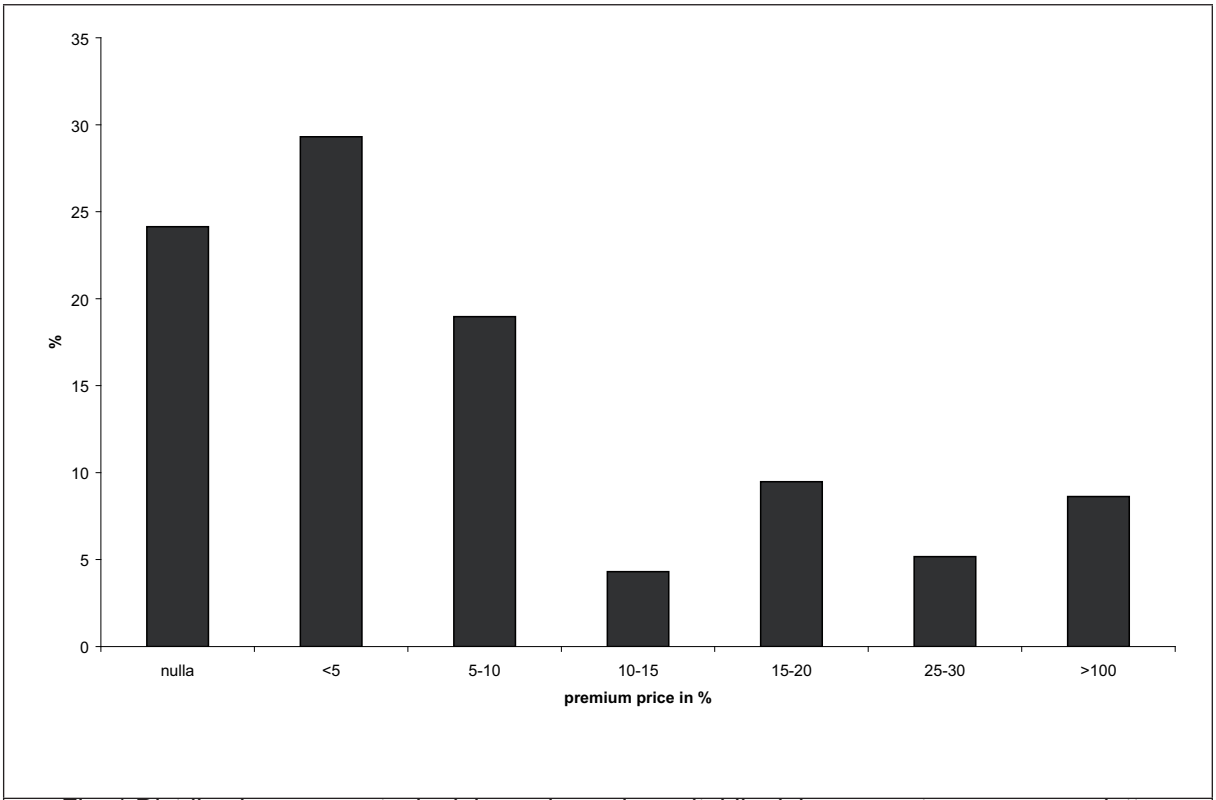

Fig. 1 Distribuzione percentuale del premium price esitabile dal consumatore per un prodotto certificato

Tab. 1 Premium price esitabile da parte del campione intervistato

Prezzo	Frequenza	Percentuale	Percentuale valida	Percentuale cumulata
nulla	28	24,1	24,1	24,1
<5	34	29,3	29,3	53,4
5-10	22	19,0	19,0	72,4
10-15	5	4,3	4,3	76,7
15-20	11	9,5	9,5	86,2
25-30	6	5,2	5,2	91,4
>100	10	8,6	8,6	100,0
Totale	116	100,0	100,0	

Risultati e discussione

L'analisi è stata condotta su un campione di 116 soggetti intervistati ai quali è stato somministrato il questionario. Il campione intervistato è stato costituito prevalentemente dal uomini, pari al 67,2% degli intervistati, appartenenti a gruppi familiari costituiti nel 44% dei casi osservati da 4 persone. Il campione intervistato, per oltre il 60% dei soggetti intervistati, si collocava nel *range* di età compreso tra 30-40 anni e a seguire nell'intervallo 40-50. La formazione culturale è risultata essere di buon livello con oltre l'85% del campione in possesso di un titolo di studio medio-alto e nel 4,3% dei casi di un titolo post laurea. La situazione reddituale degli intervistati ha evidenziato una certa eterogeneità, anche se un quarto del campione si è collocato nell'intervallo compreso tra 15.000 e i 20.000 Euro annui; tuttavia, circa un decimo del campione intervistato si è collocato su livelli di reddito alti compresi nell'intervallo 40-60.000 Euro/anno. Tuttavia, dall'analisi è emerso come permangano delle quote significative di persone intervistate, pari ad un quinto del campione, che si è collocato al di sotto dei 5.000 euro di reddito annuale. Il campione intervistato ha evidenziato un consumo abbastanza limitato del tartufo tal quale e nelle sue trasformazioni dirette e/o indirette; infatti, solo 73 soggetti su 116 hanno confermato di avere consumato nell'ultimo anno solo tartufo bianco o nero; in particolare, dall'indagine è emerso come il tartufo nero è risultato essere prevalente nei consumi da parte degli intervistati. Dall'analisi è prevalso un consumo prevalente di prodotti trasformati quali salsa tartufata (42% del campione intervistato) e paste e/o risotti con una base di tartufo. La maggior parte degli acquisti vengono effettuati nei supermercati e nei negozi specializzati; l'acquisto nelle aziende agricole o presso amici e/o conoscenti rimane essere una opzione di acquisto preferita dal campione intervistato. L'acquisto di tale prodotto attraverso l'hard discount è risultata essere un'opzione non scelta dal campione intervistato; benché in alcuni casi una parte consistente degli intervistati si sia collocata in una fascia di reddito pro capite bassa ha preferito o non effettuare il processo di acquisto oppure acquistarne in minore quantità ma in circuiti di vendita ben definiti, rifiutando i canali della Grande distribuzione organizzata. Tra i prodotti del territorio da valorizzare insieme al tartufo, per una promozione completa dello spazio rurale dell'area di studio, il campione intervistato ha indicato l'olio, il vino, i funghi porcini e la lenticchia tra i prodotti agroalimentari ritenuti essere dei buoni elementi in grado di valorizzare il territorio nella sua completezza.

Dal questionario somministrato è emerso come le nuove tecnologie informatiche non possano svolgere un ruolo positivo per la commercializzazione del tartufo verso le imprese agricole dell'area di studio, anche se il 76% del campione ha evidenziato come le *Information Comunication Tecnologies* (ICT) potranno in futuro essere uno strumento molto utile per rendere competitive le aziende agricole delle aree marginali.

L'analisi delle tabelle di frequenza della disponibilità a pagare per la certificazione di qualità del tartufo è emerso come il 34% del campione sia disponibile a pagare una percentuale aggiuntiva al prezzo di soglia inferiore al 5% e per il 28% nullo (Fig. 1); invece, solo un 10% del campione è disponibile a pagare solo un *premium price* superiore al 100% rispetto ad un prodotto non certificato. Complessivamente solo un 25% del campione è stato disponibile a sborsare un

premio di 5 euro a parziale copertura dei costi di certificazione di qualità del prodotto e un 17% degli intervistati un prezzo pari a 10 euro per certificare la qualità del tartufo (Tab. 1). A margine di ciò, è utile sottolineare come più di un quinto del campione intervistato non abbia intenzione di pagare alcun *premium price* per la certificazione della qualità del tartufo.

Tab. 2 Analisi delle crosstables e chi quadrato test tra la variabile prezzo e disponibilità a pagare (Wtp)

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1,449	1	,229		
Continuity Correction	,931	1	,335		
Likelihood Ratio	1,436	1	,231		
Fisher's Exact Test				,243	,167
Linear-by-Linear Association	1,437	1	,231		
N of Valid Cases	116				

Tab. 3 Analisi delle crosstables e chi quadrato test per acquisti prodotti di qualità certificata e disponibilità a pagare (Wtp)

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	20,567	1	,000		
Continuity Correction	18,456	1	,000		
Likelihood Ratio	28,668	1	,000		
Fisher's Exact Test				,000	,000
Linear-by-Linear Association	20,390	1	,000		
N of Valid Cases	116				

Tab. 4 Analisi delle crosstables e chi quadrato test per percentuale di *premium price* e disponibilità a pagare (Wtp)

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	54,228	6	,000
Likelihood Ratio	74,059	6	,000
Linear-by-Linear Association	12,184	1	,000
N of Valid Cases	116		

Tab. 5 Analisi delle crosstables e chi quadrato test per prezzo e disponibilità a pagare (Wtp)

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	40,327	7	,000
Likelihood Ratio	49,430	7	,000
Linear-by-Linear Association	8,274	1	,004
N of Valid Cases	116		

Tab. 6 Analisi delle crosstables e chi quadrato test per sesso dell'intervistato e disponibilità a pagare (Wtp)

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,061	1	,804		
Continuity Correction	,002	1	,962		
Likelihood Ratio	,061	1	,804		
Fisher's Exact Test				,843	,480
Linear-by-Linear Association	,061	1	,805		
N of Valid Cases	116				

Tab. 7 Analisi delle crosstables e chi quadrato test per numero componenti nucleo familiare dell'intervistato e disponibilità a pagare (Wtp)

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4,757	4	,313
Likelihood Ratio	4,788	4	,310
Linear-by-Linear Association	,063	1	,801
N of Valid Cases	116		

Tab. 8 Analisi delle crosstables e chi quadrato test per classe di età dell'intervistato e disponibilità a pagare (Wtp)

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	25,559	4	,000
Likelihood Ratio	32,452	4	,000
Linear-by-Linear Association	,093	1	,761
N of Valid Cases	116		

Tab. 9 Analisi delle crosstables e chi quadrato test per formazione culturale dell'intervistato e disponibilità a pagare (Wtp)

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	16,908	3	,001
Likelihood Ratio	23,162	3	,000
Linear-by-Linear Association	6,083	1	,014
N of Valid Cases	116		

Tab. 10 Analisi delle crosstables e chi quadrato test per classe di reddito dell'intervistato e disponibilità a pagare (Wtp)

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	58,713(a)	6	,000
Likelihood Ratio	76,495	6	,000
Linear-by-Linear Association	5,801	1	,016
N of Valid Cases	116		

La valutazione dell'esistenza di possibili interazioni tra la variabile dicotomica disponibilità a pagare (Wtp) e le altre variabili socio-economiche ha fatto ricorso, nelle tabelle di contingenza, all'analisi di alcuni indici di indipendenza derivanti da alcuni test statistici quali il chi-quadrato, l'indice di contingenza quadratica media del Phi-quadro e l'indice V di Cramer. La disamina dei dati ha consentito di poter trarre le seguenti indicazioni:

1) tra la variabile Wtp e la variabile prezzo, quale elemento condizionante le scelte di acquisto, non esiste alcun tipo di legame (Tab. 2); ciò dimostra come il campione intervistato abbia una volontà di pagare un *premium price* che è scollegata e sganciata dal ruolo svolto dal prezzo nel condizionare le scelte. Pertanto il consumatore cerca di massimizzare il proprio beneficio minimizzando i costi senza che ciò vada ad incidere sulla propria disponibilità a pagare;
2) una significativa dipendenza statistica si è venuta a creare tra la variabile disponibilità a pagare e il ruolo che una certificazione Dop e/o Igp potrebbe avere per la valorizzazione della produzione di tartufo dell'area di studio (Tab. 3). Dall'analisi è emersa una correlazione positiva tra queste due variabili, pertanto, sembra confermata la necessità di certificare le produzioni tartuficola dell'area di studio cui il consumatore sembra ben disposto a pagare un premio in più necessario per valorizzare il binomio prodotto-territorio;
3) le relazione tra la variabile disponibilità a pagare del parte del consumatore e le classi di percentuale di prezzo aggiuntivo al prezzo soglia ha confermato l'esistenza di una forte dipendenza tra le variabili (Tab. 4);
4) una conferma delle relazioni che esistono tra la variabile Wtp e le classi di prezzo proposte al campione ha confermato come esista un legame molto forte tra le variabili con dei livelli di significatività molto elevati (Tab. 5);
5) la variabile sesso del campione intervistato e la disponibilità pagare ha confermato nelle tabelle di contingenza una maggiore predisposizione dei soggetti maschili a pagare un *premium price* rispetto agli individui femminili, in analogia con altri studi effettuati su altri prodotti agroalimentari (Galluzzo, 2008), imputabile ad una maggiore frequenza del fenomeno di acquisto quotidiano svolto dalle donne rispetto agli uomini. Tuttavia, la ricerca dell'esistenza di possibili legami tra le variabili sesso del campione intervistato e Wtp ha evidenziato l'indipendenza delle due variabili e l'assenza di legami statistici significativi (Tab. 6). Ciò conferma come la frequenza del processo di acquisto e il consolidamento mentale del processo di acquisto non potranno svolgere alcun ruolo nel condizionare la disponibilità a pagare;
6) la numerosità del nucleo familiare è risultato essere indipendente dalla disponibilità a pagare del consumatore per un *premium price* (Tab. 7) il che conferma come la qualità abbia una posizione *leader* nel processo di acquisto che travalica la variabile prezzo;
7) la disponibilità a pagare del consumatore per acquistare degli alimenti di qualità certificata, pagando un premio aggiuntivo, ha evidenziato l'esistenza di uno stretto legame statisticamente significativo con la variabile età dell'intervistato (Tab. 8). Dall'analisi dei dati dell'indice R di Pearson e della correlazione di Spearman è emersa

una relazione inversa tra queste due variabili in base alle quali ad un incremento della classe di età dei soggetti intervistati abbia corrisposto una diminuzione della disponibilità a pagare;

8) tra la variabile disponibilità a pagare del consumatore e la formazione del campione intervistato è emerso dalle tabelle di contingenza una situazione abbastanza particolare in base alla quale le persone dotate di un alto livello di formazione post laurea sono quelle in grado di pagare un *premium price*; dal lato opposto le persone dotate di un titolo di studio di *default* rappresentato dalla licenza di scuola media inferiore hanno dimostrato una scarsa propensione a pagare un prezzo aggiuntivo per la qualità (Tab. 9);
9) tra la variabile disponibilità a pagare e reddito è emersa l'esistenza di una forte e significativa dipendenza (Tab. 10); inoltre, l'indice R di Pearson e della correlazione di Spearman hanno evidenziato delle correlazioni negative tra la variabile Wtp e il reddito, il che sembra confermare, come riportato nelle tabelle di contingenza, come le persone con alto reddito vogliano un prodotto di qualità certificato e verso il quale abbiano una limitata disponibilità a pagare un *premium price*, confermando un'asimmetria redistributiva nei confronti del settore primario, costituito dalle imprese tartuficole locali.

Tab. 11 Risultati emersi con l'applicazione del modello OLS

Variabili	Coefficienti	Statistica Z	P value	Significatività.
Titolo di studio	0.178480	3.268	0.00145	***
Classe di reddito	-0.0644701	-3.340	0.00114	***

*** <1%

Tab. 12 Risultati ottenuti con l'applicazione del modello Probit

Variabili	Coefficienti	Statistica Z	P value
Costante	-1.92431	0.675451	-2.849
Titolo di studio	0.495164	0.165252	2.966
Classe di reddito	-0.189583	0.0596937	-3.176
Totale osservazioni 116			
LR statistic (5df) 15.71 p value 0.007705			
PCP 67.2			

Tab. 13 Risultati ottenuti con l'applicazione del modello Logit

Variabili	Coefficienti	Statistica Z	P value
Costante	-3.03102	1.05629	-2.869
Titolo di studio	0.791532	0.265023	2.987
Classe di reddito	-0.300594	0.0957634	-3.139
Totale osservazioni 116			
LR statistic (5df) 15.44 p value 0.008624			
PCP 67.2			

Per valutare le relazioni che esistono tra la variabile dipendente disponibilità pagare per la certificazione del tartufo e le variabili indipendenti socio-economiche del campione intervistato sono stati applicati dei modelli di probabilità lineare Logit e Probit confrontandoli con il modello

econometrico OLS. L'analisi nel modello OLS ha confermato come la disponibilità a pagare sia correlata direttamente, con una significatività del 1%, al titolo di studio dei soggetti intervistati, confermando il legame di dipendenza tra variabili durante i test di indipendenza statistica compiuti sui dati. In base a ciò sembra che persone con elevata formazione culturale (titolo di studio medio-alto) abbiano una maggiore propensione a pagare un *premium price* rispetto a persone con un livello culturale minore, il che è un indicatore di come l'accesso alle informazioni possa rappresentare un discrimine sul comportamento di acquisto del consumatore (Tab. 11). La disponibilità a pagare è risultata essere correlata negativamente con la variabile indipendente classe di reddito, confermando quanto emerso nei test di indipendenza statistica (coefficienti R di Pearson e di correlazione di Spearman).

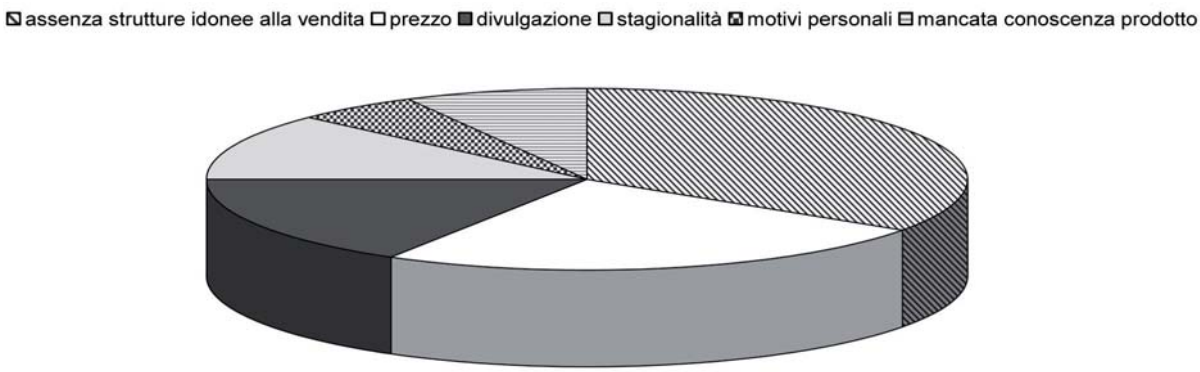

Fig. 2 Criticità evidenziate dal campione intervistato circa la valorizzazione del tartufo

L'applicazione del modello Probit e Logit ha dato luogo ai medesimi risultati con un numero dei casi previsti correttamente abbastanza significativo e pari al 67% dei casi complessivamente osservati (Tabb. 12-13). Dei bassi valori della pendenza rispetto alla media ci hanno consentito di affermare come l'errore nella misura sia stato abbastanza limitato. In base ai risultati statistici emersi è possibile definire il modello che è stato il seguente:

$$P\ (p=1) = \beta_0 + \beta_1 \text{ Titolo di studio} + \beta_2 \text{ reddito} + \varepsilon_i \quad \text{con } i=1, 2, 3\ldots n$$

Dall'analisi dei dati le variabili indipendenti titolo di studio e reddito sono state quelle capaci di agire in misura prevalente sulla disponibilità a pagare nel campione intervistato; in tal caso a classi di reddito basse corrisponde una disponibilità a pagare associata a un titolo di studio inferiore. I risultati dei modelli a scelta discreta sono risultati in linea con quelli ottenuti con l'applicazione del modello dei minimi quadrati ordinari. Il confronto dei modelli a scelta discreta, di quelli OLS e i dati di indipendenza statistica risultano esser tutti in accordo tra loro, confermando come la disponibilità a pagare non sia correlata direttamente al livello di reddito posseduto come invece la comune interpretazione vorrebbe. Dall'analisi è, invece, emerso, anche in accordo con i modelli teorici disponibili in letteratura, che il titolo di studio possa influire significativamente sulla disponibilità a pagare.

Conclusioni

L'analisi effettuata ha confermato come il tartufo si collochi ancora in un mercato di nicchia che potrà beneficiare positivamente degli effetti derivanti dall'istituzione di un consorzio che sappia valorizzare le produzioni locali e il territorio mediante campagne promozionali specifiche in grado di incrementare il livello di conoscenza del prodotto, la promozione del territorio e il sistema di certificazione. Le criticità che il campione ha evidenziato riguardano nel 35% dei casi la assenza di strutture idonee alla vendita, un prezzo ritenuto elevato e una scarsa capacità di pubblicizzare il tartufo al di fuori dei confini dell'are di studio (Fig. 2). Quest'ultimo punto appare esser in linea con quanto emerso in altre analisi condotte nella provincia di Rieti dalle quali

è emersa una enorme difficoltà nel far circolare l'informazione al di fuori del territorio e tutto ciò non consente al prodotto di uscire dalla propria nicchia territoriale. L'indagine mediante questionario ha consentito di definire il consumatore tipo del tartufo della provincia di Rieti: un individuo di sesso maschile di età giovane in possesso di un livello di formazione medio-alto e che vede nella famiglia numerosa e nelle limitate disponibilità di reddito annue (15-20.000 Euro) le criticità maggiori per pagare un *premium price* al tartufo della provincia di Rieti. In analogia con quanto riportato in ricerche simili esiste la disponibilità da parte del consumatore di pagare un *premium price* per un prodotto di qualità certificato anche se i costi di certificazione non sono capaci, sia intermini percentuali che in termini di valore monetario tal quale, di coprire i costi di certificazione e di adesione al consorzio collocandosi nella maggior parte dei casi analizzati a valori inferiori alla soglia del 20% in più su un prezzo di base. Le tecnologie informatiche non potranno rappresentare l'elemento sul quale porre le basi per un *marketplace on line* ma potranno, invece, rappresentare l'elemento trainante per promuovere il prodotto e il territorio in un'ottica di marketing territoriale integrato. In linea generale per i prodotti agroalimentari di alto target il consumatore sembra intenzionato ad andare presso l'azienda, percepito quale luogo in grado di assicurare maggiori garanzie sia per la sicurezza che per il prezzo (filiera cortissima), e perfezionare *in loco* il processo di acquisto. Il ruolo delle istituzioni sembra abbastanza chiaro, ossia quello di promuovere, mediante campagne promozionali specifiche, il tartufo e i prodotti di qualità certificati al di fuori dei confini dell'area di studio.

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TOLERANCE OF FRESH SUMMER TRUFFLES (*TUBER AESTIVUM*) TO DIFFERENT LEVELS OF O_2 AND CO_2

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Abstract

Truffles belonging to the species *Tuber melanosporum* (black truffles) and *Tuber aestivum* (summer truffles) are highly appreciated and in great demand, due to their special organoleptic properties. In order to satisfy consumer demand, it is necessary to increase the production of these ascocarps and to optimize their storage methods. Modified atmosphere packaging (MAP) can be very useful for extending the shelf life of truffles by preserving their organoleptic properties. The selection of O_2 and CO_2 concentrations that prolong the shelf life of a given fresh product is essential to the design of a successful MAP system. Thus the objectives of this work are 1) to determine the effect of different levels of O_2 and CO_2 on the microbiological and quality characteristics of truffles and 2) to establish the optimum ranges of O_2 and CO_2 for storing this product.

Fresh summer truffles were obtained from the Spanish region of Aragon, transported to the laboratory in cooled, insulated boxes and processed within a few hours. The truffles were placed in glass jars of 1-liter capacity connected to the specific atmosphere (CO_2-O_2-N_2) and stored at 4ºC for 26 days. The gas flow rate to each jar was 100 mL min^{-1}. Five atmospheres were tested: 0-5, 10-10, 5-20, 20-20 and 50-20 % v/v CO_2 and O_2, balanced with N_2 and compared to air (0-21). The beneficial effect of the atmosphere was established based on two main criteria: 1) the microbial analysis (total microbial recovery of mesophilic aerobic bacteria, Gº *Pseudomonas*, the *Enterobacteriaceae* family, moulds and yeasts as well as an investigation into *Listeria monocytogenes*) and 2) quality characterization (surface and flesh colour, texture, pH, humidity and water activity) of the truffles. These parameters were evaluated periodically during storage.

The results showed the effectiveness of the five modified atmospheres studied for decreasing microbial populations. The lowest counts for mesophilic bacteria, the *Enterobacteriaceae* family, *Pseudomonas* spp., moulds and yeasts after 26 days were obtained in truffles stored in 10% CO_2-10% O_2. The atmosphere in which the texture (firmness, cohesiveness and chewiness) of the truffles remained closest to the initial data was 5%CO_2-20% O_2. However, pH, humidity, surface colour and flesh colour (L* value) were better preserved in 10% CO_2-10% O_2 atmosphere.

From these results we can conclude that low O_2 as well as high CO_2 atmospheres are beneficial to fresh summer truffles, with lower microbial populations and longer preservation of quality characteristics. Moreover, atmospheres with 10% CO_2-10 O_2% are relatively easy to achieve when selecting the appropriate film over-wrap, and they are therefore promising for the future development of MAP storage of truffles.

Key words: *Tuber aestivum*, modified atmospheres, packaging.

Introduction

Some species of truffles, such as *Tuber magnatum*, *Tuber melanosporum* and *Tuber aestivum,* are in great demand worldwide due to their organoleptic properties, especially their taste and unique aroma (Díaz *et al*., 2003; Barbieri *et al*., 2005; Mello *et al*., 2006; Pacioni *et al*., 2007). Traditionally produced in Italy, France and Spain, truffles are considered a gourmet product and the fresh variety has the highest gastronomic value. The demand for fresh truffles has increased in the last decade, but possibilities for a wider market are still limited by their short shelf-life. The best known and most appreciated species collected in Spain are *Tuber melanosporum*

(black or winter truffles) and *Tuber aestivum* (white or summer truffles). *Tuber aestivum* is less prized than *Tuber melanosporum*, but this species also has excellent gastronomic qualities and during recent years has been in increasing demand due to the attention paid to it by chefs and researchers (Hall *et al*., 2007).
In recent decades in Spain, advances in *Tuber* spp. cultivation have led to the establishment of artificial truffle-grounds, where *Tuber melanosporum* is harvested successfully (November–March). On the other hand, *Tuber aestivum* is mainly harvested from natural truffle-grounds during the summer season (June-October). *Tuber aestivum* is found throughout Europe (Hall *et al*., 2007), while *Tuber melanosporum* is collected mainly in the Mediterranean countries. The development of the fruit bodies from these truffle species occurs at different depths: black truffle growth takes place 20-30 cm underground but summer truffles are sometimes easy to find near the surface as they grow 5-15 cm underground.
Currently, post-harvest storage and conservation technologies frequently used in other fresh products (fruits, vegetables and mushrooms) are not applied to fresh truffles. Their high market price (≈200-2000 €/kg) is one of the incentives for the development of innovative food storage methods that would extend truffle shelf-life and increase the possibilities for a wider foreign market. Little work has been done on the postharvest physiology of truffles. Studies on the respiration rate of black and summer truffles revealed a very high respiration rate for both species (Rivera *et al*., 2007). The use of modified atmosphere packaging (MAP) could be considered as an alternative for the post-harvest storage of fresh truffles, both for extending the shelf life and preserving their organoleptic properties.
The use of appropriate MAP, which can reduce the respiration rate and ethylene production, retards softening and physiological changes in most vegetable products (Kader and Saltveit, 2003). The selection of O_2 and CO_2 concentrations that prolong the shelf life of a given fresh product is essential to the design of a successful MAP system. Therefore, feasibility studies are necessary in order to establish the tolerance limits of O_2 and CO_2 for fresh summer truffles. The purpose of these studies is to determine if modified atmospheres storage can provide better quality than air storage. If the results are not favorable, then it would appear that MAP is not a suitable technology for the particular product (Yam and Lee, 1995). Thus, the objectives of this work are to determine the effect of different levels of O_2 and CO_2 on the microbiological and quality characteristics of summer truffles and to establish the optimum ranges of O_2 and CO_2 for storing this product.

Material and methods

Truffles and storage conditions

Fresh summer truffles were obtained from natural truffle grounds in the province of Teruel (Spain) by digging them out with the help of trained truffle-dogs. The ascocarps were transported to the laboratory in cooled, insulated boxes and processed within a few hours. Covering soil was removed by washing the ascocarps with tap water and brushing them with a soft brush. Qualitative selection of the carpophores was made by discarding truffles with softened texture, dipters and coleoptera larvae or those extremely damaged during the harvest (by shovel or dog's teeth). The truffles were placed in glass jars of 1-liter capacity, connected to the specific modified atmosphere (CO_2-O_2-N_2). The modified atmosphere was created using the flow-through system with a humidified gas rate of 100 mL min^{-1} and stored at 4ºC during 26 days. Table 1 shows the gas composition of the modified atmospheres used in this study.

Tab. 1 Gas concentration (%CO_2:%O_2:%N_2) of modified atmospheres used to store *Tuber aestivum* truffles.

Modified Atmosphere	CO_2 (%)	O_2 (%)	N_2 (%)
MA 1 (Air)	0	21	79
MA 2	0	5	85
MA 3	5	20	75
MA 4	10	10	80
MA 5	20	20	60
MA 6	50	20	30

Microbial analysis

The growth of the most important microbial groups probably associated with the spoilage of fresh truffles was monitored during this experiment. Each sample consisting of one ascocarp was decimal diluted in sterile distilled peptone water 0.1% (Merck, Darmstadt, Germany) and homogenized using a stomacher Lab-Blender 400 (Seward Laboratory, London, UK). The same diluent was used for subsequent serial dilutions. One ml of each dilution was pour plated on duplicate Plate Count Agar (PCA) (Merck) for mesophilic aerobic plate counts and incubated for 72 h at 30º C (ISO 4833:2003).

The *Enterobacteriaceae* family was enumerated on Violet Red Bile Glucose agar (VRBG) (Merck), then incubated at 30º C for 18-24 h. For moulds and yeast, spread plating of 0.1 ml of each dilution was carried out in duplicate on Dichloran Rose-Bengal Chloramphenicol (DRBC) agar (Oxoid, Cambridge, UK), supplemented with Gentamicin (Carlier, Barcelona, Spain), to avoid *Pseudomonas* growth, and incubated at 25º C for 5 days.

Pseudomonas were enumerated by spread plating of 0.1 ml of each dilution on *Pseudomonas* Agar Base (Oxoid) with Cetrimide Fucidin Cephaloridine (CFC) supplement (Oxoid) added, and incubated at 25º C for 24-48 h (ISO 11290-1:2004).

Twenty five grams of truffles were used for the detection of *Listeria monocytogenes*. Each sample was decimal diluted in 225 ml of Fraser Half Concentration broth (Merck), homogenized using a stomacher Lab-Blender 400 and incubated at 30º C for 24 h. A loopful of culture from the enriched Fraser broth was streaked onto *Listeria* Octaviani & Agosti agar (ALOA) (Biolife, Milan, Italy), incubated at 37º C for 24-48 h, and examined for typical *Listeria* colonies (ISO 11290-1:2004).

Physicochemical analysis

Peridium (surface) and gleba (flesh) colour was measured using a Chroma Meter (model CR-200 Minolta, Ramsey, New Jersey). The values were expressed as CIELAB colour space units and mean values for lightness (L*), red-greenness (a*) and blue-yellowness (b*) parameters were calculated for each batch of truffles. Texture parameters were evaluated by performing a TPA (texture profile analysis) test on cubes (1 cm^3), using a TA-TX Plus texture analyzer (Stable Micro Systems, Goaldming, England). The maximum force required to deform the truffles 36 mm at a speed of 0.17 mm/min was recorded. The humidity was determined as a percentage, using the Halogen Moisture Analyzer HR-73 (Mettler Toledo, USA). The pH was measured using a ph meter (Crisol Basic 20).

Microbial and physicochemical analyses were performed after 0, 5, 12, 19 and 26 days of storage. The values are means of three replicates.

Results

Previous studies have reported a total microbial load in fresh ascocarps of *Tuber aestivum* ranging from 6.00 to 7.90 logCFU (colony forming units)/g (Adamo *et al*., 2004; Rivera *et al*., 2005; Nazzaro *et al*., 2006). In this study, the initial microbial counts in fresh ascocarps were higher than the published data, averaging 8.15 logCFU/g (Table 2).

Our results show that Gº P*seudomonas* and the *Enterobacteriaceae* family were the most prevalent microbial groups in the bacterial community of summer truffles, with average counts

of 7.40 logCFU/g. These results differ from those found in *Tuber melanoporum,* where the prevalent microbial group is the *Pseudomonas* genus*,* with lower counts reported for the *Enterobacteriaceae* family (Rivera *et al*., 2008). These high initial microbial counts for the *Enterobacteriaceae* family in *T. aestivum* truffles may be due to their origin. They are collected in natural truffle grounds with greater exposure to wild fauna, such as dipters and coleoptera larvae, than ascocarps grown in artificial truffle grounds and are therefore more susceptible to microbial contamination. Another possible explanation is that *T. aestivum* truffles grow at lesser depth than other species, and that in the harvest season of *T. aestivum* (summer) the temperatures reached can increase the *Enterobacteriaceae* family development. The *Pseudomonas* genus has a dual function: it acts as mycorrhizal helper bacteria (Bedini *et al*., 1999; Gazzanelli *et al*., 1999) and also as a decay agent (Masson *et al*., 2002). These species are strictly aerobic and psychrotrophic, and produce several enzymes (proteases, lipases, quitinases, pectinases, etc) which prevent this microbial group from being a common deterioration agent in many foods. When populations of *Pseudomonas* reach 8.0 logCFU/g the spoilage is evident. In truffles there is no accurate data for *Pseudomonas* counts and no evidence of their role in truffle deterioration.

Given the prevalence of *Pseudomonas* spp. in truffles, their ability to develop under refrigeration, and their capacity for causing deterioration in fresh produce, they should be considered as potential spoilage microorganisms in truffles. Therefore, it is necessary to reduce the presence of this bacterial group during fresh truffle conservation to prolong shelf life.

Tab. 2 Evolution of microbial counts of *Tuber aestivum* samples stored in modified atmospheres at 4 ºC.

Storage time (days)	Modified Atmospheres (%CO_2:%O_2)	Microbial counts (logCFU/g)				
		TMA[1]	ENT[2]	PSE[3]	Moulds	Yeasts
0	MA 1 (0:21)	8.15	7.40	7.39	4.22	5.06
	MA 2 (0:5)					
	MA 3 (5:20)					
	MA 4 (10:10)					
	MA 5 (20:20)					
	MA 6 (50:20)					
5	MA 1	7.81	7.01	6.27	3.91	4.32
	MA 2	7.33	6.11	6.68	2.69	3.84
	MA 3	8.40	7.05	7.33	2.75	3.63
	MA 4	9.25	7.18	7.50	3.28	3.93
	MA 5	7.94	6.48	7.24	3.26	4.46
	MA 6	8.58	6.08	7.28	2.85	3.41
12	MA 1	10.16	7.90	8.23	4.22	5.32
	MA 2	9.52	7.44	7.66	4.83	5.19
	MA 3	9.07	8.13	8.94	3.74	5.82
	MA 4	8.88	7.69	8.30	2.85	3.65
	MA 5	9.59	7.51	7.40	5.34	5.13
	MA 6	9.33	8.12	8.05	4.56	4.87
19	MA 1	10.97	8.16	8.43	5.01	5.48
	MA 2	11.12	8.20	8.91	5.28	5.78
	MA 3	10.75	9.00	9.34	5.30	7.05
	MA 4	8.87	8.00	8.82	2.39	5.96
	MA 5	8.74	8.29	8.60	4.57	5.37
	MA 6	9.53	8.90	8.65	3.63	4.53

26	**MA 1**	11.62	8.99	8.89	4.50	4.37
	MA 2	9.87	7.76	8.64	3.48	4.91
	MA 3	11.25	9.36	10.05	5.26	5.77
	MA 4	9.14	7.60	7.98	2.00	3.08
	MA 5	12.33	8.99	9.19	4.15	5.50
	MA 6	11.31	9.19	8.13	3.30	4.93

[1]TMA (Total mesophilic aerobes); [2]ENT (*Enterobacteriaceae* family); [3]PSE (*Pseudomonas* genus)

Previous studies reported a 100% incidence of *Listeria monocytogenes* in ascocarps of *Tuber indicum* sold in retail markets in Zaragoza (Reyes *et al*., 2006). Given that these truffles are normally eaten raw, mainly finely sliced or grated, it was considered important to include the investigation of this microorganism in this study. However, no incidence of *L. monocytogenes* was detected in the fresh truffles analyzed in this work. Some authors have related the scarce incidence of *L. monocytogenes* in foods with the production of a fluorescent siderophore by the pseudomonads that inhibit the growth of this microorganism (Liao and Sapers, 1999).

All the modified atmospheres tested were able to maintain the microbial quality of truffles for 12 days at 4º C. Microbial counts ranged from 9.07 to 9.58 logCFU/g, except in truffles stored in air where total counts exceeded 10 logCFU/g. After 19 days of storage we observed a reduction of almost 1 logarithmic unit in the microbial counts in batches stored in high carbon dioxide concentration atmospheres (20 and 50%, atmospheres 5 and 6, respectively). However, at the end of the storage period (26 days) the truffles with the best microbial quality were those stored in low oxygen concentration atmospheres (5 and 10%, atmospheres 2 and 4, respectively). This fact is probably due to the growth inhibition of the pseudomonas, that are strictly aerobic microorganisms. Some authors (Mencarelli *et al*., 1997; Massini and Landucci, 1998; Mansantini *et al*., 2002) recommend high carbon dioxide concentrations (up to 60%) for storing *T. aestivum* truffles since these atmospheres inhibit mold and bacterial growth, slow the enzymatic activity and maintain the aroma and texture after 35 days of cold storage. However, in this study, no differences were found between samples packaged in 50% CO_2 and the truffles kept in lower carbon dioxide concentrations.

It was very difficult to detect significant differences in the firmness of truffles because of the huge individual variability of the samples but it was possible to identify a trend. The firmness was maintained close to the initial data in all the modified atmospheres tested and especially in medium content carbon dioxide atmospheres (atmosphere 3 (5% CO_2: 20% O_2) and 4 (10% CO_2: 10% O_2) (Figure 1). However, truffles stored in air showed a different behaviour since they underwent an initial and significant hardening in the first 12 days after which their firmness decreased.

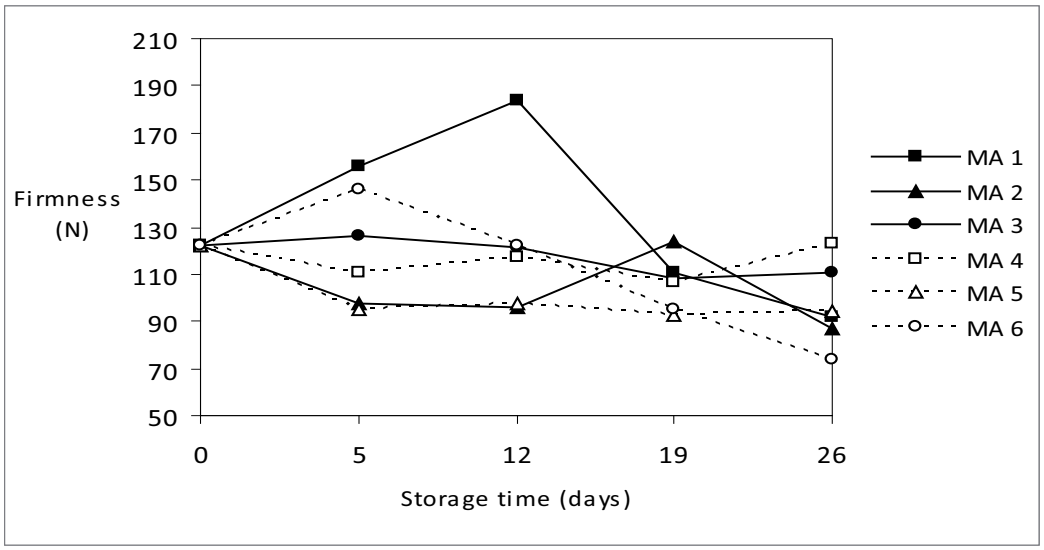
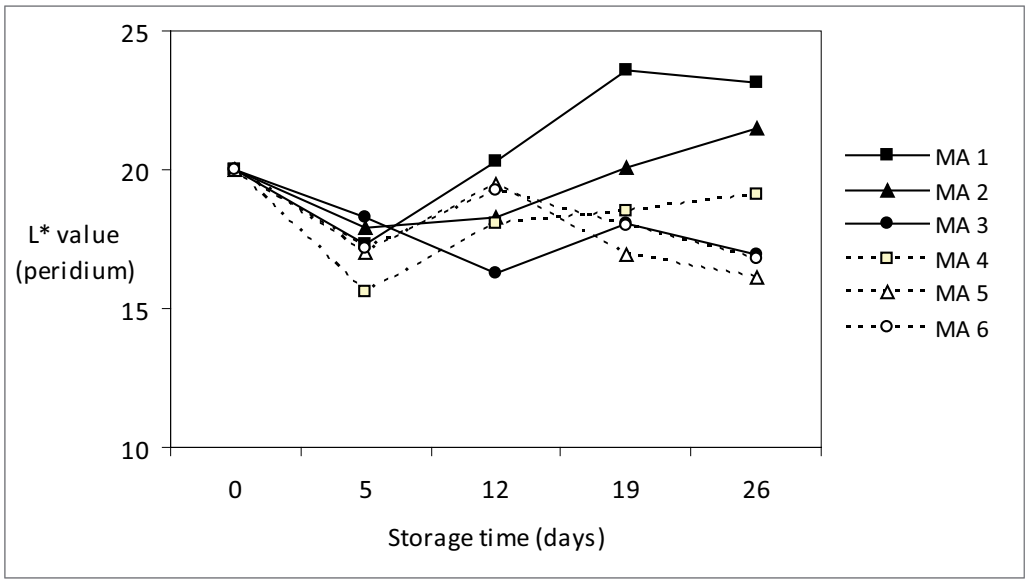


Fig. 1 Evolution of firmness of *Tuber aestivum* truffles stored in modified atmospheres at 4 ºC.

The most representative parameter for detecting differences in the colour of truffles was the lightness (L*) (Figure 2). Mean values for lightness (0=black, 100=white) of the peridium of recently harvested black truffles are near to 20 (Rivera *et al*., 2005; 2006).

All the batches of truffles maintained the lightness of the peridium close to the initial data throughout the storage period. Only in *T. aestivum* truffles stored in air and in 0% CO_2: 5% O_2 (atmosphere 2) was a slight whitening observed after 12 days.

At the end of the storage period the truffles kept in 5% CO_2: 20% O_2 (atmosphere 3) and 10% CO_2: 10% O_2 (atmosphere 4) showed the highest L* values in the gleba. This fact could be due to a minor percentage of mature spores, and in consequence to a lower degree of ripeness. The modified atmosphere 4 was considered the best for keeping the initial colour of truffles close to the initial data.



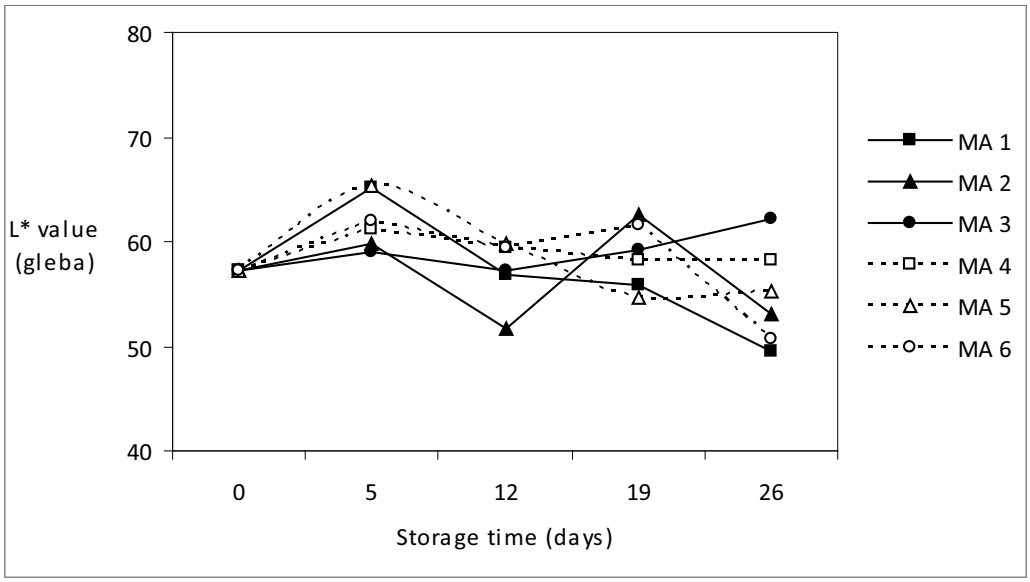


Fig. 2 Evolution of lightness (L*) values of *Tuber aestivum* truffles stored in modified atmospheres at 4 ºC

The initial pH values of truffles were slightly basic, averaging 7.25 units (Table 3). This value is higher than that obtained in the species *T. melanosporum,* where the pH mean value was near to 6.0 (Rivera *et al*., 2008).

Tab. 3 Evolution of pH of *Tuber aestivum* samples stored in modified atmospheres at 4 ºC.

Quality parameters	Modified Atmospheres (%CO2:%O2)	Storage time (days)				
		0	5	12	19	26
pH	MA 1 (0:21)	7.25	7.15	7.48	8.08	7.98
	MA 2 (0:5)		7.45	7.40	7.36	6.94
	MA 3 (5:20)		6.92	7.28	7.01	7.52
	MA 4 (10:10)		6.62	6.72	6.61	6.82
	MA 5 (20:20)		6.74	6.88	6.89	7.54
	MA 6 (50:20)		6.70	6.96	7.04	7.46

The pH of air-stored truffles increased with the storage time reaching values of nearly 8 from day 19 onward. A decrease in the pH values was detected in truffles stored in high carbon dioxide concentration atmospheres in the first three weeks of storage. However, at the end of the storage time it was observed that the pH increased parallel with the carbon dioxide concentration, showing values of nearly 8. Truffles stored in low oxygen concentrations (5 and 10%, atmospheres 2 and 3) showed a different behaviour since pH values increased during the first days of storage, decreased from day 5 onward, and reached the lowest values at the end of storage. Truffles with the highest microbial counts (atmospheres 1 (air), 3, 5 and 6) obtained the highest pH values. This fact could be related with the proteolytic metabolism of the *Pseudomonas* genus that produces ammonia as a final product and in consequence increases the pH of the truffle.

The percentage of humidity ranged from 64 to 70% (data not shown) in all batches and no evidence of dehydration was detected, probably due to the humidified gas flow maintained during storage time.

Conclusions

Truffles belonging to the species *T. aestivum*, grown in Teruel, have a high microbial load, consisting mainly of the *Pseudomonas* genus and *Enterobacteriaceae* family. According to our results, a modified atmosphere combining high carbon dioxide and low oxygen concentrations could prolong the postharvest shelf life of truffles, decreasing the microbial growth rate and maintaining the quality parameters. The optimum ranges of O_2 and CO_2 selected in this study for storing this product were 10%. These gas concentrations are easy to achieve in a traditional passive modified atmosphere packaging without initial gas injection. However, this treatment has to be applied in combination with a sanitizing agent that reduces the initial microbial populations.

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THE GROWING UP OF OREGON TRUFFLES (*TUBER OREGONENSE, TUBER GIBBOSUM, LEUCANGIUM CARTHUSIANUM*)

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Abstract
Oregon Truffles were introduced to the market beginning in 1983, but failed to impress. The best that could be said about them was that they were “chameleons”--that is, every once-in-a-while, one got a good one. But no one knew why.
Their inability to garner culinary respect was a result of the lack of respect exercised by their excavators and traders. Twenty years of such disrespect earned Oregon Truffles the title of cheap, “faux” truffles, and created a culture of excavation of almost universally site-degrading thievery.
Then, one handler, believing that to be a needless tragedy, began an investigation into what could be done about it. Drawing an unprecedented sharing of secrets by the handful of wild harvesters who had great respect and tastes for Oregon Truffles, and were the most observant, he designed experiments quickly yielding previously undiscovered keys to presenting to the market reliably excellent Oregon Truffles.
As well, one of those trufflers had proven that the commercial harvest of Oregon Truffles could be done without risk of site degradation. Thus, a passionate crusade began to save the Oregon Truffle from its headlong rush toward the compost pile of failed culinary dreams, elevating it instead to a rightful status as a world-class culinary gem.
Discovering of the techniques by this handler was partially facilitated by the fact that he had no experience with European truffles, for, as it turns out, Oregon Truffles are as different as they are similar to their European cousins. Those differences extend to their culinary uses as well, both requiring chefs to learn new truffle tricks and allowing Oregon Truffles to enter the culinary stage without displacing any other members of the cast, broadening the reach of truffle cuisine, enriching its lore and appeal. As a *Tuber melanosporum* would say to a *T. magnatum*: “Viva la difference!”
For Oregon Truffles to finish growing up, however, they must learn from the experiences of their European elders. Wholesale excavation must be replaced by the use of dogs for selective excavation, grading standards adopted, and methods developed to reliably establish plantations.
Although Dr. Lefevre’s Oregon Truffle Festival, launched relying on this new supply, has caused a sensation, the requisite fiscal and regulatory support is not yet forthcoming from government. Substantial private investment will likely be required.
Key words: Oregon, raking, history, conservation, commerce, valorization.

MICO PLANTS TRUFFLE CULTIVATION

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Abstract
"Mico Plants" truffle cultivation is a family-run Garden Centre situated in S. Demetrio of Vestini AQ - Abruzzi - that offers its very high experience in the field of truffle cultivation. The company's main activity is the production of some mycorrhized plants for truffle plantations.
The business philosophy is oriented towards the preservation of the Abruzzi truffle germoplasm; this is the reason why mycorrhized plants are obtained from certified seeds and truffles both coming from the Abruzzi. Upon request, mycorrhized plants obtained from seeds and truffles of other Italian Regions can be produced.
Since the year 2000, the product certification of the Garden Centre is issued by the Department of Applied Biology of the University of Perugia, that certifies carpophores used for the inocula and truffle-producing plantlets. Certification is based on carpophore morphological analysis and on mycorrhizal morphological and bio-molecular studies.
Production features.
The Garden Centre assortment comprises:

- *Quercus pubescens - x T. melanosporum - x T. aestivum*
- *Quercus ilex - x T. melanosporum - x T. aestivum*
- *Corylus avellana - x T. melanosporum - x T. aestivum*
- *Ostrya carpinifolia - x T. melanosporum - x T. aestivum*

After the inoculum, plantlets are bred in cells characterized by a dimension of 7x7x17 – 9x9x13 – 11x11x17 cm, made of a substrate represented by corrected and sterilized natural soil. In this way plantlets not only have an elevated degree of mycorrhization, but also a strong and expanded root apparatus.
Truffle plantations obtained with our mycorrhized plants, on suitable sites, are already productive.

LA SPECIALIZZAZIONE TERRITORIALE DELLA TARTUFICOLTURA IN UN'AREA INTERNA DELL'APPENNINO CENTRALE MEDIANTE L'APPLICAZIONE DI UN MODELLO GRAVITAZIONALE

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Abstract: The truffle territorial specialization in an inner area of the central Apennine using gravity model approach
The truffle cultivation has interested, for a long time, the areas located near the Italian Apennine, becoming an element of multifunctionality and farm diversification, able to reduce the marginalization of the rural areas. This research has studied in an Italian inner highland area, as the province of Rieti, during a long period (1990-2006), the territorial specialization of truffle cultivation; the aim of this research was to appraise the existence of some indispensable conditions for the definition and the recognition of the rural district by the political administrations. In the first part of this research it used a simple model to describe the truffle territorial specialization, as the modified index of Balassa-Hoover; in this case the specialization index was modified with the aim to standardize the statistical data in a dimensionless variation range of ± 1. In the communes of the area of study, close to the Apennine mountain ridge, some values of index of specialization have been significant, that have strengthen their position inside the rural district, even if, it has observed a substantial decrease of the authorized truffle pickers during the second period of observation. The following phase of this research has been characterized by the application of a linear gravity model, used in physics, transformed with the least ordinary squares; in this case it has been possible to estimate a linear equation that connected, in all the communes of the study area, the farm multifunctionality, represented by the dependent variable truffle pickers, with a lot of independent variables linked to the rural and socioeconomic development of the territory, highlighted the existence of some significant relationships in the statistical model. The results have affirmed as the truffle can represent a variable able to increase the socioeconomic development of the rural areas, reducing the phenomenons of marginalization. The analysis has shown as in the next years it should intervene on the rural territory with some specific cultural paths, represented by the ways of taste, and by shared marketing actions, that are able to reduce the economical crisis of rural communities, to reduce the conditions of economic isolation of rural areas and to put the truffle from a market of niche to a wide market economically interesting, utilizing completely the advantages of the rural district, shortening the brokerage costs and the chain and exploiting the territory and the rural peculiarities of the multifunctionality.
Key words: gravity model**,** territorial specialization index, index Balassa-Hoover, rural areas, rural district.

Introduzione

La coltivazione del tartufo ha interessato, da sempre, le aree pedemontane a ridosso della cimosa appenninica, diventando un elemento di multifunzionalità e di pluriattività aziendale, capace di ridurre il fenomeno di marginalizzazione delle aree rurali. Nel corso degli anni ottanta le zone interne dell'Appennino centrale si sono contraddistinte per un forte fenomeno di abbandono delle aree rurali che ha causato un impoverimento socio-economico di queste aree e che ha avuto delle conseguenze sulle aziende agricole che operavano in queste zone (Formica, 2004). Con le modifiche intervenute nella Politica agricola comunitaria (Pac) c'è stato un maggiore interessamento del legislatore verso le aree rurali e verso il riconoscimento di un'agricoltura multifunzionale che sappia salvaguardare il territorio e rilanciare l'occupazione mediante iniziative previste sia nel Piano di Sviluppo rurale 2000-2006 che nella nuova programmazione prevista nel Programma di sviluppo rurale 2007-2013 mediante una serie

di iniziative tese a promuovere la multifunzionalità (Galluzzo, 2008a). Le azioni di intervento proposte hanno avuto l'obiettivo di garantire la riforestazione di alcune aree e la messa in campo di colture arboree micorrizate in grado di assicurare la produzione di tartufi. Il riconoscimento delle strade del gusto e del distretto rurale, se attuate in sinergia, possono rappresentare un utile strumento per la valorizzazione della multifunzionalità e far uscire il tartufo da un ambito di nicchia (Galluzzo, 2004a) dove si osserva l'assenza di un mercato tradizionale nel quale la domanda e l'offerta possano incontrarsi generando un prezzo di equilibrio in assenza di fallimenti di mercato e asimmetrie informative (Kreps, 1990) e non lasciando il prodotto in una situazione di mercato informale ed estemporaneo. Tutto ciò, infatti, nel lungo periodo potrà essere dannoso, inefficace ed inefficiente per i tartuficoltori i quali, nel momento in cui ricorrono a mercati non catalogabili nel modello economico standard, perdono il loro potere di mercato, a tutto vantaggio della controparte, complessificando il mercato ed eliminando quell'unica opportunità che essi sono in grado di rappresentare come filiera diretta e cortissima.

Obiettivi

Il presente lavoro ha voluto prendere in considerazione la tartuficoltura in un'area interna dell'Appennino centrale a rischio marginalizzazione, rappresentata dalla provincia di Rieti, definendo nel lungo periodo, dall'annualità 1990 fino all'anno 2006, la specializzazione territoriale nella coltivazione del tartufo. Obiettivo di fondo di questa prima fase di indagine è stato quello di valutare se nell'area di studio ci fosse l'esistenza delle condizioni necessarie, ma non sufficienti, per la definizione e il riconoscimento del distretto rurale in via di ultimazione da parte delle amministrazioni locali della regione Lazio, valutando la specializzazione territoriale, elemento base per la definizione e delimitazione di un distretto (Galluzzo, 2005; Galluzzo, 2008b) e una serie di variabili sociale ed economiche necessarie per definire delle sottozone caratterizzate da una elevata vocazionalità nella produzione dei tartufi. Nella seconda fase è stata verificata, in maniera più generale e su tutto il territorio dell'area di studio, se l'applicazione di modelli econometrici, potesse essere uno strumento di indagine utile a fornire delle indicazioni sulle relazioni statisticamente significative che si vengono a creare tra le diverse variabili in gioco, che possono aver determinato una maggiore specializzazione territoriale nella coltivazione del tartufo; inoltre, si è inteso valutare se la tartuficoltura sia connessa alla specializzazione produttiva agricola del territorio nel quale si è riscontrato un incremento delle aree boscate montane e, a seguito dei fenomeni di abbandono delle aree interne, di un progressivo invecchiamento della popolazione. L'obiettivo specifico è stato quello di valutare, indirettamente, se la tartuficoltura possa essere una variabile *proxy* della multifunzionalità sulla quale agire per rallentare i processi di marginalizzazione delle aree montane interne.

Metodologia

Il modello utilizzato, per definire la specializzazione tartufigena territoriale, è stato tratto da quanto proposto in letteratura con l'indice di Balassa-Hoover, apportandone delle semplificazioni all'impostazione di base, al fine di normalizzare i valori entro un intervallo adimensionale di facile lettura compreso tra ± 1. Altre metodologie utilizzabili ma sempre derivanti dall'indice di Balassa-Hoover possono essere due, ossia: a) indice di specializzazione; b) indice di concentrazione, utilizzato per ambiti territoriali più limitati e per alcune ricerche compiute su coltivazioni arboree (Galluzzo, 2004; Galluzzo, 2006). Il modello di Balassa-Hoover tradizionale, infatti, risulta essere un indicatore molto reattivo e sensibile alla specializzazione della variabile che si vuole osservare; tale modello, tuttavia, ha lo svantaggio di avere dei valori che si collocano nell'intervallo limitato inferiormente a 0 ma non superiormente che può arrivare a $+\infty$. Tutto questo rende di non facile lettura e interpretazione il dato inerente la specializzazione, soprattutto se si devono creare delle classi comparative. Infatti, se il dato ottenuto è pari a zero non si crea alcun dubbio interpretativo, poiché tale valore indica una mancata presenza della variabile di specializzazione produttiva individuata; i maggiori problemi si osservano con le situazioni intermedie ossia di parziale specializzazione che appaiono essere abbastanza difficili da interpretare e spiegare. A tal fine, nella presente analisi si è preferito usare un indicatore

di specializzazione territoriale che ha reso i dati di più agevole lettura riducendo il margine di variazione e consentendo di individuare poche classi specifiche e abbastanza uniformi per il confronto intertemporale e intraterritoriale. In questo caso la banda di oscillazione varia tra +1, indice di massima specializzazione e -1, nel caso si assenza di specializzazione inerente la tartuficoltura, consentendo di definire nei valori prossimi allo zero nelle situazioni intermedie. Le serie storiche dei tartuficoltori abilitati alla raccolta nella provincia di Rieti sono state utilizzate quali variabile *proxy* della diffusione del tartufo, poiché nelle aree potenzialmente ricche di tartufi o nelle quali la tartuficoltura appare essere abbastanza connatura sul e nel territorio si è, da sempre, osservata una maggiore presenza di raccoglitori che ne traggono da tale prodotto una fonte di reddito diretta (raccolta e vendita a terzi) e/o indiretta (raccolta e autoconsumo familiare). L'analisi della specializzazione territoriale ha trovato nell'indice di specializzazione (Isp), la metodologia più adatta ad effettuare dei confronti tra diversi comuni dell'area di studio anche se la base metodologica era stata applicata in ambiti geograficamente più ampi (Bagarini *et al.,* 1993). L'indice di specializzazione produttiva tartuficola (Ispt), comparata alle aziende agricole attive sul territorio, in formule può essere così riassunto:

$Ispt = [a_{ij} – b_i] * [(1-a_{ij})+ (1-bi)a_{ij}]^{-1}\_$

$a_{ij}= x_{ij} * (\Sigma_i x_{ij})^{-1}$

$b_i= \Sigma_j x_{ij} * (\Sigma_{ij} x_{ij})^{-1}$

x_{ij} ossia il numero di raccoglitori di tartufi attivi nei diversi comuni dell'area di studio

La fase successiva è stata caratterizzata dall'applicazione di un modello gravitazionale lineare, preso in prestito dalla fisica tradizionale, ottenuto applicando i minimi quadrati ordinari ordinary least square (OLS); ciò ha consentito di definire un'equazione lineare capace di mettere in relazione, in tutti i comuni dell'area di studio, la multifunzionalità aziendale, rappresentata dalla variabile *proxy* (raccoglitori di tartufi attivi) con una molteplicità di variabili indipendenti o regressori legate allo sviluppo agricolo, rurale e socio-economico del territorio, evidenziando l'esistenza di alcune relazioni statisticamente significative nel modello. Il modello gravitazionale di base, il quale ha costituito la base della presente ricerca, è stato il seguente (Head, 2003; Karemera *et al*., 2000):

$F_{ij} = G [(M_i^{\alpha} \times M_j^{\beta}) / D_{ij}^{r}]$

F_{ij} è l'attrazione gravitazionale che si crea tra due corpi
D_{ij}^{r} è la distanza che esiste tra i due corpi
M_i^{α} M_j^{β} sono le masse dei rispettivi corpi elevate a dei coefficienti specifici
G costante di attrazione gravitazionale

Le caratteristiche del modello gravitazionale consentono di sfruttare le sue peculiarità moltiplicative ottenendo un modello lineare con l'applicazione della trasformazione logaritmica dei coefficienti α e β e ottenendo il seguente modello lineare:

$\ln F_{ij} = \alpha_0 + \alpha \ln M_i + \beta \ln M_j - \gamma \ln D_{ij} + \varepsilon_{ij}$

Nella ricerca condotta il modello applicato in due diversi contesti temporali è stato il seguente:

$\ln$ tartuficoltori attivi $= \alpha_0 + \alpha \ln$ agriturismi attivi $+ \beta \ln$ aziende agricole $- \gamma \ln$ Distanza $+ \varepsilon_{ij}$

Per valutare, successivamente, le relazioni significative che la variabile dipendente (raccoglitori di tartufi attivi), variabile considerata proxy della multifunzionalità nelle aree rurali, e alcune variabili socio-economiche si è intervenuti con l'applicazione di un modello OLS, in un congruo e

significativo intervallo di tempo (1990-2006), e che in formula può esser così schematizzato:

$$y = \alpha_0 + \alpha x_1 + \beta x_2 + \gamma x_3 + \delta x_4 + \varepsilon_{jt}$$

α_0 termine costante
x_1, x_2, x_3,x_4 variabili indipendenti utilizzate
ε_{jt} termine di errore.

Le assunzioni di fondo che sono alla base per l'applicazione del modello OLS sono le seguenti:

a) l'errore statistico ε_{jt} ha media condizionata nulla data X_i, ovvero $E(u_i \mid X_i) = 0$;
b) $(X_i, Y_i), i = 1,....,n$ sono estratti indipendentemente e identicamente distribuiti (i.i.d.) dalla loro distribuzione congiunta;
c) (X_i, ε_j) hanno momenti quarti finiti non nulli;
d) che non vi sia correlazione tra i regressori e i disturbi casuali in maniera tale che il valore tra β atteso e β stimato sia identico.

I modelli statici utilizzati hanno voluto evidenziare, in due periodi diversi (annualità 1990 comparata alle annualità 2000 e 2006):

1. le relazioni significative tra classi dimensionali delle aziende agricole attive sul territorio con lo sviluppo della tartuficoltura nell'area di studio;
2. l'esistenza di un rapporto statisticamente significativo tra incremento e tipologia delle strutture agrituristiche attive con la tartuficoltura nell'area di studio;
3. l'esistenza di relazioni statisticamente significative all'interno di un modello che avesse quale variabile dipendente la numerosità di raccoglitori di tartufi attivi sul territorio e tra le variabili indipendenti la tipologia di aziende agricole attive.

Tab. 1 Variazione dell'indice di specializzazione produttiva tartuficola nell'area di studio nei diversi anni considerati

	Anno				
Classe di ampiezza	1990	1995	2000	2003	2006
-1	42	57	49	41	33
-0,99: 0	10	0	0	0	0
0: 0,5	7	0	0	16	20
0,501: 1	16	16	24	16	20

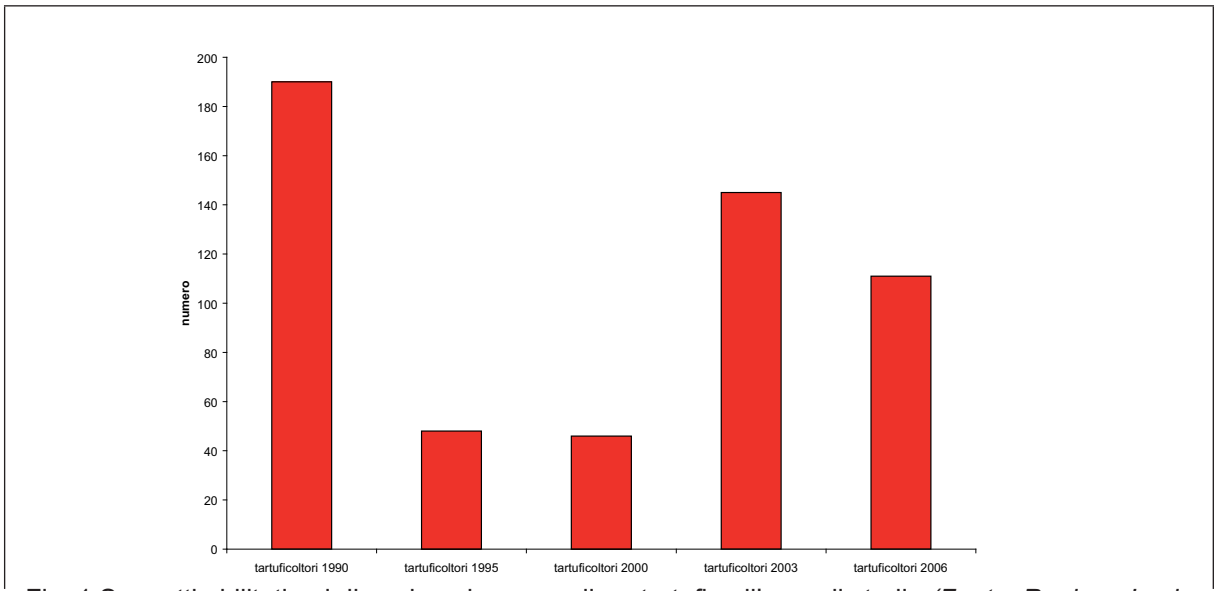

Fig. 1 Soggetti abilitati nei diversi anni a raccogliere tartufi nell'area di studio *(Fonte: Regione Lazio Area decentrata agricoltura)*

Risultati e discussione

La ricerca ha evidenziato, nell'area di studio, un significativo decremento dei soggetti abilitati a raccogliere i tartufi dal 1990 al 2006 anche se, a partire dall'anno 2003, si è riscontrato un incremento dei soggetti abilitati i quali nel triennio successivo hanno diminuito la loro consistenza numerica attestandosi su valori superiori al centinaio di unità (Fig. 1).

La specializzazione territoriale ha evidenziato come in media la provincia di Rieti sia passata da una situazione di ridotta specializzazione produttiva ad una situazione di totale indifferenza produttiva nella tartuficoltura con valori negativi ma, comunque, prossimi allo zero. Nel lungo periodo nell'area di studio si è osservata una situazione abbastanza variabile in termini di indice di specializzazione; infatti, se nell'annualità 1996 su 73 comuni dell'area di studio ben 14 presentavano un Indice di specializzazione tartuficola (Ispt) superiore a +0.50, nell'anno 2000 tale valore è incrementato interessando ben 24 comuni su 73; infine, nell'annualità 2006, in base agli ultimi dati disponibili, c'è stato un forte incremento dei comuni con Indici di specializzazione produttiva tartuficola superore a 0.50. L'indice di maggiore specializzazione, come era lecito attendersi, ha interessato prevalentemente i comuni a ridosso della catena Appeninica del Terminillo e della zona montuosa del cicolano a ridosso della catena del monte Velino (Tab. 1).

Tab. 2 Applicazione del modello gravitazionale nell'annata 1990

Variabile	Coefficiente	Errore Std.	Statistica t	p-value	Significatività
Costante	-8.04241	1.41443	-5.6860	0.00128	***
ln aziende agricole attive	1.30475	0.173944	7.5010	0.00029	***
ln agriturismi attivi	-1.66158	0.40334	-4.1195	0.00622	***
ln distanza	0.489174	0.189645	2.5794	0.04180	**
R^2 0.8643	*** <1%	** 1-5%			
R^2 corretto 0.7964					

Tab. 3 Applicazione del modello con la variabile dipendente tartuficoltori attivi nell'annata 1990 senza errori robusti rispetto all'eteroschedasticità

Variabile	Coefficiente	Errore Std.	Statistica t	p-value	Significatività
Costante	-3.41577	0.946981	-3.607	0.00059	***
Aziende agricole <1ha	0.0395164	0.0220663	-1.791	0.0777	*
Aziende agricole 1-2 ha	-0.0927936	0.0285011	-3.256	0.00176	***
Aziende agricole 2-5 ha	0.0149629	0.0442735	0.338	0.7364	ns
Aziende agricole totali	0.0513817	0.0192937	2.663	0.0966	***
R^2 0.521	*** <1%	* 5-10%			
R^2 corretto 0.493					

Tab. 4 Applicazione del modello con la variabile dipendente tartuficoltori attivi nell'annata 2006 e aziende ricettività alberghiera senza errori robusti rispetto all'eteroschedasticità

Variabile	Coefficiente	Errore Std.	Statistica t	p-value	Significatività
Costante	1.3151	1.00901	1.3034	0.20237	ns
Ricettività alberghiera	1.17108	0.421049	2.7813	0.00927	***
Ricettività esercizi complementari	0.376921	0.258544	1.4579	0.15527	ns
Agriturismi totali	1.51838	1.36161	1.1151	0.27365	ns
Agriturismo solo ristorazione	-0.444606	0.860096	-0.5169	0.60900	ns
Agriturismi solo alloggio	-2.75947	1.45494	-1.8966	0.06754	*
R^2 0.593	*** <1%	* 5-10%			
R^2 corretto 0.525					

Tabe. 5 Applicazione del modello con la variabile dipendente tartuficoltori attivi nell'annata 2006 e aziende ricettività alberghiera con errori robusti rispetto all'eteroschedasticità

Variabile	Coefficiente	Errore Std.	Statistica t	p-value	Significatività
Costante	1.3151	0.953726	1.3789	0.17812	ns
Ricettività alberghiera	1.17108	0.32679	3.5836	0.00118	***
Ricettività esercizi complementari	0.376921	0.161185	2.3384	0.02622	**
Agriturismi totali	1.51838	0.802207	1.8927	0.06808	*
Agriturismo solo ristorazione	-0.444606	0.901937	-0.4929	0.62564	ns
Agriturismi solo alloggio	-2.75947	1.08887	-2.5343	0.01672	**
R^2 0.593	*** <1%	** 1-5%	* 5-10%		
R^2 corretto 0.525					

Tab. 6 Applicazione del modello con la variabile dipendente tartuficoltori attivi nell'annata 1990 applicata alle diverse tipologie aziendali e alle diverse variabili sociali con errori robusti rispetto all'eteroschedasticità

Variabile	Coefficiente	Errore Std.	Statistica t	p-value	Significatività
Costante	0.666776	0.429451	1.553	0.12544	ns
Aziende agricole attive	-0.00647746	0.00165730	-3.908	0.00023	***
Sau a cereali	0.0176439	0.00839333	2.102	0.03948	**
Sau a foraggiere	0.00825225	0.00536932	1.537	0.12924	ns
Sau a seminativi	-0.0117619	0.00546314	-2.153	0.03510	**
Sau a prati	-0.00038944	0.0005213	-0.747	0.45783	ns
Boschi	0.00203878	0.00043100	4.730	0.00001	***
Popolazione >65 anni	-0.0003035	0.003427	-0.089	0.92970	ns
Popolazione <14 anni	0.00900635	0.00346239	2.601	0.01153	**
R^2 0.925	*** <1%	** 1-5%			
R^2 corretto 0.916					

Tab. 7 Applicazione del modello con la variabile dipendente tartuficoltori attivi nell'annata 2000 applicata alle diverse tipologie aziendali e alle diverse variabili sociali

Variabile	Coefficiente	Errore Std.	Statistica t	p-value	Significatività
Costante	0.347265	0.229956	1.510	0.13601	ns
Aziende agricole attive	-8.27 10^{-5}	0.0006650	-0.124	0.90136	ns
Sau a cereali	0.00215461	0.00615751	0.350	0.72757	ns
Sau a foraggiere	-0.004381	0.004497	-0.974	0.33365	ns
Sau a seminativi	0.0021055	0.004347	0.484	0.62982	ns
Sau a prati	0.0006252	0.0001759	3.553	0.00073	***
Boschi	-0.000178850	0.000166227	-1.076	0.28606	ns
Popolazione >65 anni	-0.00329314	0.00154027	-2.138	0.03640	**
Popolazione <14 anni	0.00434537	0.002039	2.131	0.03702	**
R^2 0.617	*** <1%	** 1-5%			
R^2 corretto 0.568					

L'applicazione del modello gravitazionale ha evidenziato dei dati abbastanza interessanti esclusivamente nell'annualità 1990, invece nell'annualità 2006 nel modello non è emersa alcuna relazione statisticamente significativa. Il modello gravitazionale applicato ai dati dell'annualità 1990 ha dimostrato come la diffusione dei raccoglitori di tartufi risenta positivamente, con una significatività del 5%, della distanza e, con una significatività dell'1%, delle aziende agricole attive; le relazioni tra aziende agrituristiche attive e raccoglitori di tartufo appare essere indiretta anche se significativa a valori inferiori all'1% (Tab. 2).
Nel primo modello statistico utilizzato, inerente la relazione tra la numerosità delle classi dimensionali delle aziende agricole attive nell'area di studio e la presenza di raccoglitori di tartufi (variabile dipendente), riferito a due istanti ben definiti e successivi, è emerso come negli anni novanta la maggiore presenza di raccoglitori di tartufi abbia risentito positivamente della numerosità aziendale complessiva (aziende attive) e negativamente delle aziende di piccole dimensioni, ossia di quelle aziende agricole che si sono collocate nella classe dimensionale inferiore alla media italiana e, in particolare, di quelle che si sono collocate nella classe dimensionale compresa tra 1 e 2 ettari e in quella inferiore ad un ettaro con una significatività statistica inferiore all'1% (Tab. 3). Ciò sembra confermare come nel secolo scorso l'attività di raccolta dei tartufi sia stata messa in atto nei comuni dell'area di studio nei quali è prevalsa l'attività agricola e, nello specifico, l'attività agricola svolta in aziende di piccole dimensioni. L'applicazione dello stesso modello nei dieci anni successivi ha evidenziato una riduzione dell'R^2 e dell'R^2 corretto e l'assenza di relazioni significative tra il numero di raccoglitori attivi sul territorio e la dimensione aziendale delle aziende; pertanto, la variabile dipendente costituita dal numero di raccoglitori di tartufo è risultata essere sganciata sia dalla presenza delle aziende agricole attive (in diminuzione) che dalla loro dimensione aziendale. Nell'annualità 2006, non essendo disponibili per l'annualità 1990 dati statistici adeguati, è stato possibile applicare un modello per verificare se la multifunzionalità, individuata nella variabile indipendente agriturismo e la diffusione di alberghi nell'area di studio abbia influito sul numero di raccoglitori di tartufi attivi sul territorio al fine di valorizzare in maniera integrata le aree rurali della provincia di Rieti. L'analisi dei dati, con errori standard non robusti rispetto alla eteroschedasticità, ha consentito di evidenziare come ad un incremento della ricettività alberghiera abbia corrisposto sul territorio dell'area di studio un incremento dei raccoglitori di tartufi; una situazione opposta si è riscontrata nelle strutture agrituristiche che effettuano solo ospitalità. Nel territorio dell'area di studio, infatti, si è osservato un incremento delle strutture agrituristiche in grado di offrire servizi di sola ospitalità, rispetto alle strutture agrituristiche in grado di effettuare ospitalità e ristorazione, cui è corrisposto, come era logico attendersi, una riduzione dei raccoglitori di tartufi. L'applicazione del modello OLS

con errori standard robusti rispetto all'eteroschedasticità ha confermato le correlazioni indicate nel precedente modello, anche se un incremento della ricettività complessiva e delle strutture agrituristiche sul territorio, indipendente dal servizio prevalentemente offerto (ristorazione vs ospitalità) ha determinato un incremento dei raccoglitori di tartufo (Tabb. 4-5).
Per valutare se i raccoglitori di tartufi si collochino in comuni a rischio marginalizzazione, ossia in comuni con una elevata presenza di popolazione non più in età lavorativa (sopra i 65 anni e sotto i 14 anni), caratterizzate da aziende agricole estensive, ossia con animali allevati allo stato brado e da ampie superfici pascolative e boscate, è stato applicato un modello OLS, che, in tre differenti momenti, annualità 1990, annualità 2000 e annualità 2006, ha cercato di indagare le variazioni verificatesi e le relazioni statisticamente più significative, con l'obiettivo di evidenziare se esiste il rischio concreto di una marginalizzazione delle aree rurali studiate e la capacità della tartuficoltura di limitarne il declino dei comuni dell'area di studio. L'analisi dei dati dell'anno 1990 ha evidenziato, con e senza l'applicazione degli errori robusti rispetto all'eteroschedasticità, ha evidenziato come ad un elevato numero di aziende agricole e ad una elevata presenza di superficie agricola utilizzabile destinata a seminativo, come era lecito attendersi, abbia corrisposto una ridotta presenza di raccoglitori di tartufi (Tab. 6). L'attività di raccolta dei tartufi sembra non esser un qualcosa che vada ad interessare comuni dell'area di studio a rischio marginalizzazione; infatti, nei comuni dove è presente una popolazione giovane si è riscontrata una maggiore diffusione di un'attività rivolta alla raccolta dei tartufi. Nell'area di studio nel 1990 il modello ha evidenziato come l'attività di raccolta interessi comuni dotati di una superficie coltivata a cereali elevata e con una buona dotazione della superficie coperta da boschi; quindi nei comuni dell'area di studio caratterizzati da superfici boscate ampie è corrisposto un incremento dei soggetti abilitatati a raccogliere il tartufo. Da questa prima simulazione è emerso come negli anni novanta l'attività di raccolta tartufi abbia interessato comuni, nei quali non si evidenziano sintomi di marginalizzazione statisticamente significativi, con popolazioni giovani nelle quali l'attività agricola si è iniziata a rarefare concentrandosi su superfici agricole utilizzate per la coltivazione dei cereali e con una elevata boscosità.
L'analisi dei dati dell'anno 2000 ha evidenziato nel modello OLS, ottenuto senza l'applicazione degli errori robusti rispetto all'eteroschedasticità, l'esistenza di relazioni statisticamente significative e indirette con il regressore popolazione oltre i 65 anni di età; pertanto, nei comuni dell'area di studio a rischio marginalizzazione con una popolazione non più in età lavorativa è stato possibile evidenziare una minore presenza di raccoglitori di tartufi, il che può essere un buon indicatore di come la multifunzionalità, estrinsecatasi e individuata nella variabile dipendente numero di raccoglitori di tartufi, possa rappresentare un buon deterrente per ridurre la marginalizzazione di queste aree (Tab. 7). L'analisi ha confermato il rischio marginalizzazione dei comuni dell'area di studio poiché si è registrato una diminuzione della popolazione giovane inferiore ai 14 anni con un conseguente decremento dei raccoglitori di tartufi; nelle aree a rischio marginalizzazione la diminuzione della superficie agricola utilizzabile è stata sostituita dai prati e dai boschi cui è seguito un incremento dell'attività tartuficola. Il modello ha, quindi, confermato come nell'area di studio si assista ad un processo di marginalizzazione cui la multifunzionalità, estrinsecatasi attraverso la tartuficoltura, può svolgere un ruolo di prevenzione e presidio delle aree rurali. Tale affermazione viene ad esser corroborata dalle risultanze del modello applicato nell'annualità 2006 dalla quale è emerso come l'attività di raccolta tartufi abbia interessato dei comuni con elevate dotazioni a superfici prative e pascolive, ossia i territori di montagna e/o di alta collina, anche se la funzione della tartuficoltura sembra, sempre di più, essersi sganciata dalla ruralità in senso ampio divenendo un'attività autonoma dal territorio e dalla ruralità in senso ampio. L'applicazione della trasformazione in logaritmo naturale dei regressori considerati, al fine di evitare un'eccessiva disomogeneità nei valori delle variabili considerate, nel modello teso a valutare l'esistenza di relazioni statisticamente significative tra la variabile dipendente, rappresentata dal numero di raccoglitori autorizzati nei tre diversi anni di confronto, e le variabili indipendenti rappresentate dalla popolazione non più attiva sul mercato del lavoro, dalle aziende agricole attive e dalla superficie coltivata, ha evidenziato per l'annualità 1990 e per l'annualità 2000 una sostanziale stabilità e concordanza dei risultati ottenuti nel modello senza l'impiego

della trasformazione logaritmica dei dati. Il modello per l'annualità 2006 ha, invece, evidenziato come la tartuficoltura abbia risentito positivamente dell'incremento della superficie boscata e in maniera inversamente proporzionale delle aziende agricole attive. I dati analizzati confermano come la tartuficoltura sia una attività che parzialmente rappresenta una concreta opportunità per le aree rurali di proteggersi dalla loro marginalizzazione, dovuta ad un calo delle aziende agricole attive e nelle quali la superficie boscata risulta essere molto diffusa.

Conclusioni

Questa breve analisi ha dimostrato come nella provincia di Rieti la produzione di tartufo si è abbastanza rarefatta negli ultimi anni con la conseguente riduzione degli operatori autorizzati alla raccolta, i quali hanno determinato una maggiore specializzazione del territorio nelle aree a ridosso della cimosa appenninica, determinando delle condizioni necessarie per l'istituzione formale-operativo e il riconoscimento di un distretto rurale della montagna che veda nei prodotti del bosco e del sottobosco l'elemento cardine della ruralità e della multifunzionalità. L'analisi degli indici di specializzazione, infatti, ha confermato l'esistenza di comuni nei quali la vocazionalità nella tartuficoltura appare abbastanza consolidata.

L'applicazione dei modelli statistici ha evidenziato come la tartuficoltura possa rappresentare una variabile utile per valutare la multifunzionalità del territorio e delle aziende agricole cui andrà erogato, trattandosi di una esternalità positiva, un compenso economico congruo che i Programmi di Sviluppo Rurale 2007-2013 non hanno previsto in maniera diretta ed organica.

Per i prossimi anni sarebbe auspicabile una maggiore collaborazione tra l'attività agrituristica e/o la ricettività alberghiera del territorio, al fine di generare un ambiente coeso che si estrinsechi sia nel distretto della montagna che nella istituzionalizzazione della strada del gusto del tartufo, al fine di generare dei percorsi enogastronomici-naturalistici integrati con l'agriturismo, necessari per il rilancio socio-economico delle aree rurali a serio rischio marginalizzazione, come dimostrato dall'incremento della superficie boscata e prativa (estensivizzazione aziendale-agricola), condizione necessaria ma non sufficiente per l'impoverimento socio-economico-agronomico delle aree rurali, cui la tartuficoltura ha cercato di contrastare il trend di marginalizzazione.

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IL CENTRO SPERIMENTALE PER LA TARTUFICOLTURA DI PORTO VIRO (ROVIGO) E LA VALORIZZAZIONE DELLE COLTURE TARTUFIGENE NEL VENETO (ITALIA)

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Abstract: The experimental centre of the mycorrhization and production of mychorrhized plants in Porto Viro (Rovigo) and the valorization of truffle plantation in the Veneto region (Italy)

The protection and the restoration of the natural truffles heritage are important purposes to achieve in our country. The Region of Veneto promoted the L.R. 30/88 to increase the natural truffle productions. Within the limits of the law, the Forest and Regional Service of Padua and Rovigo realized a new Experimental Centre of Mycorrhization and Production of young mycorrhized plants. The greenhouse and laboratory for the investigation of mycorrhizae of the Centre are located in Porto Viro (Rovigo). The Centre produces, sells and uses the small plants in reforestation areas to encourage a wider distribution of tree plants associated with every commercial truffle species into Veneto territory.

The increasing percentage of plants is very important both for ecological-environmental improvement and for economic profits deriving from the cultivation and the valorization of truffle plantations.

The authors reported the production results of mycorrhizations obtained in the hothouse and the greenhouse of the nursery Centre from 2000 to 2007.

They noticed that:

1) The production percentage of mycorrhized plants associated with different species of *Tuber* has increased about 30 points and is maintaining a constant trend till nowadays.
2) The production of mycorrhized plants such as *Quercus robur* L., *Corylus avellana* L. and *Ostrya carpinifolia* Scop. associated with *Tuber borchii* Vittad., *T. brumale* Vittad., *T. melanosporum* Vittad., *(T. aestivum* Vittad.*)* has raised about 5-10 points using spores, seeds, shoots of local ecotypes.

Moreover they also present the growth and production data of plantations with *Tuber melanosporum* Vittad. planted out in four different experimental truffle fields in the district of Rovigo.

In particular they observed that, after 10-12 years planting, four thick unproductive plants began to produce truffles again after despite they were subjected to cuts and strong prunings.

Key words*:* cultivation, mycorrhized plants, experimental truffle fields.

La tartuficoltura nel Veneto

La salvaguardia e il ripristino del patrimonio naturale tartufigeno sono obiettivi importanti da raggiungere per il nostro territorio. La Regione del Veneto ha promulgato la L.R. 30/88 per la tutela e la riqualificazione del patrimonio tartuficolo esistente sia naturale che coltivato. Nell'ambito di tale legge il Servizio Forestale di Padova e Rovigo ha realizzato un centro sperimentale per la micorrizazione e produzione di piantine micorrizate, al fine di promuovere la diffusione di piantagioni di piante micorrizate con tutte le specie di tartufo commerciabili nel Veneto.

Poiché nella coltivazione dei tartufi è importante porre le piante tartufigene in condizioni

pedoclimatiche e colturali idonee, pur essendo disponibili diverse specie simbionti si è cercato di utilizzare negli impianti le specie arboree che contraggono una simbiosi micorrizica simile a quella che si riscontra allo stato naturale.

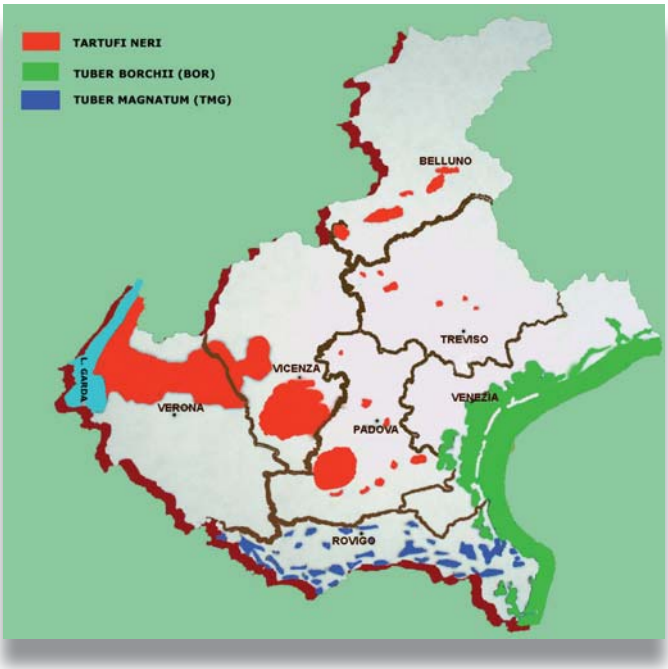


Fig. 1 Aree principali del patrimonio tartufigeno naturale del Veneto

L'indagine del patrimonio naturale tartuficolo del Veneto ha evidenziato che la Regione può essere suddivisa nelle seguenti tre macrozone di vocazione tartufigena (vedi Fig.1) nel cui intorno si consiglia di realizzare gli impianti.
La prima macroarea comprende le province di Rovigo e di Padova nelle quali si raccoglie prevalentemente il *Tuber magnatum* Pico (tartufo bianco d'Alba). Nel rodigino il tartufo si rinviene soprattutto lungo l'asta fluviale del Po, dall'alto Polesine fino all'inizio geografico del Delta Padano. Nell'area patavina si rinviene a sud dei Colli Euganei e nei parchi di antiche ville.
La seconda macrozona che comprende la fascia costiera delle provincie di Rovigo e Venezia è caratterizzata dalla presenza quasi esclusiva del *Tuber borchii* Pico. In essa, le aree naturali tartufigene sono distribuite all'interno o ai margini delle pinete litoranee in aree di duna stabilizzata e fossile.
La terza macrozona comprendente l'area tipica dei tartufi neri in cui si raccolgono tutte le specie commerciabili. Di essa fanno parte le aree veronesi e vicentine nelle quali si rinvengono *Tuber melanosporum* Vittad. cioè le colline moreniche del lago di Garda e le basse pendici del Monte Baldo (Gregori, 1991). Nell'area vanno annoverati anche i Colli Berici, i Monti Lessini e il Monte Baldo che sono vocati al *T. aestivum* Vittad.. La parte di territorio regionale dei tartufi neri comprende poi le zone trevigiane pedemontane confinanti con la provincia di Vicenza fino a Maser e quelle confinanti con la provincia di Venezia in cui si ritrova la specie *Tuber brumale* Vittad. Nella provincia di Belluno le specie frequentemente segnalate sono *T. aestivum* Vittad., *T. brumale* Vittad., *T. uncinatum* Chatin, *T. mesentericum* Vittad. e T. *macrosporum* Vittad..
I ritrovamenti più consistenti sono stati registrati nei comuni dislocati più a sud in particolare Arsiè, Feltre, Lentiai, Cesiomaggiore, Sedico e Belluno.
L'avvio di politiche, che puntino all'incremento degli impianti artificiali tartuficoli è nelle priorità dell'amministrazione regionale, infatti negli ultimi tre anni ha riattivato il relativo capitolo di bilancio. Naturalmente la pubblica amministrazione persegue finalità più generali incrementando la produzione di piante arboree micorrizate da utilizzare non solo per la tartuficoltura ma anche nelle opere estensive di miglioramento e sistemazione idraulico-forestale ai sensi della L.R. del Veneto 52/78.

Il Centro sperimentale per la tartuficoltura di Porto Viro

Il Servizio Forestale Regionale di Padova e Rovigo gestisce da circa quindici anni il Centro Sperimentale per la Tartuficoltura di Porto Viro (RO) (Fig. 2), tramite personale specializzato presso il Centro Studi per la Micologia del terreno del CNR di Torino e il Centro di Ricerca sul Tartufo di S. Angelo in Vado (PU).

Fig. 2 Il Centro sperimentale per la tartuficoltura di Porto Viro

Si tratta dell'unica struttura pubblica presente in Veneto nel settore. Usufruisce di appositi finanziamenti regionali che garantiscono gli attuali livelli produttivi.

L'obiettivo della politica regionale nel settore della tartuficoltura è di diffondere, attraverso il Centro di Porto Viro, l'utilizzo delle piantine micorrizate sia nelle opere di rimboschimento e che nelle aree marginali. La valorizzazione di queste ultime deriverebbe dal fatto che, al materiale di propagazione micorrizato è riconosciuto un valore ecologico aggiuntivo in termini di capacità di accrescimento e di arricchimento biologico dei suoli.

Fig. 3 Serra riscaldata di micorrizazione (interno)

Il Centro svolge l'attività vivaistica di coltivazione delle piante tartufigene destinate alla vendita per la realizzazione di tartufaie coltivate da parte di soggetti privati e per il ripristino delle aree a vocazione tartufigena degradate, mediante la gestione di tartufaie controllate. Esso può svolgere attività di divulgazione e assistenza tecnica per tutti gli acquirenti di piante prodotte dal Centro. La struttura di Porto Viro gestisce inoltre tartufaie coltivate attraverso contratti e convenzioni appositamente stipulate con i proprietari.

Fig. 4 Serra riscaldata di micorrizazione (esterno)

Fig. 5 Tunnel di allevamento delle giovani piante (esterno)

Fig. 6 Il controllo dei lotti micorrizati nel Tunnel di allevamento

Per le micorrizazioni e per l'allevamento delle piante tartufigene ci si avvale rispettivamente di una serra riscaldata (Fig. 3 e fig. 4) e di un tunnel (Fig. 5 e fig. 6).
Il Centro produce attualmente circa 6000 piantine annue. Le quantità limitate consentono il controllo accurato di ogni singola pianta così da garantire un'elevata percentuale di micorrizazione degli apparati radicali (Fig. 7).

Fig. 7 Il controllo degli apici radicali in laboratorio

Il relativo esiguo numero di piante prodotte rimane molto inferiore rispetto alle potenzialità della struttura, giacché le richieste finora avanzate rimangono ancora numericamente inferiori rispetto alla produzione offerta. (Fig. 8)

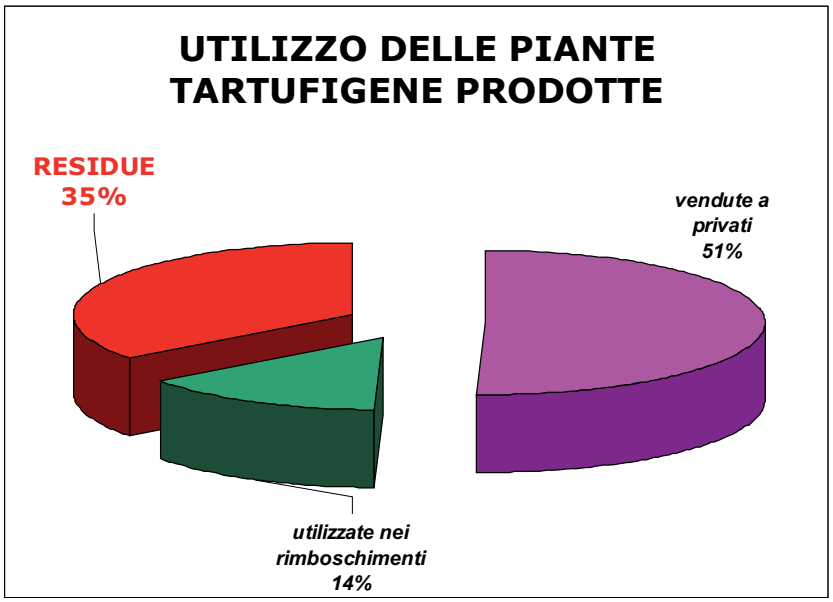


Fig. 8 Utilizzo delle piante tartufigene prodotte

La produzione di piante micorrizate
Gli autori riportano i risultati ottenuti dal 2000 al 2007 relativi alla produzione di piante micorrizate idonee alla messa a dimora.
Le metodiche standardizzate nel Centro per l'ottenimento della simbiosi micorrizica sono essenzialmente due: l'inoculazione della semente tramite sospensione sporale (*metodo Mannozzi-Torini, 1970*), e l'inoculazione di giovani piante per contatto tra il loro apparato radicale e quello micorrizato di una pianta madre (*metodo per Trasmissione radicale*).
Tra le angiosperme tartuficole il Centro produce in particolare le latifoglie arboree delle specie

Quercus robur L., *Q. pubescens* Willd., *Q. cerris* L., *Quercus petraea* (Matt.) Liebl, *Tilia cordata* Miller, *Carpinus betulus* L. associate a *Tuber borchii* Vittad., *T. brumale* Vittad., *T. melanosporum* Vittad., *T. aestivum* Vittad., *T. mesentericum* Vittad. e *T. macrosporum* Vittad..
Tra le arbustive invece si ottiene l'essenza *Corylus avellana* L. associata a tutte le specie precedenti eccetto il tartufo liscio e il bianchetto e la specie *Ostrya carpinifolia* Scop. micorrizata con lo scorzone estivo e invernale (*T. aestivum* Vittad. e T. *uncinatum* Chatin).
Tra le sempreverdi tartuficole si producono inoltre *Quercus ilex* L., *Pinus pinea* L. e *Pinus pinaster* Aiton associate a *T. borchii* Vittad. (Tab. 1)

SPECIE TARTUFICOLE PRODOTTE

	ALBERI E ARBUSTI SIMBIONTI	TUBER INOCULATO						
		Tartufo nero pregiato (Tuber melanosporum Vitt.)	Tartufo scorzone (Tuber aestivum Vitt.)	Tartufo uncinato (Tuber uncinatum Chat.)	Tartufo brumale (Tuber brumale Vitt.)	Tartufo mesentericum (Tuber mesentericum Vitt.)	Tartufo macrosporum (Tuber macrosporum Vitt.)	Tartufo bianchetto (Tuber borchii Vitt.)
LATIFOGLIE ANGIOSPERME	Corylus avellana (nocciolo)	*	*	*	*	*		
	Ostrya carpinifolia (carpino nero)		*	*				
	Carpinus betulus (carpino bianco)						*	
	Quercus cerris (cerro)	*	*					
	Quercus petraea (rovere)	*	*		*			*
	Quercus pubescens (roverella)	*	*		*		*	*
	Quercus robur (farnia)	*	*		*			*
	Tilia cordata (tiglio selvatico)	*			*		*	
ALTRE	Quercus ilex (leccio)							*
	Pinus pinaster (pino marittimo)							*
	Pinus pinea (pino domestico)							*

Tab. 1 Piante prodotte nel Centro Sperimentale

La produzione è cominciata all'inizio del '90. In quegli anni si inoculavano semi e piante acquistate anche da fornitori che non erano in grado di certificare l'esatta provenienza del materiale vegetale messo in vendita. Pure l'impiego dei substrati era d'incerta provenienza, anche se si utilizzavano terreni rispondenti ai parametri ottimali per la crescita delle plantule.
Infine i miscugli di tartufo utilizzati nelle sospensioni sporali degli inoculi, provenendo da varie regioni d'Italia, si diversificavano nel germoplasma e quindi non davano sempre la stessa probabilità di successo ai lotti omogenei sottoposti a verifica della micorrizazione.
Durante i primi anni la sperimentazione della produzione di piante tartufigene dava risultati piuttosto deludenti. Le piante micorrizate dichiarate idonee secondo il "*Metodo di valutazione delle piante micorrizate con funghi del genere Tuber basato sulla caratterizzazione morfologica delle micorrize (12/06/1995)*", erano in media circa il 35% di quelle inoculate. La percentuale citata è sensibilmente migliorata in seguito ai vari accorgimenti, di seguito descritti, attuati a partire dagli anni 2000-2001.
In primo luogo si è provveduto all'accurata selezione delle simbionti. La scelta delle specie botaniche è stata orientata esclusivamente sull'utilizzo di materiale autoctono e proveniente da aeree boscate naturali delle zone venete, direttamente raccolte dagli operatori del Centro.
In secondo luogo si è deciso di allestire i substrati su cui allevare le piantine micorrizate

prelevando la terra o dalle tartufaie naturali, o da tartufaie coltivate produttive, o da zone vicine alle une o alle altre per accertarsi della sicura idoneità dal punto di vista chimico e fisico.
Infine per ciò che riguarda la scelta del materiale fungino ci si è orientati sull'acquisto di carpofori forniti dai raccoglitori locali delle aree tartufigene naturali venete.
Con il nuovo modo di operare la percentuale di piante che ha superato l'esame durante la fase di controllo è stata duplicata. Dal 2002 la produzione è rimasta stabile aggirandosi intorno a valori compresi tra il 63,6% del 2002 e il 79,1% del 2005. La flessione del 2003, anno in cui la produzione si è arrestata al 13,3%, è da ricercarsi a problemi legati alla difficoltà, in seguito superata, di controllare l'elevata temperatura raggiunta nella nuova serra di micorrizazione.
Il grafico della fig. 9 rivela proprio come la produzione sia aumentata di diversi punti percentuali grazie all'utilizzo di spore, semi e germogli autoctoni e al nuovo sistema di allestimento dei terreni (63,5% - media ponderata).

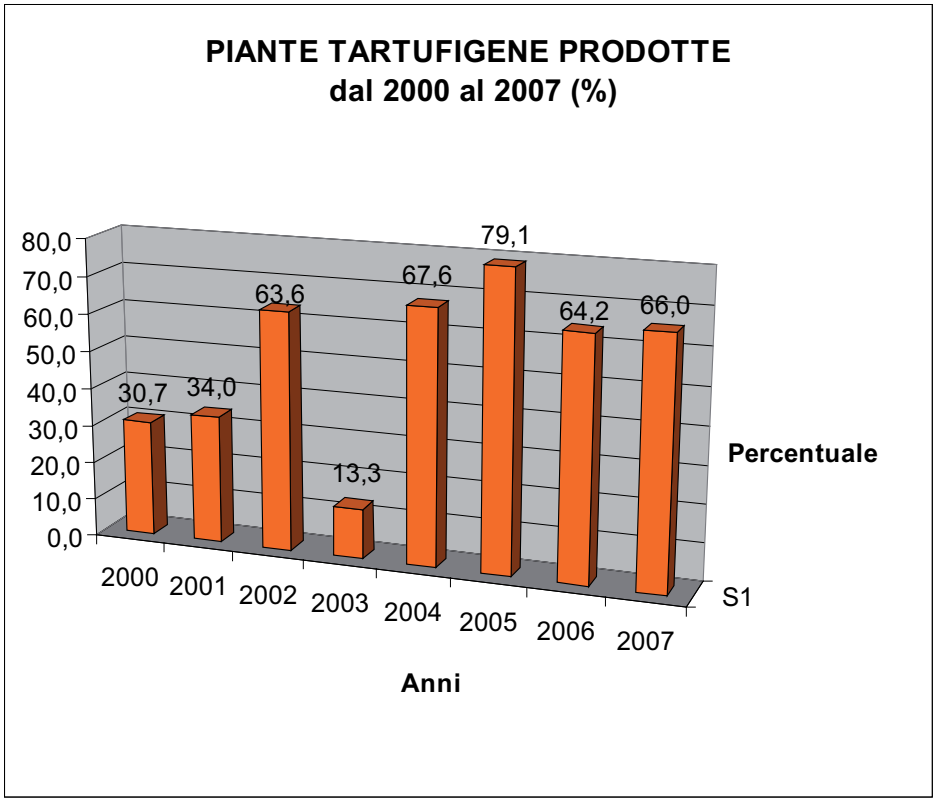


Fig. 9 Grafico della produzione di piante tartufigene dal 2000 al 2007

La gestione delle quattro tartufaie coltivate sperimentali: RO-6; RO-7; RO-8; RO-9
Fin dai primi tempi, oltre alla produzione di piante micorrizate destinate alla realizzazione di nuove tartufaie, ci si è occupati della manutenzione e gestione di impianti già presenti sul territorio. Si riportano i risultati della gestione dei quattro campi sperimentali coltivati in provincia di Rovigo allo scopo di mostrare le possibili tecniche colturali utili a favorire e ottimizzare la produzione di tartufi. Di codesti impianti si riportano di seguito i dati di localizzazione, composizione, gestione e quelli sulle lavorazioni degli impianti.
La gestione degli impianti è stata affidata al Servizio Forestale Regionale per le province di PD-RO secondo le modalità previste da contratti di comodato, da forme legali di sottomissione e dall'esecuzione di progetti redatti nell'ambito di specifiche misure di finanziamento regionale e comunitario (Regolamento CEE 2078/92 e PPSSRR), che i proprietari dei terreni hanno stipulato con la Regione del Veneto. I cicli di gestione e monitoraggio pattuiti hanno durata decennale o quinquennale e sono eventualmente rinnovabili. Alcuni proprietari dei fondi si sono impegnati a seguire le indicazioni colturali impartite dai tecnici del Servizio Forestale anche successivamente la scadenza delle convenzioni.

Impianto sperimentale Fratta RO-6

La tartufaia è localizzata nel comune di Fratta Polesine (RO) in area pianeggiante a valle dell'argine maestro del Canal Bianco. L'impianto è stato realizzato dal Servizio Forestale nel 1991 su una superficie di 18.000 mq. E' costituita da una piantumazione di roverelle e noccioli micorrizate con *Tuber melanosporum* che circonda su tre lati una seconda piantagione caratterizzata da cerro, farnia e nocciolo micorrizate con *Tuber magnatum*. Tali piante tartuficole sono state acquistate da produttori del Centro Italia. Il sesto di impianto (5X4), nonostante sia adeguato più per il *T. magnatum* che per il *T. melanosporum* è stato scelto in via sperimentale.

La tartufaia è stata seguita dal momento della piantumazione fino al 10° anno. Dall'undicesimo anno (2002) la Regione Veneto ha seguito l'impianto raccogliendo unicamente i dati relativi alla produzione.

Nella Tab. 2 sono riassunte le cure colturali eseguite. Durante il primo decennio è iniziato il ripristino delle fallanze (primo anno). Le irrigazioni di soccorso, i controlli degli apici radicali e i primi sfalci delle infestanti sono stati implementati il 2°-3° anno. Il terzo anno sono state praticate le potature d'impostazione, le spollonature e sramature proseguite poi negli anni successivi.

Dal 2002 la tartufaia è lasciata alla naturale evoluzione.

TARTUFAIA -RO-6

6.a	SITO:	PIANURA
6.b	LOCALITA':	FRATTA POLESINE
6.c	ANNO:	1991
6.d	SESTO:	M. 4X5
6.e	SUPERFICIE:	HA 01.80.00
		NOCCIOLO,CERRO, ROVERELLA, FARNIA
6. f	SIMBIONTE:	(900)
6.g	TARTUFO:	TML - TMG

INTERVENTI	ANNO										
	91	92	93	94	95	96	97	98	99	00	01
ETA' TARTUFAIA		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
SOSTITUZIONE FALLANZE		X			X						
DISERBO MECCANICO		X	X	X	X	X	X	X	X	X	X
POTATURA-SPOLLONATURA			X	X	X	X	X	X	X	X	X
IRRIGAZIONE DI SOCCORSO		X	X								
FITOSANITARI											
CONTROLLO APICI RADICALI			X	X							

Tab. 2 Impianto sperimentale RO-6 di Fratta Polesine

Impianto sperimentale Adria RO-7

La tartufaia è localizzata nel comune di Adria (RO) in area pianeggiante nei pressi del Canal Bianco su terreni precedentemente coltivati a mais. E' stata realizzata direttamente dal proprietario nel 1986 su di una superficie di 2000 mq ed è costituita da piante di roverella e cerro micorrizate con *Tuber melanosporum* e disposte in un sesto quadrato di 2,5 m di lato.
La tartufaia è stata seguita dal 1991 (5° anno) fino al 2000 (14° anno).
Nella Tab. 3 sono riassunte le cure colturali eseguite. Inizialmente su diverse piante sono state prelevate alcune porzioni di radice per verificare il tipo di micorrize presenti. Oltre alle stagionali operazioni di sfalcio delle infestanti, sono stati operati alcuni interventi di diradamento e potatura su piante non produttive e poco produttive (8°-9° anno).
Dal 2000 la tartufaia è lasciata alla naturale evoluzione.

TARTUFAIA -RO-7

7.a	SITO:	PIANURA
7.b	LOCALITA':	ADRIA
7.c	ANNO:	1986
7.d	SESTO:	M. 3X3
7.e	SUPERFICIE:	HA 0,2.00.00
7. f	SIMBIONTE:	CERRO (100), ROVERELLA (100)
7.g	TARTUFO:	TML

INTERVENTI	ANNO									
	91	92	93	94	95	96	97	98	99	00
ETA' TARTUFAIA	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
SOSTITUZIONE FALLANZE										
DISERBO MECCANICO	X	X	X	X	X	X	X	X	X	X
POTATURA				X	X					
IRRIGAZIONE DI SOCCORSO										
FITOSANITARI										
CONTROLLO APICI RADICALI	X									

Tab. 3 Impianto sperimentale RO-7 di Adria

Impianto sperimentale Adria RO-8

La tartufaia è localizzata nel comune di Adria (RO) in area pianeggiante vicino all'argine sinistro del Canal Bianco. L'impianto è stato realizzato dal conduttore nel 1995 su un ex terreno agrario di 5.000 mq con un sesto quadrato (3X3). La tartufaia è collocata in un territorio legato tradizionalmente alle coltivazioni agricole a ciclo annuale e di cui si ignorava la vocazione tartuficola; contrariamente alle aspettative il sito si è rivelato particolarmente idoneo.

E' costituita da una piantumazione di roverelle micorrizate con *Tuber melanosporum* e da un filare di piante di Pino marittimo micorrizate con *Tuber borchii*. Tali piante tartuficole sono state acquistate da una ditta produttrice del Veneto. La tartufaia è stata seguita dal 2000 a tutt'oggi, in forza di un rapporto di collaborazione di natura scientifica.

Nella Tab. 4 sono riassunte le cure colturali svolte dal proprietario su indicazione dei tecnici del Servizio Forestale.

Sono stati effettuati numerosi controlli sulle micorrize per verificare l'effettiva specie micorrizata. Sono state impartite indicazioni precise su sfalci, potature e diradamenti ponendo l'attenzione sulle modalità e la periodicità con cui devono essere attuati. Il proprietario ha eseguito periodicamente il diserbo meccanico soltanto in una direzione dell'interfilare e nell'anno 2003 importanti interventi di potatura sulla quasi totalità delle piante. Il diradamento consigliato non è invece ancora stato attuato.

Il Servizio Forestale ha inoltre provveduto alla raccolta del prodotto dalla stagione 2000/01 a ora.

TARTUFAIA -RO-8

8.a SITO: PIANURA
8.b LOCALITA': ADRIA
8.c ANNO: 1995
8.d SESTO: M. 3X3
8.e SUPERFICIE: HA 05.00.00
8. f SIMBIONTE: ROVERELLA (478), PINO MARITTIMO
8.g TARTUFO: TML

INTERVENTI	ANNO												
	96	97	99	99	00	01	02	03	04	05	06	07	08
ETA' TARTUFAIA	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
SOSTITUZIONE FALLANZE													
DISERBO MECCANICO INTERFILARE PARZIALE (INDICAZIONI)					X	X	X	X	X	X	X	X	X
POTATURA (INDICAZIONI)								X					
IRRIGAZIONE DI SOCCORSO													
FITOSANITARI													
RICERCA E RACCOLTA TUBER						X	X	X	X	X	X	X	X
CONTROLLO APICI RADICALI						X							

Tab. 4 Impianto sperimentale RO-8 di Adria

Impianto sperimentale Polesella RO-9

La tartufaia è localizzata nel comune di Polesella (RO) in area pianeggiante vicino all'argine sinistro del Po. L'impianto è stato realizzato dal conduttore nel 1994 su una superficie di circa 4 ettari su terreni precedentemente coltivati; presenta un'alternanza di piante di roverelle e noccioli micorrizate con *Tuber melanosporum* disposte in un sesto a quin-quonce.

Tali piante tartuficole sono state acquistate da una ditta produttrice dell'Emilia Romagna. La tartufaia è stata gestita fino al 2006 dal proprietario e da allora è oggetto di interventi selvicolturali progettati dal Servizio Forestale nell'ambito di un atto di sottomissione sottoscritto dal proprietario a favore del Servizio Forestale stesso per interventi di manutenzione ordinaria e controllo dell'impianto.

Nella Tab. 5 sono riassunte le cure colturali eseguite sia dal proprietario che dal Servizio Forestale.

Gli interventi colturali più significativi consistono nei diserbi meccanici iniziati al quinto anno dalla messa a dimora delle piante. Le potature sono iniziate nel 2006 interessando dapprima le querce e negli anni successivi anche i noccioli.

Sono stati prelevati numerosi apici radicali per la verifica delle micorrize.

Il Servizio Forestale provvede alla ricerca del prodotto dal 2002 e ha raccolto i primi carpofori nel 2006. La tartufaia è meta delle escursioni didattiche degli studenti dei corsi di Micologia e Tartuficoltura dell'Università di Padova - Facoltà di Agraria.

TARTUFAIA -RO-9

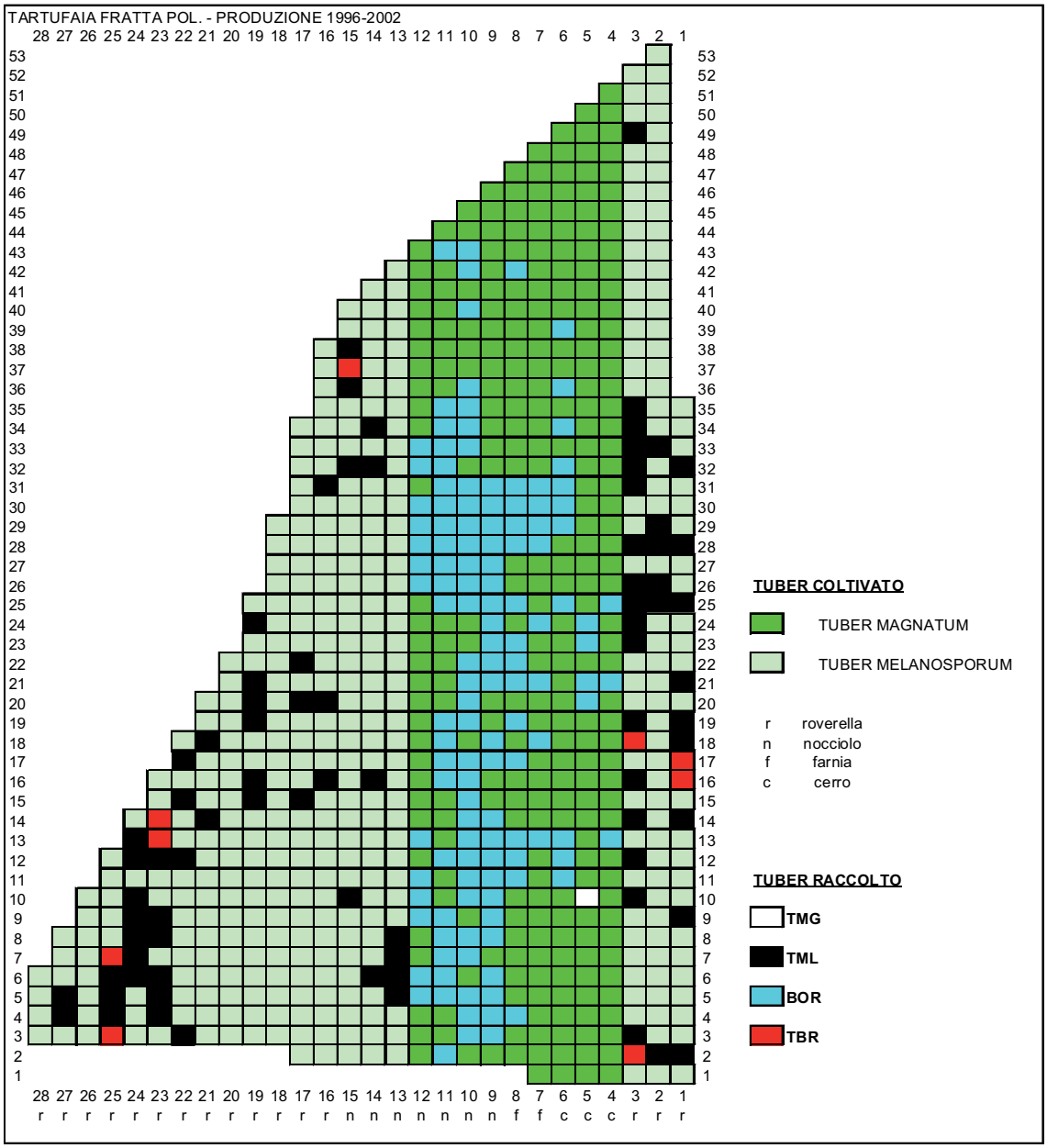
9.a	SITO:	PIANURA
9.b	LOCALITA':	POLESELLA
9.c	ANNO:	1994
9.d	SESTO:	M. 6X6
9.e	SUPERFICIE:	HA 04.05.85
9. f	SIMBIONTE:	ROVERELLA (190), NOCCIOLO (168)
9.g	TARTUFO:	TML

INTERVENTI	ANNO									
	99	00	01	02	03	04	05	06	07	08
ETA' TARTUFAIA	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
SOSTITUZIONE FALLANZE										
DISERBO MECCANICO	X	X	X	X	X	X	X	X	X	X
POTATURA								X	X	X
IRRIGAZIONE DI SOCCORSO										
FITOSANITARI										
RICERCA E RACCOLTA TUBER				X	X	X	X	X	X	X
DIDATTICA									X	X
CONTROLLO APICI RADICALI						X			X	

Tab. 5 Impianto sperimentale RO-9 di Polesella

Risultati
Si illustrano i dati qualitativi e quantitativi sulle produzioni delle quattro tartufaie coltivate sperimentali descritte precedentemente e seguite negli anni dal Centro Sperimentale per la Tartuficoltura del Servizio Forestale.

Effetti degli interventi selvicolturali sulla produzione -Impianto RO-6-
Osservando lo schema delle aree produttive riportate nella Tab. 6, si nota che nella zona della tartufaia coltivata a nero pregiato oltre alla raccolta di carpofori di *Tuber melanosporum* (TML) sono stati rinvenuti anche tartufi di *T. brumale* (TBR). Nell'area piantumata a bianco pregiato solo un piccolo numero di piante ha prodotto *T. magnatum* (TMG) mentre nella maggioranza delle rimanenti arboree si è raccolto solo *T. borchii* in abbondante quantità (BOR).
L'improduttività della parte coltivata a *T. magnatum* è legata al fatto di aver utilizzato piante poco o affatto micorrizate, cosa accertata solo in seguito dopo i controlli eseguiti sugli apparati radicali. Si ricorda che negli anni in cui l'impianto è stato realizzato gravava non solo l'incertezza sul riconoscimento e sulla determinazione delle micorrize del *T. magnatum* ma anche la grande difficoltà di ottenere piante inoculate con tale specie pregiata da parte dei vivai (Granetti *et al*., 2005).
I primi carpofori di *T. melanosporum* sono stati raccolti il quinto anno, nel 1996 e il loro ritrovamento è proseguito costantemente fino al 2002.
Nei dieci anni di monitoraggio oltre la raccolta di *T. melanosporum* si evidenzia quella di *T. brumale*. Pur essendo difficile stabilire i motivi del ritrovamento della specie meno pregiata, condividiamo il parere di altri autori nel ritenere che ciò sia attribuibile alla presenza di alcuni corpi fruttiferi di *Tuber brumale* sfuggiti al controllo durante la fase di micorrizazione (Vezzola, 2004).



Tab. 6 Schema delle aree produttive dell'impianto sperimentale RO-6

Nei grafici della fig. 10 si nota come la produzione delle due specie di *Tuber*, iniziata nel 1996, sia proseguita fino al 2002 in linea con le produzioni nazionali. Successivamente a quella data la produzione è calata in maniera progressiva fino a diventare così trascurabile da rendere la tartufaia improduttiva.

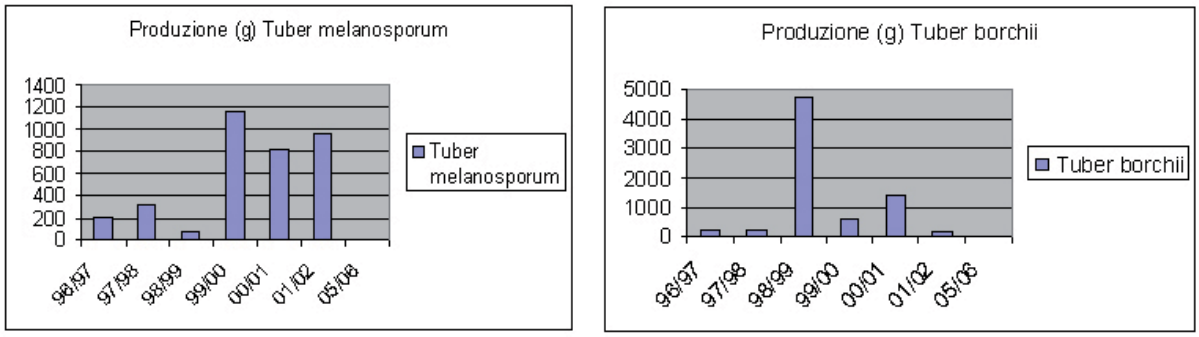
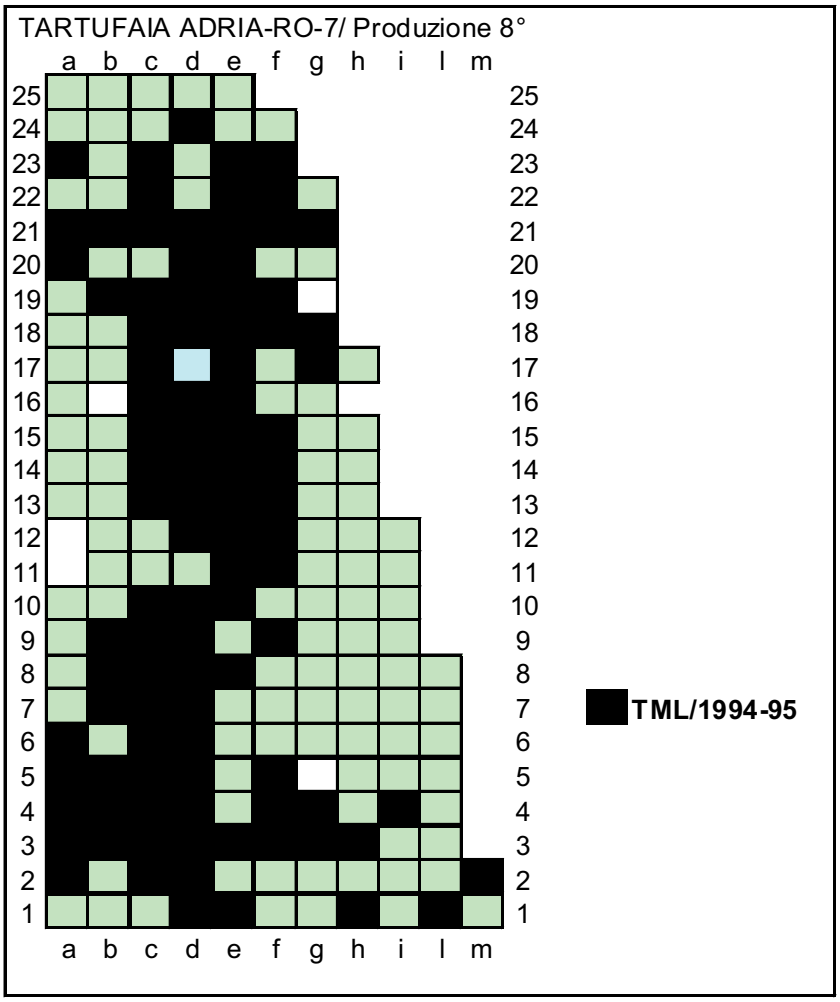


Fig. 10 Produzione dell'impianto sperimentale RO-6 nei due settori

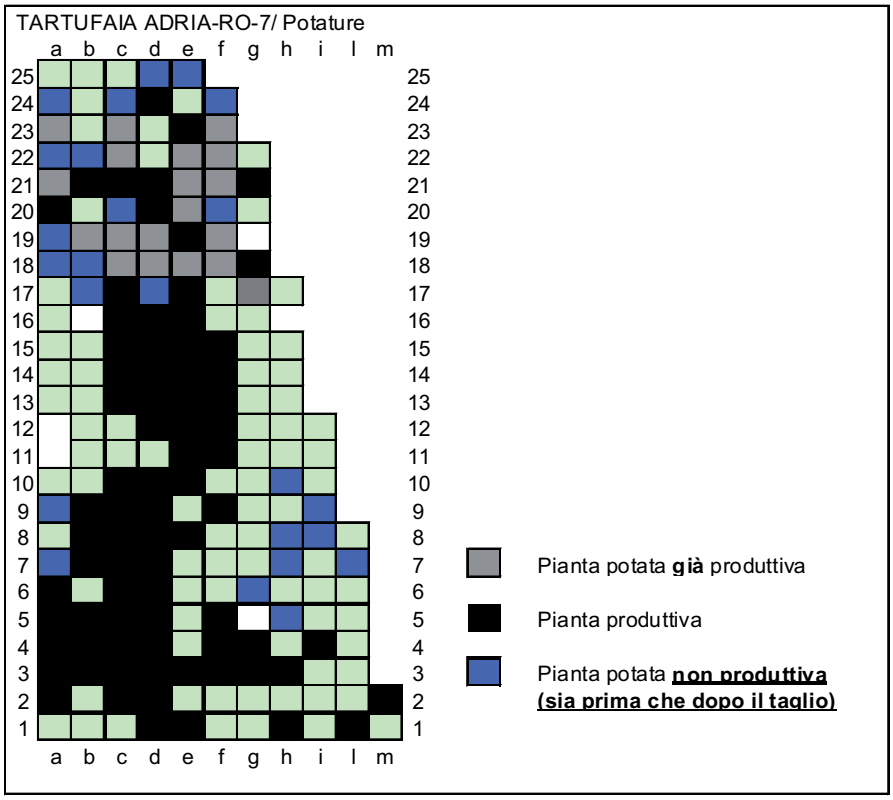
In questo caso, come in altri da noi osservati (qui non riportati) il calo e il successivo arresto della produzione è da attribuire all'abbandono dell'impianto piuttosto che all'esaurimento fisiologico delle piante simbionti data la giovane vita della tartufaia.
Lo stato di abbandono, provocando infatti un eccessivo deposito di materia organica e di infoltimento delle simbionti modifica le condizioni ideali di fruttificazione che erano state mantenute artificialmente, con le cure gestionali praticate fino al 1999.

Effetti degli interventi selvicolturali sulla produzione -Impianto RO-7-
La tartufaia ha iniziato a produrre prima del settimo anno e ha raggiunto livelli notevoli di produzione nell'ottavo anno soprattutto nei suoi settori settentrionale e meridionale, vedi Tab. 7.



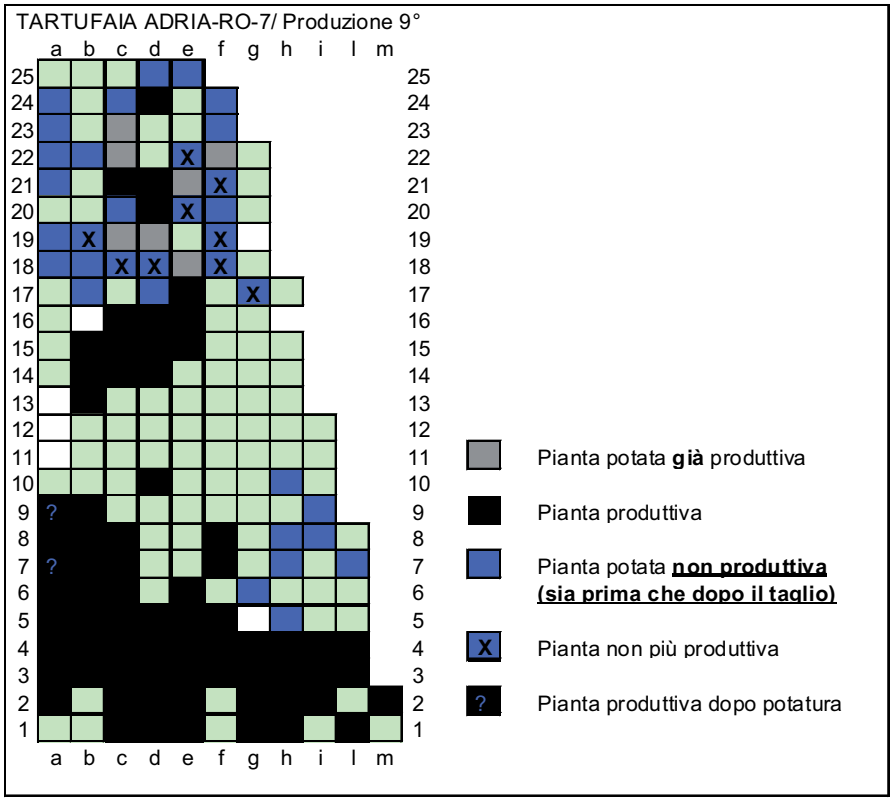
Tab. 7 Schema delle aree produttive concentrate nel settore nord e sud dell'impianto sperimentale RO-7 all'8° anno

La quantità di tartufi raccolta ha subito un progressivo decremento a causa dell'eccessiva copertura e quindi della mancanza di luce e arieggiamento del terreno. Per ricreare le condizioni ottimali è stato necessario intervenire correggendo il sesto d'impianto.
Si è deciso di effettuare un intervento sperimentale consistente in potature e diradamenti operato solo sull'area più settentrionale della tartufaia coltivata, lasciando invece la parte meridionale alla libera evoluzione. Nella Tab. 8 si vede inoltre che le potature hanno interessato sia le piante improduttive che quelle scarsamente produttive.

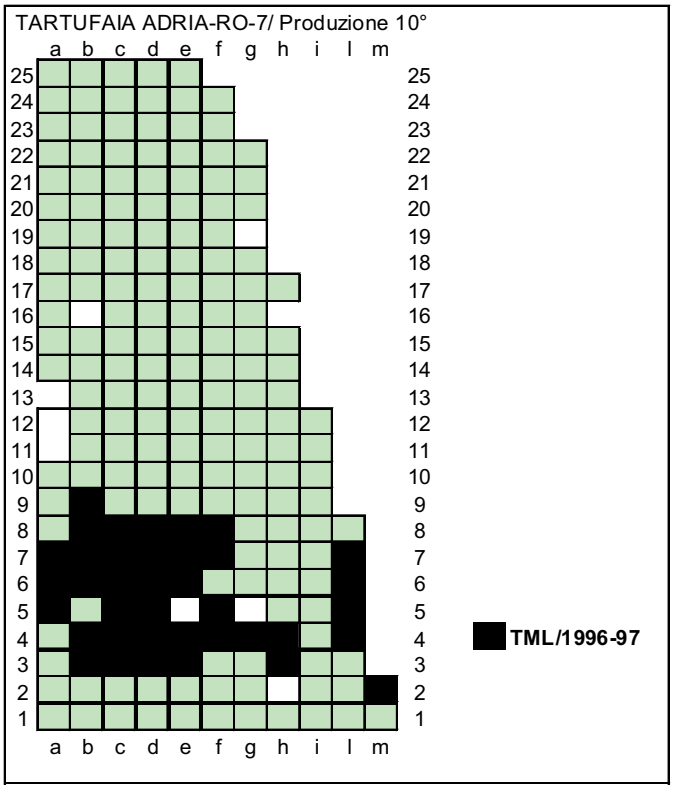


Tab. 8 Schema dell'area dell'impianto sperimentale RO-7 all'8° anno sottoposta ad intervento

Negli anni immediatamente successivi all'intervento si è assistito alla flessione della produzione fino al suo completo arresto (vedi Tabb. 9 e 10).

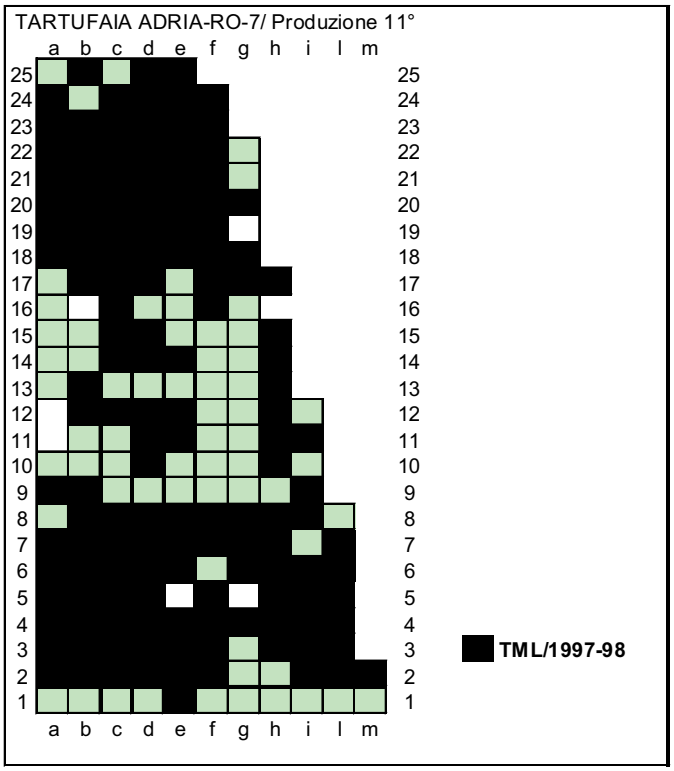


Tab. 9 Reazione di iniziale flessione della fruttificazione dell'impianto sperimentale RO-7 al 9°anno (in seguito all'intervento)



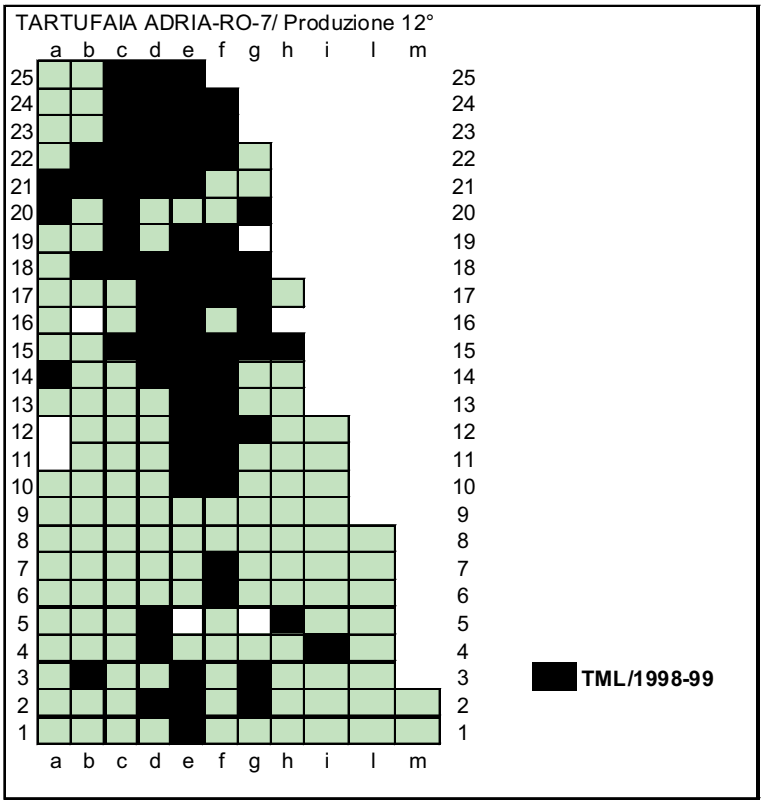
Tab. 10 Reazione di totale arresto della fruttificazione dell'impianto sperimentale RO-7 al 10°anno (a due anni dall'intervento)

Nella Tab. 11 (vedi settore nord), è schematizzata la ripresa della produzione dopo tre anni dall'intervento. Oltre alle querce potate hanno iniziato a produrre tartufi anche le piante che fino a quel momento (11° anno) risultavano non produttive.



Tab. 11 Ripresa della produzione del settore nord (dopo tre anni dalla potatura)

Mentre il settore settentrionale, negli anni successivi all'intervento, ha subito un incremento della produzione, il settore meridionale ha avuto una diversa evoluzione. Le piante concentrate nelle aree meridionali sono andate incontro ad una diminuita produzione fino alla completa cessazione (Vedi Tab.12).



Tab. 12 Decremento della produzione nel settore meridionale (non sottoposto ad intervento)

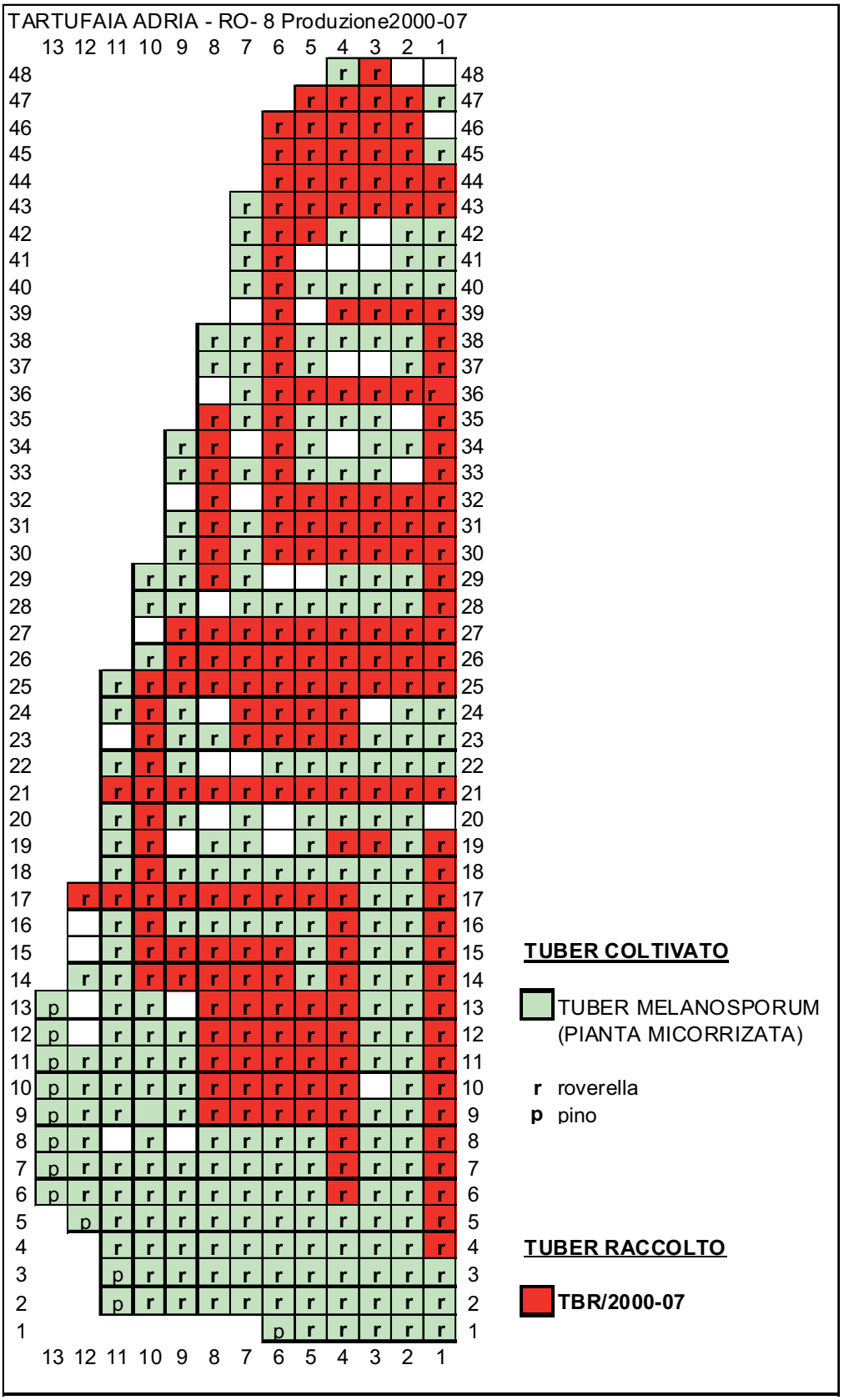
Effetti degli interventi selvicolturali sulla produzione -Impianto RO-8-

La tartufaia è entrata in produzione il sesto anno (2001), quindi abbastanza precocemente. Allo stato attuale le piante produttive sono oltre la metà di quelle messe a dimora (vedi Tab. 13).

Come si evince dal grafico della Fig. 11, le quantità di tartufo raccolto sono progressivamente aumentate raggiungendo 7250 g nella stagione 2007/2008. Il grafico evidenzia pure che la specie raccolta (TBR) sia diversa da quella dichiarata in fase di scelta della coltivazione (TML).

La produzione di brumale degli anni successivi al 2001 conferma le aspettative, poiché nonostante le piante di roverella fossero state dichiarate micorrizate con *T. melanosporum*, l'esame delle micorrize post-impianto (effettuato sin dal 2001) aveva registrato la presenza di *T. brumale* su quasi tutte le piante analizzate. Il fatto di non aver raccolto nero pregiato è attribuibile alla probabile errata micorrizazione iniziale dovuta all'utilizzo di carpofori di *Tuber brumale* nell'inoculazione effettuata nel vivaio di provenienza.

La produzione sta registrando a tutt'oggi un incremento decisivo e continuo grazie agli interventi di potatura eseguiti su tutto l'impianto dal proprietario su indicazione dei tecnici del Centro.



Tab. 13 Piante produttive dell'Impianto sperimentale RO-8

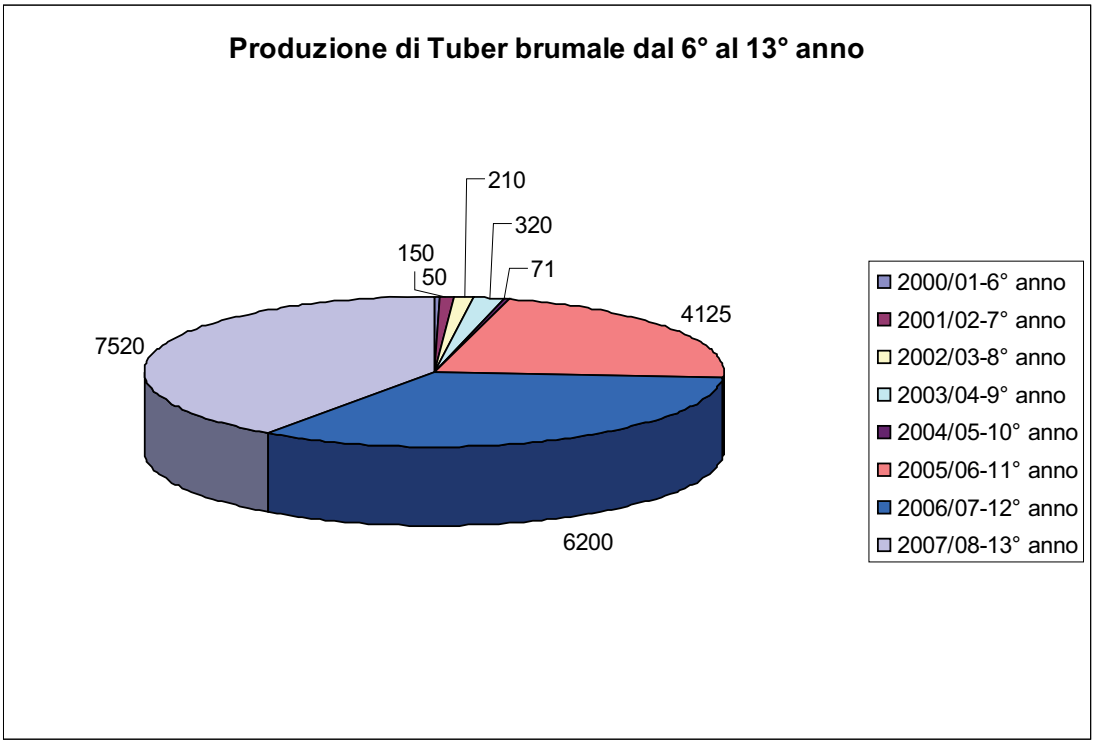


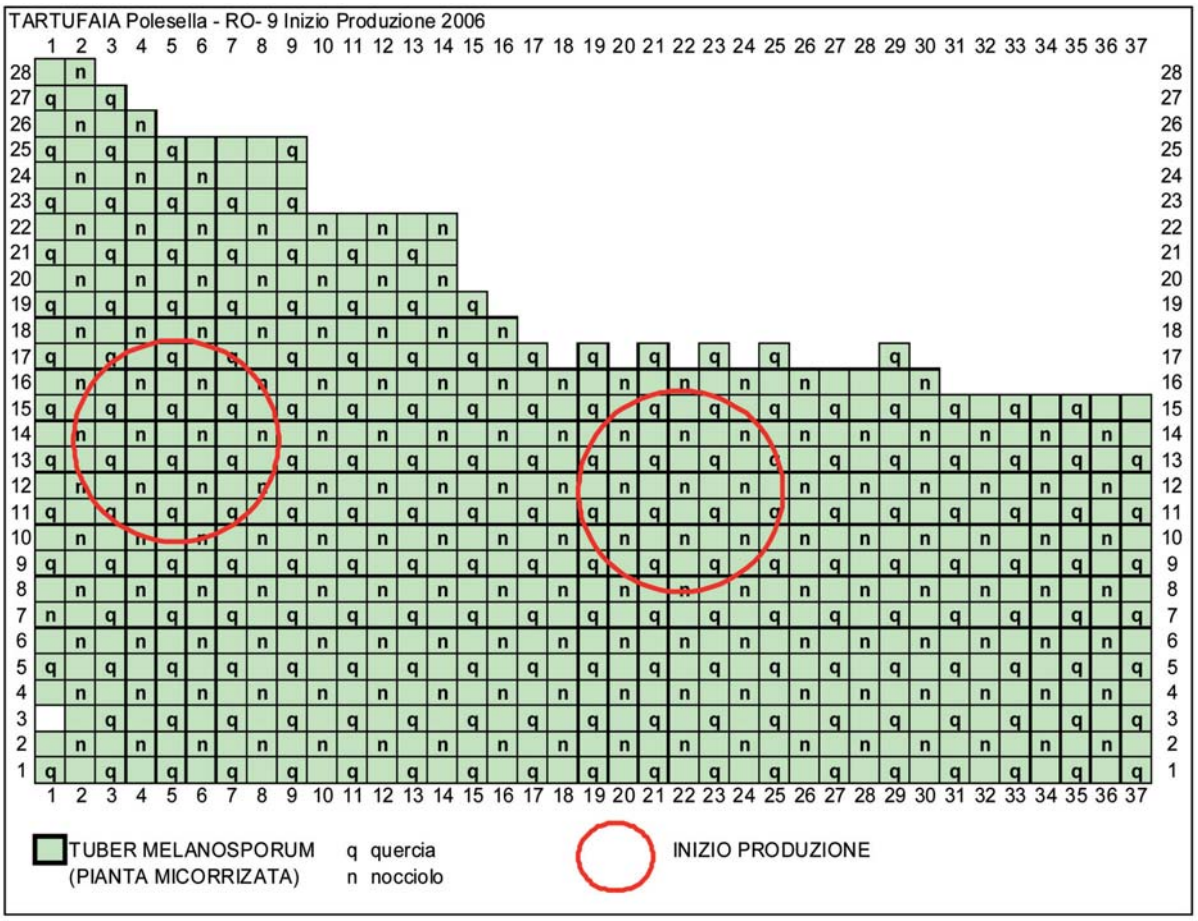
Fig. 11 Produzione dell'Impianto sperimentale –RO-8

Le qualità organolettiche del prodotto raccolto tuttavia in questa tartufaia non sono apprezzabili probabilmente anche a causa della mancata eliminazione delle infestanti e della scarsa lavorazione del terreno attorno alle piante. L'implementazione di lavorazioni finalizzate all'ottenimento di un prodotto di maggior qualità sarà sperimentata nei prossimi futuri interventi.

Effetti degli interventi selvicolturali sulla produzione -Impianto RO-9-

Questo impianto, rimasto improduttivo fino al 12° anno dalla messa a dimora delle piante, non era mai stato sottoposto ad alcun intervento selvicolturale. Constatata la presenza di micorrize di *T. melanosporum* in seguito alle analisi degli apici radicali effettuata nel 2004, il Servizio Forestale ha deciso di prendere in gestione l'impianto. Dal 2006, anno in cui il Servizio Forestale ha iniziato a gestire l'impianto, è stato implementato un programma di recupero della tartufaia attraverso la progettazione di una serie di interventi consistenti in lavorazioni del terreno e decise potature sull'intera piantagione. La prima fase progettuale, già conclusa nello stesso anno 2006, è consistita nella potatura delle querce. L'intervento è stato realizzato eliminando tutti i rami prossimi al suolo, le ramificazioni più grosse e talvolta quelle principali di tutte le arboree, conferendo loro un aspetto slanciato e armonico.

La seconda fase iniziata nel 2008 e non ancora conclusa è consistita nella progettazione degli intereventi sui noccioli. Le potature dei noccioli hanno portato all'eliminazione decisa della maggior parte dei polloni conferendo alla pianta il classico aspetto di cono rovesciato.



Tab. 14 Aree di raccolta primi carpofori nell'Impianto sperimentale RO-9

Gli interventi sono stati mirati a creare le condizioni ecologiche di minor ombreggiamento e buona insolazione ideali per la fruttificazione del tartufo nero pregiato.
A tre anni dalla potatura delle querce (2008) la tartufaia ha iniziato a rispondere positivamente. Nelle due aree indicate nella tab. 14, è stato possibile infatti raccogliere i primi carpofori di piccole dimensioni dopo 14 anni dalla messa a dimore delle piante.
La progettazione degli interventi futura prevede oltre alla chiusura dei cicli di potatura dei noccioli anche delle lavorazioni meccaniche mirate a rendere il suolo più soffice in modo da favorire l'ossigenazione degli apparati radicali.

Conclusioni

L'esperienza quindicinale maturata dal Centro Sperimentale per la Tartuficoltura di Porto Viro del Servizio Forestale di Padova e Rovigo, è un contributo utile al fine di approfondire le conoscenze nei settori della vivaistica delle piante micorrizate, della coltivazione del tartufo nel territorio regionale e delle applicazioni della tartuficoltura nelle politiche di conservazione ambientale.
Per quanto concerne la produzione vivaistica, sono stati discussi i dati che hanno portato alla selezione del materiale vegetale, dei tartufi e dei tipi di terreni da utilizzare nell'allestimento degli inoculi. Si commentano i dati vivaistici che hanno permesso di ottenere piantine micorrizate di elevata qualità da utilizzare sia negli impianti che nei rimboschimenti, delle aree in cui opera l'ente regionale.
Rivelatasi insoddisfacente la consueta prassi di acquistare sementi di provenienza incerta, si è deciso di raccogliere il materiale di propagazione vegetale dopo aver selezionato alcune piante con accentuata capacità tartuficola.
All'acquisto di tartufo di varia provenienza si è preferito il reperimento di carpofori raccolti nelle aree di produzione locali.

Operando in questo modo si ha la certezza di utilizzare materiale vegetale e fungino autoctono ovvero avente le caratteristiche genetiche degli ecotipi rappresentativi delle specifiche aree geografiche venete. Il risultato conseguito è stato quello di ottenere il 63,5% di piante micorrizate sul totale delle inoculate, quasi duplicando una percentuale che era rimasta ferma al 35%, da anni.
Per quanto riguarda la tartuficoltura è stata posta l'attenzione sulle sperimentazioni di alcune cure colturali (come potature e diradamenti) e sulle osservazioni relative alla risposta delle simbionti e del tartufo negli impianti sperimentali di *Tuber melanosporum* gestiti.
E' stato dimostrato che ai fini della produzione di tartufo, nel caso in cui la tartufaia sia giovane sono necessari interventi di potatura d'impostazione precisa per querce e noccioli intervenendo con tagli leggeri e continui anche in piante produttive. Nel caso in cui la tartufaia sia improduttiva o lasciata da tempo alla libera evoluzione sono richiesti interventi decisi come diradamenti e pesanti potature sulla maggior parte dell'apparato subaereo della pianta al fine di recuperare o instaurare le condizioni ecologiche ideali di fruttificazione del tartufo.
Nella nuova progettazione è stata introdotta inoltre la pratica di realizzare i progetti di rimboschimento prevedendo l'utilizzo di piante forestali micorrizate nelle piantumazioni. Ciò è finalizzato alla conservazione della biodiversità, caratterizzata nei popolamenti boschivi naturali, anche dalla presenza di piante tartufigene dato che molte aree della nostra regione sono vocate alla produzione dei tartufi, come storicamente documentato.
Inoltre l'introduzione di piantine ectomicorrizate nelle opere di recupero delle aree marginali, come quelle confinanti con i boschi lungo i pendii della bassa montagna e le superfici agrarie non utilizzabili, implementa modelli di gestione selvicolturale sostenibile, assecondando le potenzialità ecologiche ed economiche di siti destinati altrimenti a un probabile dissesto idrogeologico.

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LA FILIERA DEL TARTUFO E LA SUA VALORIZZAZIONE IN TOSCANA E ABRUZZO

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Abstract: The chain of truffle and the valorization of the product in Toscana and Abruzzo
Through this poster we want to present the multi-regional research project: "Initiatives of search and development related to the sector of the truffle", promoted by ARSIA for the regions: Abruzzo, Emilia Romagna, Molise, Toscana with Decree No. 321, 25-09-2007. The poster refers to the subproject B) and it is focalized on the "Study of the chain of truffle", with particular attention on the possibility of valorization of the product and on the analysis of investments for the construction of specialized woody plant in symbiosis with mycorrhizal truffle. Partners who won the public research project for the subproject B) are: DEART – Department of Agricultural and Land Economics – University of Florence (Coordinator); DSA – Department Environmental Science – University of Aquila; URATT – Unione Regionale Associazioni Tartufai Toscane; Associazione Tartufai delle Colline Samminiatesi; Associazione Tartufai Senesi; Centro di Ricerca per la Selvicoltura di Arezzo. The project is made up of two objectives. Objective 1 regards the analysis of investments for construction of specialized plants (valuation of the potentialities and limits of the development) and it is articulated in two actions. The first action wants to reconstruct the universe of the subjects that plays a role in the truffle market through the partners involvement which are directly in contact with many of the existing operating units on the territory; the second action focalizes on the knowledge of the operators involved in the chain through the study of socio-economic aspects, the farm structure analysis, the choice model behavior of the gatherers or producers, the description of the environmental area of production, on the productive system and on the production and harvest costs. The aim of this first part of the analysis is the investment valuation (profit of the systems, different type of enterprises, actual criticalities). Objective 2 concerns a deep analysis of the truffle chain (in particular protection and valorization of the product). Also in this case are planned two different actions. The first one is referred to the data collection. The second one regards the implementation of a survey based on a collection of samples with the aim of underline the gatherer profile, the characteristics of the market and of the product. The results attended for this objective are those to underline a profile of the gatherers, to analyze the costs of carrying out this activity, to verify the consistence of the market structure and to value the volume of product that transits from the chain. The project, besides, is finalized to the evaluation of the economic relapses that can be foreseen for the territory in terms of increase of the production or in terms of cost of the resources directly or indirectly linked to the truffle activities, of increase of the defense of the environment and the activity of safeguard and environmental improvement, of activation of paths of valorization for the product and for the territory.
Key word: Conservation, commerce and valorization.

Il comparto tartuficolo è stato, ed è tuttora, oggetto di regolamentazione in Italia da parte degli Enti a questo preposti: lo Stato, le Regioni e le Province autonome. Le Leggi emanate hanno lo scopo di dare principi chiari e comuni per tutte le attività legate al tartufo – raccolta, produzione, commercializzazione di prodotti freschi o conservati – e di offrire tutela ai consumatori per quanto concerne le frodi, all'ambiente per quanto riguarda l'utilizzo del territorio e ai coltivatori o proprietari di tartufaie controllate per quanto riguarda il furto. A livello nazionale, la normativa di riferimento è la Legge Quadro n. 752 del 16 dicembre 1985 "in materia di raccolta, coltivazione

e commercio dei tartufi freschi o conservati destinati al consumo", in seguito integrata dalla Legge n. 162 del 17 maggio 1991. Esiste anche un'ampia legislazione regionale in cui, in parte si riprendono le prescrizioni dettate dallo Stato, ed in parte si promuovono attività, dettano divieti o norme nuove, la cui motivazione va ricercata nella specificità della situazione territoriale.
Allo stato attuale il settore del tartufo mostra alcune rilevanti carenze rispetto alle quali il trasferimento delle conoscenze scientifiche agli operatori del settore potrebbe generare ampi vantaggi per questi ultimi e per l'economia del territorio. Queste carenze possono riassumersi all'interno di due principali problematiche: l'incertezza dei risultati produttivi ottenibili dall'attività di raccolta e di coltivazione del prodotto e l'efficiente loro collocazione sul mercato.
Se si ritiene che la raccolta/coltivazione del tartufo possa essere considerata una attività che va oltre la semplice pratica hobbistica, come fanno pensare le numerose associazioni tra gli operatori e le economie di mercato che il prodotto è in grado di promuovere, è necessario contribuire alla soluzione dei due problemi sopra evidenziati.
Nel primo caso potrebbe essere utile raccogliere tutti i dati attualmente disponibili sulle capacità produttive sia dei siti naturali sia di quelli coltivati, in funzione dei parametri che il mondo scientifico e l'esperienza diretta indicano come influenti sul livello produttivo. Tale elaborazione potrebbe consentire di individuare alcuni parametri utili alla definizione delle caratteristiche vocazionali minime di un territorio ai fini dell'espressione di un giudizio di convenienza economica dell'investimento che il raccoglitore/coltivatore decide di intraprendere.
La metodologia da adottare in questo caso è quella dell'analisi del costo di produzione e dei ricavi. L'elemento innovativo che vorremmo introdurre con questa ricerca è quello di individuare diverse tipologie di raccoglitori/coltivatori in quanto i costi sostenuti per l'investimento sono rilevabili come costi opportunità e di conseguenza estremamente variabili in relazione alle caratteristiche di colui che effettua l'investimento. Dal lato dei ricavi sarà sicuramente più difficile individuare valori certi, data la naturale tendenza a non svelare la propria capacità reddituale. Sarà però interessante mirare a conoscere una molteplicità di indicazioni sulla capacità produttiva dei singoli siti in relazione a parametri naturali e non, attualmente individuati in letteratura, attraverso la costruzione di un apposito database di raccolta dei dati rilevati.
Come risulta dalla letteratura esistente sull'argomento è spesso difficile arrivare a definire una stima precisa della produzione di un determinato territorio, mentre è più facile stimare le variazioni produttive che si verificano di anno in anno e che risultano molto elevate nell'ambito della tartuficoltura. La conoscenza di tali variazioni e della loro ciclicità ci consentirà però di valutare un ricavo medio annuo che, anche se non dichiarato, dovrà essere almeno pari al costo di produzione sostenuto nel caso di profitto nullo. L'informazione risulterà utile sia per il soggetto privato, che saprà di dovere sopportare costi che non verranno ripagati annualmente (valutazione della capacità di sopportare l'eventuale fabbisogno finanziario dell'operazione), ma solo in periodi poliannuali e, al contempo, consentiranno alle amministrazioni una maggiore capacità nella scelta di redistribuzione delle risorse che deriva sia dalla valutazione delle esternalità prodotte dall'attività tartuficola (di cui in questo progetto non ci occuperemo) sia dalla conoscenza dell'ammontare dei costi di produzione sostenuti dai soggetti coinvolti in questo tipo di attività.

Non meno importante risulta il secondo problema evidenziato che deriva dalla consapevolezza che la conoscenza della domanda e dell'offerta di un bene costituiscono elementi imprescindibili per una efficiente organizzazione del mercato. Lo studio di filiera, sequenza dei procedimenti e delle lavorazioni che dalla materia prima portano al prodotto finito rappresenta sicuramente un utile strumento di conoscenza per la corretta collocazione del prodotto sul mercato.
Lo studio di filiera, oltre ad individuare tutti le componenti che la caratterizzano e la loro articolazione, necessita di esprimere tali relazioni in termini di valore e/o di quantità. Come è noto per il tartufo tali conoscenze sono assenti e i singoli saperi di coloro che in qualche misura partecipano al processo di filiera sono del tutto insufficienti per la sua costruzione. Il tartufaio sa esattamente quanto tartufo cava, immagina più o meno la quantità che cava il suo amico o concorrente, pensa di poter stimare la quantità prodotta nel suo territorio, non ha idea del

valore e della quantità che caratterizza la filiera tartufo a livello regionale o nazionale. Lo stesso può dirsi del grossista, del trasformatore del prodotto, del commerciante, dell'amministratore che vorrebbe in qualche modo svolgere la sua funzione di riequilibro nelle possibili distorsioni del mercato.
La possibilità di valorizzare il prodotto attraverso le normative a difesa e sostegno delle produzioni di qualità o tipiche non può prescindere dall'esigenza di conoscenza della filiera, necessaria per intraprendere qualsiasi azione di sviluppo e di valorizzazione del prodotto.
In particolare risulta rilevante approfondire i seguenti aspetti:
- La conoscenza del prodotto deve essere caratterizzata dalla visibilità delle produzioni tipiche definendone:
    - natura (riconoscimento genetico…)
    - standard di riferimento
    - caratteristiche proprie e inequivocabili
- La definizione dei costi del riconoscimento:
    - valutare quanto è disposto a spendere in più il consumatore sapendo che il tartufo è certamente proveniente da un determinato territorio;
    - valutare quanto è necessario spendere per determinare le caratteristiche del tartufo A rispetto a quelle del tartufo B;
    - valutare quanto è necessario spendere per informare il consumatore di tali differenze;
    - valutare il costo della certificazione;o valutare l'incremento di prezzo e l'incremento di quantità venduta e se l'incremento di valore è sufficiente a coprire i costi di cui sopra.

Operando in tal modo potrebbero sorgere delle effettive difficoltà di sostenibilità economica dei costi e a tal fine sarebbe necessario preventivamente sviluppare una seria ricerca di mercato.
Così come sarebbe necessario che il decisore pubblico, che abbiamo visto non è attore secondario nell'organizzazione di questo processo di filiera, valutasse l'impatto economico complessivo per decidere se questo è compatibile con le sue politiche ed allo stesso tempo finanziariamente sostenibile.
Anche in questo caso è difficile ipotizzare di arrivare ad una quantificazione del prodotto che interessa la filiera nelle due regioni oggetto dell'indagine, ma è sicuramente possibile individuare le diverse componenti della filiera e le relazioni tra di esse. In particolare si pensa di individuare, attraverso le rispettive associazioni di categoria, gli esercizi che commercializzano il tartufo (alimentari, grande distribuzione, ristoranti delle regioni Toscana e Abruzzo) e le industrie di lavorazione, conservazione e commercio all'ingrosso del prodotto (il rapporto RAFT della Regione Toscana – ARSIA ne individua 13), a cui sottoporre un questionario mirato ad individuare le relazioni che legano tali soggetti. Anche in questo caso sarà difficile pervenire ad una stima attendibile delle quantità di prodotto della filiera, ma sarà possibile individuare i punti di forza e di debolezza della filiera soprattutto in un'ottica di valorizzazione del prodotto. Inoltre, soprattutto nei riguardi delle industrie di trasformazione, i questionari che saranno somministrati ai soggetti sopra individuati saranno mirati ad individuare quali sono i prodotti che vedono il tartufo come principale ingrediente. L'esistenza di canali distributivi diretti suggerisce di approfondire anche questo aspetto attraverso interviste da somministrare direttamente ai tartufai e attraverso l'osservazione di quanto avviene nelle principali sagre, fiere e manifestazioni del settore.
Le attività ipotizzate possono riassumersi nelle operazioni di seguito elencate:
- raccolta di tutti i dati disponibili attualmente in letteratura;
- valutazione degli investimenti nell'attività di coltivazione e miglioramento delle tartufaie in funzione delle caratteristiche dei territori oggetto di studio e della tipologia di imprenditore (indagine campionaria);
- analisi quali – quantitativa dei comparti caratteristici della filiera del tartufo (indagine campionaria);
- analisi quali – quantitativa della domanda di mercato a livello regionale ed extra regionale (per la stima della domanda si farà riferimento ai dati disponibili nelle statistiche ufficiali relative

al consumo dei prodotti a base di tartufo);
- analisi dell'impatto del miglioramento delle conoscenze sia nei riguardi delle mondo operativo sia nei confronti della difesa e del miglioramento dell'ambiente naturale.
La finalità ultima della ricerca può riassumersi in due principali obiettivi:
- valutare gli investimenti dell'attività di raccolta/coltivazione;
- acquisire maggiori e più approfondite conoscenze sulla filiera tartuficola.
Per il primo obiettivo sono previste le azioni di seguito descritte:
1.1. Il coinvolgimento dei partner che operano sul territorio, e che rappresentano un campione molto significativo dell'universo, ci consentirà di attuare indagini che vanno al di là della semplice raccolta di dati relativi alla produttività e al costo di realizzazione degli impianti. Tali impianti sono infatti spesso oggetto di sperimentazione e pertanto non sufficientemente rappresentative dell'universo da analizzare. Allargare la numerosità dei dati rappresenta uno dei principali obiettivi e il coinvolgimento di partner che sono a contatto con molte delle realtà operative esistenti sul territorio ci consente di lavorare su dati molto più significativi rispetto a quelli che scaturiscono dalla sperimentazione.
La difficoltà consisterà nel rendere omogenee le informazioni assunte e nel cercare di trovare relazioni scientificamente significative tra i livelli produttivi ottenuti nell'ambito delle tartufaie e le principali variabili che la letteratura ci indica come influenti sui livelli produttivi.
1.2. La rilevazione dei dati sopra descritti dovrà essere accompagnata dall'osservazione dei caratteri distintivi degli operatori. Come suggerito dal bando si ipotizza di riuscire a prendere in considerazione i sotto indicati aspetti: caratteri anagrafici e sociali del tartuficoltore, struttura aziendale in cui opera il tartuficoltore, motivazioni che l'hanno portato a intraprendere l'attività, caratteristiche dell'ambiente in cui è stato realizzato l'impianto, caratteristiche dell'impianto, reperimento materiale vivaistico, operazioni colturali all'impianto e negli anni successivi, costi sostenuti, attività di raccolta e commercializzazione, finanziamenti ottenuti, aspettative, motivazioni in merito a tale attività, problemi di assistenza tecnica.
La finalità dell'acquisizione di questi dati converge nella concreta possibilità di valutare gli investimenti, la redditività degli impianti, l'individuazione delle differenti tipologie di impresa e le eventuali criticità presenti.
Per il secondo obiettivo le azioni previste possono riassumersi in:
2.1 Acquisizione dei dati esistenti in letteratura e nelle statistiche ufficiali relativamente alla situazione del mercato nazionale e internazionale
2.2. In ciascuna delle regioni aderenti al bando sarà sviluppata una indagine campionaria mirata a delineare un profilo della figura del raccoglitore e la sua evoluzione rispetto al passato; saranno a tal fine utilizzate indagini compiute nello scorso decennio che hanno individuato precise tipologie di tartufai riconducibili ad una serie di variabili come caratteri anagrafici e sociali, tradizione familiare, specie di tartufo raccolte.
2.3. In ciascuna delle regioni aderenti al bando sarà sviluppata una indagine campionaria mirata ad evidenziare le caratteristiche del mercato regionale del tartufo fresco, conservato e trasformato. Il campione prescelto dovrà tener conto della struttura del mercato e tenere distinte le informazioni relative al mercato alla produzione, al mercato all'ingrosso e al mercato al consumo. In tale fase della ricerca i questionari saranno predisposti in maniera tale da evidenziare possibili relazioni tra tipologie di tartufai e mercati serviti. Obiettivo principale è quello di individuare tutti i soggetti coinvolti nella filiera e il loro ruolo, come obiettivo secondario si pone quello di stimare i quantitativi, i prezzi, la loro formazione in relazione alla variabilità della domanda e dell'offerta e delle tipologie di prodotto. L'universo di riferimento sarà quello suggerito dal bando:

- aziende del settore dei prodotti trasformati;
- sagre, mostre mercato, manifestazioni fieristiche;
- ristorazione, grande distribuzione, negozi alimentari e specializzati.

Come in precedenti indagini la conoscenza del profilo dei tartufai e dei costi legati allo svolgimento della loro attività ci consente di effettuare delle prime stime sulla consistenza del mercato del tartufo.

La conoscenza più approfondita, attraverso l'analisi di mercato, della struttura e del volume della filiera potrebbe consentirci di effettuare valutazioni sulle ricadute economiche per il territorio, sulla opportunità di attivare percorsi di valorizzazione del prodotto. Le ricadute sul territorio potranno essere osservate non solo sotto l'aspetto di incremento della produzione al costo dei fattori dei diversi comparti coinvolti in maniera diretta, indiretta o indotta dall'attività tartuficola, ma anche in termini di incremento della difesa dell'ambiente visto lo stretto legame tra produzioni tartuficole e condizioni di naturalità degli ambienti vocati e attività di salvaguardia e miglioramento ambientale promosso dalle stesse associazioni di tartuficoltori.
I risultati ottenuti potranno offrire un contributo ad una serie di soggetti interessati che pensiamo possano essere individuati in:

- **attori della filiera**
  La conoscenza della filiera e la valutazione di convenienza economica degli investimenti costituiranno degli ottimi strumenti a difesa delle prodotto locale e di qualità, limitando l'impatto negativo legato all'introduzione di prodotti surrogati al tartufo e all'uso di eccipienti di natura sintetica nella preparazione di tutti i prodotti a base di tartufo.
- **imprese**
  per lo sviluppo di strumenti di mercato che consentano alle imprese di beneficiare del riconoscimento di qualità e tipicità del prodotto attraverso una maggiore trasparenza della filiera.
- **comunità locali**
  Lo sviluppo delle attività tartuficole, in quanto legato all'incremento del reddito delle popolazioni e alla salvaguardia ambientale del territorio non può non avere impatti positivi sull'economia del territorio.
- **istituzioni**
  per la elaborazione di politiche che possano essere di aiuto alla promozione del prodotto e per l'adozione di strumenti a supporto delle esternalità positive prodotte dalla salvaguardia dei territori tartufigeni.

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UMBRAFLOR S.R.L. - VIVAIO CERTIFICATO ISO 9001-2000 PRODUZIONE DI PIANTE TARTUFIGENE CON L'UTILIZZO DI TARTUFI DI PROVENIENZA LOCALE E DI SEMENTI AUTOCTONE CERTIFICATE

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Abstract: Umbraflor s.r.l. – ISO 9001 certified garden centre - Truffle-producing plant production, using local truffles and native seeds, certified according to European (dir. 1999/105/CE), national (D.lgs 386/2003) and regional laws
The nursery "Il Castellaccio", managed by UmbraFlor s.r.l., has been making truffle-producing plants for 30 years, relying on the careful experience of its workers and of 6 graduates in Agronomy and Forest Science, present in the Company.
UmbraFlor s.r.l. is also well known for other garden products, such as in particular cancer-resistant cypresses, Dutch Elm Disease-resistant elms and walnut trees grafted with fruiting cultivars.
UmbraFlor s.r.l. has put much interest in the truffle-producing plant line production, due both to the local and national interest in this field, and to the direct effects on its turnover. All our greenhouses, recently renewed, have been designed to maintain strict sterile conditions, with air filtration systems and internal environmental parameters controlled by automated electronic stations. The average annual production is of about 30.000 plants, belonging to various forest plants and truffle species more suitable to the Italian habitats, with the exclusion of *Tuber magnatum* Pico.
All the production line, from the beginning to the final plant delivery to the customer, is daily monitored according to the ISO 9001 certification, which the Garden Centre has acquired several years ago.
To obtain truffle-producing plants, exclusively native seeds are utilized, certified on the basis of present rules (Dir. 1999/105/CE – D.lgs. 386/2003 – Regional laws and regulations in force in specific areas). The origin of both seeds and carpophores reflects the different Italian regions where UmbraFlor s.r.l. has its main markets.
All the carpophores, prior to the *inoculum*, are sectioned one by one and microscopically analysed by the University of Perugia, who verifies and guarantees the identity of the truffle species required by the nursery. In October and November, all the mycorrhized plants are controlled by the Department of Applied Biology of the University of Perugia, who certifies the plant quality for truffle cultivation.
This certification is issued on the basis of mycorrhizal morphology characterization, according to the agreement made in 1995 between ten Regions and eight University and Research Institutes. Following the Umbria Region Reg. 8/2007, this certification is granted also on the basis of biomolecular analyses, extended to all the marketed truffle-producing plants.
Key words: UmbraFlor, Garden Centre, truffle, truffle-plantation, mycorrhized plants.

Il vivaio "Il Castellaccio" di Spello (PG), gestito dalla UmbraFlor s.r.l. Azienda Vivaistica Regionale, produce piante tartufigene da oltre 30 anni. Per questo oggi può vantare una esperienza senza eguali, accumulata nel tempo sia dalle oltre n. 20 maestranze presenti a livello operativo in vivaio che dallo staff tecnico, organizzativo e di assistenza alla clientela formato da ben n. 6 agronomi e dottori forestali. UmbraFlor s.r.l. riveste una posizione non secondaria anche in altre specializzazioni produttive riferite al settore vivaistico. In particolare la struttura è ben conosciuta per la produzione di cipressi resistenti al cancro brevettati con i

nomi di '*Bolgheri*' - '*Agrimed 1*' - '*Italico*' e '*Mediterraneo*', nonché per la produzione, con licenza di vendita in esclusiva per il Centro e Sud Italia, degli olmi resistenti alla grafiosi brevettati dal C.N.R., meglio conosciuti come olmo '*Plinio*' ed olmo '*San Zanobi*'.

UmbraFlor s.r.l. si è affermata nel tempo anche per la produzione di noci innestati da frutto, sia di origine italiana che delle migliori cultivar selezionate in Francia e negli Stati Uniti. Grazie alle specializzazioni produttive accennate UmbraFlor s.r.l. ha partecipato, quale partner italiano, ad alcuni progetti INTERREG insieme ad istituzioni ed organismi di ricerca di Francia, Grecia, Malta, Marocco, Portogallo, Spagna, Tunisia e Turchia.

Fig. 1 Serra con ombreggiamento e filtri dell'aria

L'Azienda Vivaistica Regionale UmbraFlor s.r.l. segue con molta attenzione ed impegno la produzione di piante tartufigene, sia per l'importanza che il settore riveste a livello regionale e nazionale, sia per le ricadute positive che ne derivano sul proprio bilancio. Per questo, negli ultimi anni, ha avviato un programma di rinnovamento delle strutture e dei macchinari destinati a tali produzioni che ha portato allo smantellamento di alcune serre già utilizzate per la produzione di piante tartufigene fin dagli anni '70 ed alla loro ricostruzione dotandole di impianti del tutto innovativi. Le nuove serre, da poco messe in opera, sono state progettate per garantire una elevatissima condizione di asepsi all'interno, almeno per i primi 4-5 mesi dopo l'avvio del processo di micorrizazione. Dentro tali ambienti, le cui pareti sono formate da policarbonato alveolare, circola esclusivamente aria sottoposta a filtraggio, quale salvaguardia contro l'ingresso di spore indesiderate. Inoltre tutti i parametri ambientali relativi a temperatura, umidità, irrigazione, ecc, sono controllati da centraline elettroniche dedicate che ne rendono automatico il mantenimento ai valori prefissati.
Tali accorgimenti consentono di limitare al minimo indispensabile l'ingresso del personale

all'interno delle serre, riducendo così i rischi di veicolare possibili inquinanti dall'esterno. Nel caso in cui si rendesse comunque indispensabile entrare nei locali mantenuti in condizioni di sterilità, i dipendenti incaricati dispongono di abbigliamento del tipo "usa e getta". Il cambio di vestiario avviene nel disimpegno di pre-ingresso (avanserra), realizzato allo scopo di eliminare o ridurre gli scambi diretti tra lo spazio di coltivazione e l'ambiente esterno.
Tutte le cure colturali alle piante tartufigene, dall'avvio della produzione fino alla consegna alla clientela, sono individuate ed eseguite secondo le procedure previste dalla certificazione ISO 9001-2000, acquisita dal vivaio già da alcuni anni. Il controllo di qualità riguarda anche l'approvvigionamento delle sementi e dei carpofori utilizzati per la micorrizazione. Le rigide regole di procedura interna prevedono che per produrre le piante tartufigene vengano utilizzati esclusivamente semi di piante forestali di provenienza autoctona, certificati in base alle normative in atto (Direttiva 1999/105/CE – D.lgs. 386 del 2003 - Leggi e regolamenti regionali vigenti nelle singole zone). Le sementi vengono raccolte esclusivamente sotto il controllo del Corpo Forestale dello Stato e certificate dalle Amministrazioni pubbliche locali delegate dalle Regioni di competenza. Le diverse provenienze delle sementi e dei carpofori di *Tuber* utilizzati per la micorrizazione tengono conto delle varie regioni italiane nelle quali UmbraFlor s.r.l. ha i principali sbocchi di mercato.

Fig. 2 Semenzai con semi appena germinati

Nel vivaio "Il Castellaccio" di Spello (PG) vengono utilizzati ogni anno, per l'inoculo delle circa 30.000 piante tartufigene prodotte, oltre 70 kg di carpofori delle varie specie di *Tuber*. Tutti i carpofori delle varie partite vengono sottoposti, uno ad uno, a sezionamento ed a controllo al microscopio ottico da parte della Università degli Studi di Perugia. Con tale verifica vengono selezionati i soli tartufi rispondenti tassativamente alla specie richiesta dal vivaio, a condizione che siano sani, maturi e dotati di adeguata presenza di spore. Solo quelli che superano il controllo passano alla fase successiva per essere trattati e conservati in laboratorio.

Fig. 3 Interno di una serra a primavera

Fig. 4 Micorrize di *Tuber aestivum* Vittad. su nocciolo

Durante tutte le fasi di allevamento in vivaio le piante tartufigene restano sotto il controllo delle maestranze di UmbraFlor s.r.l che registrano, a cadenze regolari sulle schede previste dalla certificazione ISO 9001, le operazioni effettuate, le anomalie riscontrate ed i rimedi posti in opera. Nei mesi di ottobre e novembre di ciascun anno il Dipartimento di Biologia Applicata della Università degli Studi di Perugia provvede a controllare tutte le piante disponibili, sia quelle prodotte nell'anno che quelle degli anni precedenti, distinguendo i lotti riconosciuti idonei alla tartuficoltura, che vengono immediatamente certificati, da quelli rinviati ai controlli successivi.
Fino alla stagione silvana 2007/2008 la certificazione era rilasciata secondo la metodologia di valutazione delle piantine micorrizate con funghi del genere *Tuber*, basata sulla caratterizzazione morfologica delle micorrize, metodologia disciplinata dalla convenzione stipulata nel 1995 fra dieci Regioni e otto Istituti universitari e di ricerca. Dopo l'emanazione del Regolamento

della Regione Umbria n. 8/2007 tale certificazione è rilasciata anche sulla base del controllo biomolecolare esteso a tutte le piante poste sul mercato da UmbraFlor s.r.l..

Fig. 5 Interno di una serra a metà e a fine estate

Riassumendo quanto fin qui detto si può sicuramente affermare che nessuna struttura vivaistica può oggi fornire, tutte insieme, le medesime garanzie e certificazioni di qualità che UmbraFlor s.r.l. offre alla propria clientela per le piante tartufigene:

1. certificazione delle sementi di provenienza autoctona;
2. controllo al microscopio ottico di tutti i carpofori utilizzati per l'inoculo con garanzia della specie di *Tuber* di provenienza locale;
3. certificazione delle piante tartufigene su base morfologica;
4. certificazione aggiuntiva con analisi biomolecolare;
5. certificazione ISO 9001-2000.

In fase di programmazione della produzione di piante tartufigene viene posta molta attenzione al rispetto delle esigenze delle diverse specie di *Tuber*, prevedendone l'abbinamento con le piante forestali che manifestano necessità simili in fatto di condizioni pedologiche ed ambientali. Le specie di *Tuber* più utilizzate per la micorrizazione presso il vivaio della UmbraFlor s.r.l. a Spello (PG) sono le seguenti:

- *Tuber melanosporum* Vittad. - Tartufo nero pregiato (tartufo nero di Norcia o di Spoleto);
- *Tuber brumale var. moschatum* De Ferry – Tartufo moscato;
- *Tuber uncinatum* Chatin – Tartufo uncinato;
- *Tuber aestivum* Vittad. – Tartufo scorzone estivo;
- *Tuber borchii* Vittad. – Tartufo bianchetto o marzuolo.

A loro volta i tartufi sopra elencati sono disponibili in simbiosi con le principali piante arboree ed arbustive riportate di seguito:

- *Cistus incanus* L. - Cisto
- *Corylus avellana* L. - Nocciolo
- *Ostrya carpinifolia* Scop. - Carpino nero
- *Pinus halepensis* Miller - Pino d'Aleppo
- *Pinus pinea* L. - Pino domestico
- *Quercus cerris* L. - Cerro
- *Quercus ilex* L. - Leccio
- *Quercus pubescens* Willd. - Roverella
- *Quercus robur* L. - Farnia
- *Tilia cordata* Mill. - Tiglio

L'Azienda Vivaistica Regionale UmbraFlor s.r.l., pur garantendo la migliore qualità possibile delle piante tartufigene è consapevole che, per ottenere risultati soddisfacenti in campo, è necessario tener conto anche di una serie di altri fattori tra i quali si ritiene opportuno annotare i seguenti:

- necessità di accertare, con analisi chimiche e con sopralluoghi sul posto da parte di tecnici specializzati, se il terreno destinato alla tartuficoltura è idoneo a tale scopo;
- disponibilità, da parte di chi investe nel settore della tartuficoltura, di tempo e di risorse da dedicare alle cure della tartufaia, secondo le esigenze che emergeranno man mano, dal momento della messa a dimora delle piante fino a tutta la loro vita produttiva;
- dedizione al settore, quale garanzia per un assiduo aggiornamento tecnico, tale da consentire l'acquisizione e l'applicazione delle nuove conoscenze che scaturiranno dalla continua evoluzione della ricerca;
- capacità organizzativa ed economica per far fronte ai seppur minimi interventi necessari, con particolare riferimento a:
    - irrigazioni almeno di soccorso per la sopravvivenza delle piante;
    - cure colturali al terreno per garantire il necessario arieggiamento del suolo in superficie e per mantenerlo sgombro dalla vegetazione indesiderata e dalle infestanti;
    - protezione delle piante, a livello individuale o sull'intera superficie, per evitare danneggiamenti da animali selvatici;
    - difesa della tartufaia per evitare la sottrazione dei tartufi ed il deterioramento delle cave da parti di estranei;

Fig. 6 Tartufaia realizzata nel 1980

A conclusione vogliamo affermare che nel settore della tartuficoltura non esistono e non esisteranno mai delle tecniche universali da utilizzare in tutte le zone ed in tutte le condizioni. Infatti, per ottenere dei risultati soddisfacenti, è necessario impiegare un approccio focalizzato sul microambiente naturale della propria tartufaia, adattando le conoscenze tecniche alle particolari condizioni di ciascun luogo. E' evidente che un tale orientamento presuppone, nella pratica, il possesso di una buona conoscenza delle esigenze delle piante, delle necessità delle diverse specie di tartufo e delle caratteristiche edafiche e climatiche del sito ove si opera.

NOT ONLY A CULINARY TREASURE. TRUFFICULTURE AS AN ENVIRONMENTAL AND AGRO-POLITICAL ARGUMENT FOR REFORESTATION

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Abstract
Among the numerous wonders of what in Italian is called *il sottobosco* (i.e. the 'subforestal' world), mushrooms and especially truffles might belong to the highest-ranking. However, like other natural resources, truffles are increasingly threatened with extinction. This is not only due to rather recent phenomena like overharvesting and climatic changes, but also to long-term factors, such as for example the more general disregard of forestry and agriculture evident since the industrialisation of French and Italian societies.
In the case of the truffle, the enduring disinterest cannot be reduced to the decline of rural life, but should also be correlated to the general view of truffles as a *niche*-product, only affordable for the happy few and virtually devoid of any extra-culinary relevance. Apart from the fact that (cheaper) truffles can nowadays be obtained by nearly everybody, the misconception that truffles are of no avail outside of the kitchen is, as I hope to demonstrate, a persistent one.
Drawing upon a combination of insights from modern mycology and from the historical documentation on the trufficulture programme of the region of Vaucluse in the 19th century, I would like to give some illustration of the ecological value of truffles. By doing this, I hope to make people and especially the experts from the field of forestry sensible of the environmental potential of trufficulture as an additional argument for reforestation. And even more so, because an ecological approach towards trufficulture might obtain greater resonance in the Germanic, Anglo-Saxon, and Scandinavian world and consequently enhance the agro-political latitude. It is argued, then, that by convincing agricultural decision makers of the environmentally beneficial effects of trufficulture, it will be achievable to develop a truly European truffle policy.
Key words: Reforestation, Mycoforestry, Mediterranean basin, Ecological valorization, European truffle policy.

It is generally known that wild salmon is delicious, but it is less known that the very presence of wild salmon can be considered an indicator of ecological quality. In much the same way, truffles are – due perhaps to their overwhelming scent and their extravagant status – usually solely perceived in terms of their gastronomic qualities. As a result, the notion that mycelia not only play a crucial and unique role in ecosystems, but can also be adopted in order to enhance forest health is at best spread among specialists.
Without denying, obviously, the truffle's superb culinary properties, this article will rather explore its various ecological uses within the larger framework of agro-forestal management.

More precisely, it will point out that reforestation in view of trufficulture will have, besides the obvious environmental advantages of planting new forests, at least three beneficial effects on the sustainable development of the Mediterranean basin:

- reforesting Southern-Europe with oaks and other truffle-producing broadleaves may substantially reduce the risk of forest fires, erosion, inundations, and other ecological disasters, as the large-scale reforestation annex trufficulture programme in the region of Vaucluse around 1875 clearly shows;
- as products of a very complicated and highly fragile ecosystem truffles are known as reliable indicators of the ecological quality of a specific area, which can render the tuber a useful instrument for pedological monitoring as well as for mycoforestal aims;

- ❖ trufficulture can at least in the intermediate period be accompanied by the cultivation of crops like, for instance, grapes and grain and thus enhance the economic possibilities of regions whose resources are rather scarce.

Vaucluse, the slopes of the Mont Ventoux (1912 m.), which since time immemorial and at least since Petrarch's legendary conquest always has been remembered for their abundant vegetation and very green appearance, were around 1866 described in a rather apocalyptic way: "I would like to have the trumpet of the Last Judgment in order to mobilise the people from the Vaucluse (…) and to shout them with a voice that would wake up the deaths: 'You (…), who are so proud on your splendidly cultivated plains, go, cross your desolate mountains; they are nearly all since a long time stripped off their decoration; their slopes and plateaus have lost their cover of humus (…); the beds of the creeks and the rivers, once attractive and fresh valleys, are now devastated; where you once saw vital woods and magnificent forests, they are nowadays only some scarce bushes and shrivelled trees left."'(1)

Because of its particular windy position (*Mons Ventosus*) and microclimatological extremities, the "stripped" Mont Ventoux simultaneously had catastrophic repercussions for the surrounding regions: the recurrent torrential rains led to inundations and erosion and this, of course, accelerated the process of deforestation. Significantly, the denuding of the Mont Ventoux was first and foremost induced by human activity. During the revolutionary period the French forests experienced, as it seems, a full-blown anarchy, and this massive and reckless chopping could only be stopped by a more repressive policy under Napoleon. However, at that time it was already too late to repair the damage, also because shepherds and bee-keepers in the meantime had already got used to herbal instead of arboreal vegetation and especially the shepherds turned out to be very reluctant to change their pasturing practices. The notorious mistral, then, did the rest to accomplish the ecological disaster…

It was just a question of time and the desertification had also reached the lower parts of the mountain, where some mixed agriculture was practiced. However, with a *circulaire*, written by the prefect of the *département du Vaucluse*, Durand Saint Amand, a radical change set in. The prefect had already tried to convince the local councils of the economic advantages of reforesting unexploited areas, but since the World Exhibition of Paris 1855 he had a new trump.

One of the awardees who exposed at this *Exposition* was Auguste Rousseau from Carpentras, obtaining a *médaille de première classe* for the invention of a new method of canning truffles. However, a real landmark was another test done by Rousseau, whose stunning preliminary results he might have brought to public attention during the World Exhibition in Paris. It must be said that Rousseau was, after all, the third to experiment with the planting of *chênes truffiers* (i.e. with the acorns of truffle-producing oaks), but he was actually the first who monitored meticulously the results and who decided, unlike his predecessors Monsieur de Monclar and Joseph Talon, not to hide his innovation.

On the contrary, it seems that Rousseau deliberately informed the Academy of Sciences which presumably notified the prefect about this breakthrough. Durand Saint Amand for his part did not hesitate and on 6 November 1856 sent a *circulaire*, advising the local municipalities to reforest unexploited areas with *chênes truffiers*. In order to remove all possible restrictions, he instantly authorised the town administrations to use for this aim the *crédit des dépenses imprévues* or do in any case their utmost best to find ways of financing this project.

The reforestation programme soon yielded rich rewards. Within a couple of years the Vaucluse faced nothing less than a metamorphosis: in 1875 the reforested areas exceeded 60,000 hectares, which meant that almost a sixth of the department's surface was covered by oak plantations. The total truffle harvest increased accordingly, obtaining 450 tons in 1875 against 380 tons in 1868. By the start of the 20th century only the region of Vaucluse seems to have produced more than 700 tons of *Tuber melanosporum* yearly, whereas the current annual harvest of France fluctuates between 20 and 28 tons! This result was even more startling, if we consider that these fields previously lay fallow and that the trufficulture simultaneously could also generate other agro-forestal resources, like e.g. acorns, grapes (at least in the

intermediate stage), rye, and firewood. The cultivation of truffle-oaks offered consequently a very attractive perspective for a region, whose resources were rather scarce or even annihilated by the phylloxera aphid.
Even though the calculations of tonnage made around the end of the 19th century are no doubt too high, and those concerning present statistics somewhat underestimated, the decline remains just as abrupt. Nevertheless, national truffle associations and transnational interest groups like the GETT spare no pains to reverse the negative trend. However, the accomplishment of a *tour de force* illustrated by the case of Vaucluse would require huge financial investments, and as is generally known fundraising in view of large-scale trufficulture tends to be particularly difficult for different reasons. First and foremost, because trufficulture entails an enduring financial commitment, whereas budgets are usually bound up with (short) periods of government. So there is something true about the Provencal proverb 'The mistral, the parliament and the (river) Durance are the three plagues of the Provence'. In addition, given the result-driven research regime, the financing of such a Rubicon-venture often turns out to be barely viable.
But there might be more at stake here than mere budget and research policy. The best possibility for long-term funding, namely the fleshpots of Brussels, as yet remain closed due to lack of interest within the EU Commission. However, this reluctance does not necessarily mean indifference but rather manifests an intra-European cultural gap. In spite of the truffle's commercial diffusion all over Europe, there is still a kind of truffle-equator at this continent. What I mean is an imaginary mental boundary which more or less coincides with Romanesque cultures on the one hand and Nordic-Germanic cultures on the other. The northern hemisphere is characterised by a rather negative truffle perception which goes back to medieval botanists such as, amongst others, Hildegard von Bingen (1098-1179). This repugnant attitude undeniably is correlated with the over presence of harmful truffle types in the northern part of Europe. What is more important here, remnants of this hydno- and mycophoby are still present in the collective unconscious of Nordic people and they might explain the overt lack of a Europe-wide support for the truffle.
Paradoxally, the best way to gain political approval and commitment in the Northern European countries would be to renounce the seductive dimension of truffles and to put it the other way around, emphasising its largely unknown ecological utility value. In other words, the European management with regard to truffles should be presented under the guise of an intensive reforestation programme, as the Northern EU member states might be more susceptible and propitious to a truffle policy, which is based on ecological arguments rather than on gastronomic and economic arguments.
Here different arguments in a unique way converge and can be synthesised. From a scientific point of view an environmental approach of trufficulture might at least be justifiable, since Paul Stamets has argued that mycelia are very useful instruments to enhance the health of ecosystems in general (concept of mycorestoration) and of forests (concept of mycoforestry) in particular. In addition, this mycoforestal model might more than ever appeal the Germanic, Anglo-Saxon, and Scandinavian world, as the truffle frontier progressively shifts towards the boreal sphere. The environmental approach may find special attention in Germany, since truffles are a protected species by German law. Significantly, the first German truffle association, which was founded 2005 in Sinzig in the Lower Rhine Valley, was called "Association for the Promotion and Conservation of Truffles in Germany". Last but not least, another propitious circumstance may be that this mycoforestal concept basically has already been anticipated by an initiative of the honorary president of the GETT, Domenico Bigioni, to found a so-called *Federazione Europea Ambiente Antincendio Arboricoltura Simbionte* (FEAAAS).
Only by convincing agricultural decision makers of the environmentally beneficial effects of trufficulture, will it be possible to develop a truly European truffle policy, which will not identify trufficulture instantly with an elitist lobby of gourmets but rather be concerned with these subterranean mushrooms as a unique European forestal resource. For the truffle's radius of action is much wider than 'merely' tickling the palate. That truffles eventually end up in the stomach should therefore neither be attributed to their provenance, nor be an excuse for

neglecting their matrix.
This proposal is part of a postdoc research project on "The Quest for the 'Holy Spores': Exploring the truffle in early modern European science", financed by the Alexander von Humboldt-Foundation, and conducted at the *Institut für europäische Geschichte* (IEG) Mainz and the *Arbeitsstelle Historische Kulturforschung* (AHK) in Saarbrücken (*Universität des Saarlandes*, Chair of Early Modern History, prof. Wolfgang Behringer).

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"Je voudrais avoir la trompette du jugement dernier pour rassembler les Vauclusiens (…) et leur crier d'une voix à réveiller les morts: Vous (…) qui êtes si fiers de vos plaines si bien cultivées, allez, parcourez vos montagnes désolées; elles sont presque toutes depuis longtemps dépouillés de leur parue; leurs croupes et leurs plateaux ont perdu leur couches d'humus (…); les lits des torrents et des rivières, qui furent jadis de riantes et fraiches vallées, sont dévastés; là où se montraient de vigoureux taillis, des forêts magnifiques, on ne voit plus que des ronces, de rares broussailles, des arbres rabougris."Cited from: *Rapports sur les truffières artificielles de M. Rousseau (….)* (Carpentras 1866), p. 32.

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ONLY THE SKY IS THE LIMIT OF THE SOIL. MANIFESTATIONS OF TRUFFLE MANIA IN NORTHERN EUROPE IN THE 18TH CENTURY

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Abstract
Despite their subterranean existence in undisclosed and hidden locations, truffles have left their marks in the history of European civilisation since Greek and Roman Antiquity. However, the fascination with truffles has fluctuated strongly from one era to another. One of the periods of increased attraction was the eighteenth century, as became manifest in the numerous frenetic experiments with this tuber. The high expectations of tests to grow them, but also the many truffle dog missions above the Alps indicate a feverish perception of this luxury item all over Europe. Especially Northern Europe, it seemed, was under the spell of nothing less than a truffle mania. Drawing upon hitherto unknown historical documents from archives in Turin, Vienna, London and Berlin, I would like to describe some of these manifestations of truffle fever and try to explain this sudden and almost obsessive interest in the truffle against the background of the socio-cultural climate of that time. It will be argued that this excessive fascination was inextricably bound up with the historical phenomenon of what the German sociologist Norbert Elias has coined as “court society”.
With this research I intend to demonstrate that there is actually a long tradition of pan-European interest in the truffle. This implies that the actual economic-agricultural truffle policy can not be regarded as an issue of marginal importance, as it merely concerns – as is usually said – a *niche* in the economy of some Southern European countries. Truffles have always been estimated as a unique element of European natural-cultural heritage and, if only for that reason, they should be fostered by a truly European policy.
Key words: Truffle mania, Northern Europe, 18th century, court society, Piedmont.

Why not at Windsor? After many years of belligerent existence, Prince William, Duke of Cumberland, for his roughness also known as “Butcher Cumberland”, obviously longed for a distinct leisure activity. Having heard of the incredible expertise of Piedmontese dogs, he desired to verify whether Windsor and its surroundings did not in fact secrete truffles.
As one of the sons of King George II, he exploited, of course, privileged diplomatic channels and in this way he submitted in August 1751 his request directly to Charles Emmanuel II, King of Savoy. At 4 September the Foreign Minister in Turin, Ossorio, replied “The King, after having learnt that the Duke of Cumberland wished to get trained truffle dogs and a man who knows how to conduct them and how to instruct other dogs in England, (...) has seized this occasion to satisfy the wish of His Royal Highness.” (1) His Majesty immediately ordered eight truffle dogs and two experienced *trifulau* via Paris to be sent to the English court.
For the state administration in Turin, such a query was anything but a novelty, as at least two other truffle dog expeditions had already left Piedmont some decades before: around 1720 a team of three truffle dogs, accompanied by one hunter, arrived with the Prussian King, whereas some years later another group, consisting of four dogs and one supervisor left for the French court. According to some historical Italian documents, the King of Poland also submitted such a request. This fact remains unverified, however, since no other indications have been found. Be that as it may, the reputation of the Piedmontese truffle dogs and hunters eventually became so well-known that the most influential German encyclopaedia, *Zedlers Universallexikon* (published from 1749 onwards), made mention of “these dogs come from the region of Turin to Augsburg and other parts of Germany.” (2)

Nevertheless, the request of Duke Cumberland differed significantly from the previous demands, as he explicitly asked for a man who was not only experienced in guiding truffle dogs, but also acquainted with the training of unlearned dogs. Unlike the impulsive French and Prussian sovereigns, "Butcher Cumberland" obviously had a long-term planning: to comb the whole Island of Britain for the presence of the precious mushroom. For what reason did he think that the British soil could contain this treasure? Why all these painstaking efforts of ordering dogs, transporting this troop of dogs and their masters during a long and wearisome travel to Britain (one dog actually died on the road), lodging them at Windsor Castle, clothing the hunters according to the local etiquette, bringing them back home? Just for the minuscule chance of finding a miserable tuber? No, they were rather possessed by a kind of fever, as if they were gambling or playing or investing masses of others' money in credit default swaps. Mania, whether tulip, gold, truffle, or investment mania, essentially means a nearly destructive loss of self-control without being aware of it. A maniac is like someone with high fever who thinks that he is able to mount the roof of his house in order to sweep the chimney.

After having discussed some manifestations of truffle fever and, in a nutshell, the very notion of mania, the question arises: What were the specific backgrounds which explain this emerging obsessive interest in the truffle during the 18th century? A first important factor was, as it seems, the notable progress in the mycology *avant la lettre*. The first decade of the 18th century witnessed some significant developments in the field of truffle research.

In 1711, the French scholar Claude-Joseph Geoffroy firstly drew attention to the truffle's interior, where he with the aid of a microscope observed something "that seemed to be a transparent tissue, composed of vesicles." (3) Fifteen years later, his English colleague, Richard Bradley, the first person to successfully reproduce a truffle, confirmed Geoffroy's presumption that the tuber had "Seed-Vessels towards the Center*"* even though he had to admit that he had not yet "(..) been able to discover any" such seed-vessels. Simultaneously, the study of the biogeography of the truffle also made progress: whereas the famous English botanist John Ray still denied the presence of truffles in "our British Soyl", his friend Tancred Robinson triumphantly gave "account of the *Tubera Terrae*, or Truffles found at Rushton in Northamptonshire."

A rather unexpected outcome is that these novelties rapidly circulated within Europe. These messages arrived in the palaces and urged sovereigns to reflect upon the potential presence of culinary pearls on their own territory. Budgetary and gastronomic concerns played more than a minor role in prompting the need to verify these assumptions. According to a German document, the Prussian King usually ordered his truffles, "which were served as a delicious delicacy on the Royal table", (4) from Italy, paying 40 Reichsthaler per pound. However, after having learnt, that they might grow in his own country, he decided to order an Italian truffle hunter (whom he paid 13 Reichsthaler monthly), hoping that the indigenous soil would provide him with fresh truffles. Homegrown truffles would be a great advance, since the Italian ones were preserved in wine vinegar, which of course drastically deteriorated their taste.

Both trends, the evolution of truffle research as well as its nascent gastronomic exploitation outside traditional countries like France and Italy, were closely connected to, and essentially generated by the social-cultural substrate of the court society, which reached its zenith in the period between the Westphalia Peace Treaty (1648) and the French revolution. The sudden and Europe-wide interest in the truffle as an object of study and as a culinary treasure is inextricably bound up with this social model.

Since most early modern scholarly research was fostered and financed by a sovereign, these funders habitually monitored the progress of the investigations. As a result, the output of the research frequently circulated, not only within these micro societies, for example, by way of institutions like the Royal Academies of Sciences, but also between different courts. In the case of the truffle, the interest obviously exceeded a mere scientific curiosity. In fact, its exceptional gastronomic properties and its symbolic value rendered the tuber a product with unique multiple promotional possibilities, as the rulers of Savoy increasingly became aware of. Besides the "export" of truffle dogs and the casual product promotion through the organization of truffle hunting sessions in Piedmont for foreign aristocratic visitors, there was, of course, the

exploitation of the culinary qualities of the truffle.
All these applications perfectly corresponded with the following features of the court society: the search for distinction, well-being (at that time rather described as *bien-être* or *douceur*), and splendour. No matter what the cost, what pushed the early modern political elites in general and the newly arrived royalty of Savoy in particular, was the urge to demonstrate pre-eminence and prestige. After a very long and wearisome diplomatic struggle, the Dukes of Savoy in 1714 finally were bestowed with a crown. However, the international recognition of its royal status was but the beginning of a new big project: the organisation of an administrative apparatus and court entourage, which should meet the requisites of a modern state. One of the priorities was the establishment of a fully-fledged *corps diplomatique* in the main capitals of Europe.
Strikingly, the use of the truffle, amongst other regional victuals like Piedmontese cheese, wines, tobacco, and game as a promotional gift in diplomatic affairs accompanied and occasionally, as it seems, facilitated the acknowledgement of the Savoyard state. For example in the diplomatic relations with the Imperial court in Vienna, the truffle became a *point fixe*, which at a rather informal level contributed to the gradual reduction of the political tension between both courts. The Empress suspected that the Kings of Savoy wanted to expand their territory at the expense of the Imperial reign. The Piedmontese diplomatic staff soon discovered that the Viennese nobility could be easily accommodated by using Savoyard delicacies, and Maria Theresa turned out to have a very particular *faible*: she was eager of truffles. Charles Emmanuel III and Victor Amadeus III did not hesitate and sent masses of truffles to Vienna, as the following table shows.
As the table above demonstrates, the quantities of truffles offered substantially increased between 1740 and 1770, and this steep curve simultaneously shows why truffles were extremely successful in gift exchange. As the sovereign, the Empress, of course, had the *primeur* of receiving truffles, and since she consumed these delicacies deliberately in a flamboyant way in high nobility company, her example triggered off a sequence of imitative and competitive behaviour within the court society. At a later stage, the Viennese diplomats of the house of Savoy frequently were provided with truffles for their own private distribution, as they proved to be a magic medium to “get more familiar with some houses that would be useful to have contact with”(5), as a Savoyard ambassador put it in a letter from 1774.

Tab. 1 Quantities of truffles sent from Turin to Vienna (1738-1774)

year*	quantity of offered truffles**	information source (AST, L.M. Austria, Mazzo [= M.])
1738	-quelques tr. -quelques livres de tr.	M. 65, Jan. 13, 1738 M. 65, Dec. 18, 1738
1739	4 boîtes de tr.	M. 66, Nov. 18, 1739
1740	24 livres = 9,1 kg	M. 67, Dec. 3, 1740 (15 livres de tr.) M. 67, Dec. 10, 1740 (9 livres de tr.)
1766	42 livres = 16 kg	M. 88, Dec. 20, 1766
1767	84,5 livres = 32,1 kg	M. 89, Jan. 3, 1767: 38,5 poids d’ici M. 89, Dec. 19, 1767: 46 livres de tr.
1768	176 livres = 66,9 kg	M. 90, Jan. 2, 1768: 56 livres de tr. M. 90, Dec. 14, 1768: 70 livres de tr. M. 90, Dec. 24, 1768: 50 livres de tr.
1769	56 livr. = 21,3 kg	M. 90, Dec. 2, 1769
1773	28 livr. = 10,6 kg	M. 94, Dec. 22, 1773
1774	44 livr. = 16,7 kg	M. 94, Jan. 3, 1774: 44 livres de tr. M. 94, Dec. 3, 1774: no truffle quantity indicated

* Understood as calendar year (and not as truffle harvest season, which goes from October till January). Decisive are the date and the weight of the transport on the day they were sent.
** 1 livre equals approximately 380 g

Truffles turned out to be a rewarding gift for different reasons: Firstly, in terms of distinction they could be perfectly integrated into an aristocratic life style, which was governed by the quest for prestige. Secondly, the charm of the white Piedmontese truffles essentially derived from the fact that they, as delicacies, largely contributed to the state of physical happiness, which the court nobility actually pursued. It was anything but casual that gastronomy as a *savoir faire* emerged from the courtly entourage, nor was it coincidence that the first properly culinary exploitation of truffles started in this context. In the third place and perhaps even preeminently, truffles could delight immensely the recipient because of their unexpectedness and eccentricity. Startling and peculiar presents are likely to be highly effective, especially in a gift overburdened court society, where gifts, very often, were merely ritually exchanged. At that time, as we have seen, the truffle undeniably was a novelty and curiosity.
The massive evidence of truffle mania in the northern parts of Europe clearly demonstrates that at least since the early 18th century this cryptogam has been considered a unique European treasure and if only for that reason it should be cherished as a irreplaceable soil resource.

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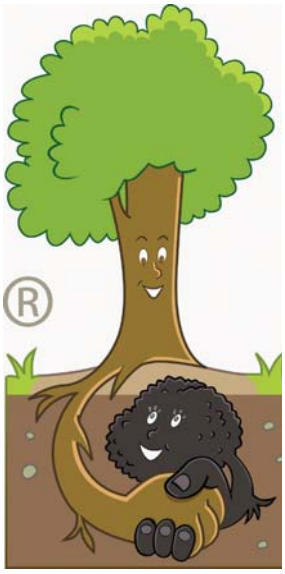
PRODUCTION OF HIGH QUALITY TRUFFLE PLANTS UNDER ISO 9001 QUALITY LABEL AND IN THE FRAME OF SPECIAL GROWING CONTRACTS



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Robin innovation
propose three major and unique ways of considerable improving truffle cultivation.
Key words: Mycorrhizal plants, Truffle, Growing agreements, nursery.

The ROBIN tree nursery company is involved in controlled mycorrhization of forests plants since 1960 and in large scale production of mycorrhizal plants including edible mushrooms since 18 years. The ROBIN nursery is the first European nursery for the production of mycorrhizal plants under ISO 9001 quality label. The nursery has been involved in several European research programs addressing mycorrhizal symbiosis related to three main goals: reforestation purpose, selected mycorrhizal seedlings for phytoremediation of contaminated area, and establishment of EDIBLE MUSHROOM ORCHARD®.These participations allowed the nursery to get a considerable experience both at mycorrhizal plants production. A staff specially trained for mycorrhizal studies manages all steps of production of truffle plants from controls and selections of fruitbodies, to final control before delivery, according to schedule defined under ISO 9001 label aimed for high quality products. The truffle plants can be produced according to the special request of our customers in the frame of a special growing contract using selected batches of seeds and truffles supplied by the customers (ie: Angellozzi special growing contract for the production of truffle plants especially adapted to the Italian region of the Marche). Each batch of seeds and truffles we receive is well identified in order to keep track of these batches all along the steps of production. Each truffle we received is controlled under microscope with the aim to identify their spores. This control allows us to check the product supplied and to evaluate the quality of our inoculum. These truffle plants are produced in patented ROBIN ANTI SPIRALLING® containers. In specially designed green houses, the production of the truffle plants is daily monitored by trained staff in order to survey the watering and to avoid eventual trouble (diseases, insect damages). The development of the mycorrhizal association is regularly followed in order to maintain the permanence of this association. At the end of the growing season each batch of this production is sampled using statistical law. Each sample done is observed under stereoscopic microscope by trained staff in order to evaluate the mycorrhizal rate of the batch (in house control). After that, the final control (Certificate of mycorrhizal guarantee) is performed first by National French institute INRA in France and on the second hand by University of Perugia according to the regional rules for contracts in the Italian

region of Marche. The control of the mycorrhizal rate is done laying particular stress on the good distribution of the mycorrhizas of the inoculated truffle along the root system and on the amount of these mycorrhizas and on the lack of other contaminant fungus.
High performance CHAMPION TREE® seems to be another way to improve truffle cultivation and production.
This program designed by our nursery has began in 1995-1999 by the identification and selection in natural truffle orchards of “best producer trees“ and their truffles and the beginning of the multiplication of these subject from mother trees stock.
During the year 2000-2001 the nursery in collaboration with INRA Clermont Ferrand has set up 15 experimental plots in France in various geographical, climatic and soils conditions using CHAMPION TREE® inoculated with their own truffle and other truffles in comparison with normal trees obtain from seeds and inoculated by these selected truffles and the normal ones (control).
These experimental plots have been followed in order to analyse the development of the CHAMPION TREE® in comparison with the normal ones and the mycorrhizal status of each plot.
At the beginning of the year 2005 the first truffle is produced under a CHAMPION TREE®.
During the year 2006 truffles were collected only under CHAMPION TREE® in several plots.
In 2007-2008 the number of CHAMPION TREE® producing truffles and the quantity of truffles harvested increased a lot. The first production of truffle under traditional occurred.
You can see on the table “Evolution of the truffle production under CHAMPION TREE® at Valensole plot“ that such preliminary results are very promising and tend to prove a better productive capacity of the CHAMPION TREE®.

Evolution of truffle production under CHAMPION TREE® at Valensole plot

Year (Harvest)	Location	Identification	Name	Weight/g
05/06	C5L5	I1	Chave V Tournayre	330
06/07	C4L6	Qp3	SJ/SJ	260
	C3L6	Qp3	SJ/SJ	220
	C4L5	I1	Chave V Tournayre	130
07/08	C5L4	Qp3	SJ/SJ	550
	C5L5	I1	Chave V Tournayre	820
	C4L6	Qp3	SJ/SJ	680
	C4L5	I1	Chave V Tournayre	280
	C4L3	Qp1	SEL2/Tournayre	240
	C3L1	I4	Std/Tournayre(témoin)	490
	C3L2	Qp5	SJ/Bernard	420
	C2L1	Qp1	SEL2/Tournayre	280
	C2L2	Qp2	SJ/Tournayre	420
	C6L5	Qp1	SEL2/Tournayre	640
	C6L7	I1	Chave V Tournayre	280

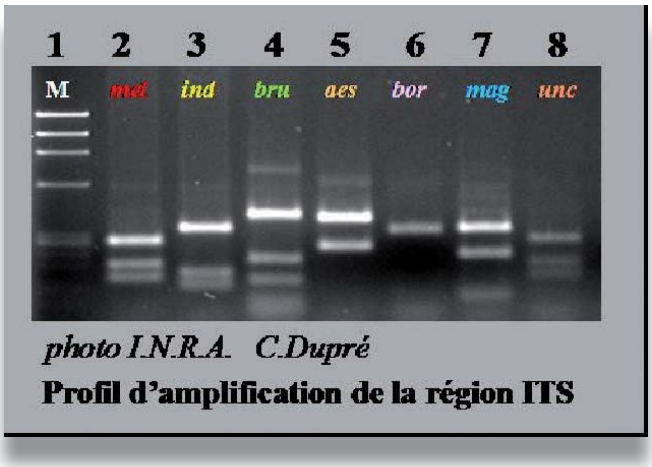
Valensole experimental plot

	C1	C2	C3	C4	C5	C6	C7
	*I	*I	*I	*I	*I	*I	*I
		Bloc1					
L1	*I	Pub1	Ilex4	Pub6	Ilex3	Pub5	*I
L2	*I	Pub2	Pub5	Pub7	Pub4	Ilex4	*I
				Bloc3			
L3	*I	Ilex1	Ilex3	Pub1	Ilex2	Pub6	*I
L4	*I	Pub3	Pub4	Pub2	Pub3	Pub7	*I
						Bloc5	
L5	*I	Ilex2	Ilex2	Ilex1	Ilex1	Pub1	*I
L6	*I	Pub4	Pub3	Pub3	Pub2	Pub2	Pub7
L7	*I	Ilex3	Ilex1	Ilex2	Pub1	Ilex1	Pub6
					Bloc4		
L8	*I	Pub5	Pub2	Pub4	Pub7	Pub3	Ilex4
L9	*I	Ilex4	Pub1	Ilex3	Pub6	Ilex2	Pub5
			Bloc2				
L10	*I	Pub6	Pub7	Pub5	Ilex4	Pub4	Ilex3
	*I	*I	*I	*I	*I	*I	*I

CHAMPION TREE® *Quercus ilex*
Chave V

CHAMPION TREE® *Quercus pubescens*
Super Jarjayes

The research achieved by INRA Clermont Ferrand (Chevalier *et al.*,) using molecular tools allowed a better knowledge, of the white Piedmont truffle *Tuber magnatum* and particularly on the identification of the mycorrhizae of this truffle.
Several years of close collaboration between INRA and ROBIN nurseries lead to a new process INRA /ROBIN for the production of guaranteed *Tuber magnatum* truffle plants.
This production allowed our nursery to set up different experimental plots in optimal ecological conditions, in Italy in close collaboration with IPLA Torino and in France within the frame work of interreg alcotra III A VERCHAMP project 2003-2006.



Biomolecular tool

experimental plot with *Tuber magnatum* truffle plant

Mycorrhizae of *Tuber magnatum*

CULTIVATION SESSION

LES IMPLICATIONS DE 40 ANNEES DE RECHERCHE SUR LES TRUFFES: PERSPECTIVES D'AVENIR

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Abstract: Implications of 40 years of research on truffles: future prospects.
All the phases of truffle cycle (spore germination, mycelium development, mycorrhization, fructiferous induction, fruiting bodies development and maturation) were studied according to very different approaches.
Controlled mycorrhization has been correctly carried out, except for *Tuber magnatum.*
The most important progress in truffle cultivation resulted from the massive utilisation of mycorrhized plants, which made possible the introduction of *T. melanosporum* into countries, such as Australia, Israel, New Zealand, U.S.A. and recently Morocco, where it didn't exist. Mycorrhized plants are under improvement by means of different combinations of high-performances tree clones and truffles from different geographical sources, in respect of plant-fungi biodiversity.
According to some new molecular biology works, truffle is heterothallic but this character doesn't affect plant suitability to truffle production as they are inoculated by a combination of spores.
The nutritional method of mycelium and ascocarp is very important to be well studied, as cultivation depends on it. *T. melanosporum* has a symbiotic behaviour on new roots, but on old roots it is more saprophytic or even a weakness parasite.
Nutritional mechanism of ascocarp is still under discussion, as the theory of fructiferous body independence has been brought into question. The implications on truffle fertilization are relevant.
The mechanism of burn formation is badly known: we only know that truffle "prepares its nest" by emitting volatile herbicide substances and strongly degrading minerals and soil organic matter.
In opposition to mycorrhized plant, truffle culture techniques are little developed but news are now proposed, thanks to fungal biology and fungal ecology studies: indirect control of rootlets net by tree pruning and root cutting, management of root growth, soil decompaction, stimulation of burn formation, organic fertilisation.
Future research will face a better knowledge of crucial points in fungal biology and ecology: intraspecific variability, saprophytic nutrition, burn formation mechanism, fructiferous induction, influence of hydro-thermic stress, soil eutrofisation by nitrogen, in order to improve cultivation practices.
Key words: truffle, *Tuber*, biological cycle, mycorrhizal plant, cultivation.

Résumé: Les différentes phases du cycle de reproduction des truffes: germination des spores, développement du mycélium, mycorhization, induction fructifère, développement et maturation des corps fructifères, ont été étudiées de manière très inégale.
La mycorhization contrôlée de *Tuber melanosporum* et *T. aestivum/uncinatum* est maintenant bien maîtrisée. Les résultats obtenus avec *Tuber magnatum* sont prometteurs. Le progrès le plus important en trufficulture découle de l'utilisation massive du plant mycorhizé; elle a permis d'introduire *T. melanosporum* dans des pays où elle n'existait pas: Australie, Israël, Nouvelle-Zélande, U.S.A. et récemment Maroc. Le plant mycorhizé est encore en cours d'amélioration par utilisation de différentes combinaisons de clones d'arbres performants et de truffes d'origine géographique variée, dans le respect de la biodiversité de l'association plante-champignon.
Des travaux récents de biologie moléculaire concluent que la truffe est hétérothallique, caractère qui n'influe pas sur l'aptitude des plants à produire des truffes, puisqu'ils sont inoculés avec des mélanges de spores.

La connaissance du mode de nutrition du mycélium et de l'ascocarpe est essentielle, car elle conditionne les façons culturales. *T. melanosporum* a un comportement symbiotique les premières années, puis saprophytique, voire de parasite de faiblesse. Le mécanisme de la nutrition de l'ascocarpe est toujours l'objet de discussions. La théorie de l'indépendance du corps fructifère vient d'être remise en question. Les implications sur la fertilisation des truffières sont importantes.
Le mécanisme du brûlé est mieux connu: on sait maintenant que la truffe «prépare son nid» en émettant des substances herbicides volatiles et dégradant fortement les minéraux et la matière organique du sol.
Contrairement au plant mycorhizé, les techniques de trufficulture ont peu évolué: de nouvelles sont maintenant proposées; elles prennent davantage en compte la biologie et l'écologie du champignon: contrôle indirect du chevelu racinaire par élagage des arbres et direct par taille racinaire, gestion de la croissance racinaire, modification des propriétés physico-chimiques des sols (décompactage des sols, allègement par apport de sables ou de granulats) apport d'amendements calcaires, fertilisation organique), stimulation de la formation des brûlés.
Les recherches à venir doivent viser à une meilleure connaissance de points-clés de la biologie et l'écologie du champignon: variabilité intraspécifique, nutrition saprophytique, rôle du calcium, fonctionnement du brûlé, induction fructifère, influence des stress hydriques et thermiques, eutrophisation des sols (azote), pour mieux orienter les pratiques culturales.

Introduction
La recherche sur la truffe est jalonnée par quelques dates-clés importantes.

- 1962: en examinant les mycorhizes qui dominent, en pépinière, dans le Piémont, sur les systèmes racinaires de pins Weymouth (*Pinus strobus*) abondamment producteurs de petites truffes blanches (*Tuber maculatum*), Fassi et De Vecchi, à l'I.N.P.L. de Turin, décrivent pour la première fois les mycorhizes d'un *Tuber*.
- 1967: Fassi, à l'I.N.P.L. et Fontana, à l'Université de Turin, réalisent la première synthèse mycorhizienne en pot entre une truffe (*Tuber maculatum*) et un arbre (*P. strobus*).
- 1968: les jeunes pins Weymouth mycorhizés en 1967 produisent des corps fructifères en pots. Toujours en 1968, lors du premier congrès international sur la truffe de Spoleto, Fontana décrit pour la première fois les mycelia de différents *Tuber* en culture.
- 1969: Palenzona, à l'I.N.P.L., réalise en pot les synthèses mycorhiziennes entre le noisetier commun (*Corylus avellana*) et trois espèces de *Tuber*: *T. melanosporum*, *brumale* et *aestivum*, en améliorant la technique de Mannozzi-Torini (1956).
- 1970: à l'I.N.R.A. de Clermont-Ferrand, G. Chevalier obtient, à son tour, les premières mycorhizes entre *T. melanosporum* et diverses espèces forestières.
- 1973: les premiers plants mycorhizés produits à grande échelle sont commercialisés à l'automne 1973 par la Société AgriTruffe, à Saint-Maixant (Gironde).
- 1977: les premières truffes (*Tuber melanosporum*) sont obtenues en décembre 1977, sous noisetier, en Bourgogne, à partir de plants produits à l'échelon industriel (noisetiers AgriTruffe plantés en 1973).
- 1979: les premières truffes (*Tuber uncinatum* et *T. mesentericum*) sont obtenues, sous chênes et noisetiers, 5 ans après plantation, dans une truffière de la Meuse (environs de Commercy).

L'avènement du plant mycorhizé est, sans aucun doute, l'évènement le plus important pour la trufficulture dans ces cinquante dernières années. Malheureusement, l'application des découvertes de la recherche sur la truffe s'est mal faite et les techniques de trufficulture n'ont pas suivi.

Les bases d'une trufficulture moderne
Les ouvrages de base sur la trufficulture sont ceux de Chatin (1869), De Bosredon (1887) et Pradel (1914). La première vraie méthode de culture de la truffe proposée aux trufficulteurs

est française. Elle a été conçue par De Bosredon, homme politique de la région de Sarlat et présentée dans son «Manuel de trufficulture» (1887). Trois points sont originaux, par rapport à la méthode utilisée par Talon, dans le Vaucluse (1810): l'utilisation de plants de chênes «bien racin» élevés en pépinière, au lieu de semis de glands en plein champ; le travail manuel du sol autour des arbres; la taille des arbres en cône inversé.

Le piochage en 1887
(De Bosredon)

en 1914
(Pradel)

et de nos jours
(Angellozzi)

En 1972, puis 1974, Grente et Delmas présentent un ouvrage «Perspectives pour une trufficulture moderne» qui établit les principes d'une nouvelle méthode de trufficulture. Elle s'inspire fortement de celle de De Bosredon mais la nouveauté est l'ensemencement des sols par l'intermédiaire de plants préalablement mycorhizés, et non plus de plants quelconques élevés en pépinière au champ.
Les bases sont:

- 1/ le choix judicieux et éventuellement l'aménagement du MILIEU: climat, topographie, altitude; sol (propriétés physiques, chimiques, biotiques),
- 2/ l'ensemencement du sol par l'intermédiaire de PLANTS MYCORHIZES artificiellement par la truffe,
- 3/ le choix de l'ESSENCE TRUFFIERE la mieux adaptée au milieu retenu,
- 4/ le maintien des CONDITIONS DE MILIEU dans un sens favorable à la mycorhization puis la fructification.

LE MILIEU

Le climat

De nombreuses études ont été effectuées. Les résultats ont été présentés dans les fascicules «Trufficulture et expérimentation en France», ainsi que dans les actes du congrès international sur la truffe tenu en février 2008 à Ménerbes, France (Chevalier & Wehrlen, Gregori, Olivier). Elles ont abouti à une meilleure connaissance des besoins en eau de la truffe et à une meilleure maîtrise des techniques d'irrigation des truffières et de paillage.

Le sol

Garcia-Montero *et al.,* (2007) soulignent à juste titre le peu d'intérêt dans le monde, au cours de

ces dix dernières années, pour les recherches sur l'environnement naturel et les plantations.
Du point de vue physique, la truffe (*Tuber melanosporum*) est capable de se développer dans une grande variété de sols, des sols sableux aux «pierriers» (tas de cailloux aux interstices pourvus de matière organique provenant de la décomposition des feuilles). Tout le monde s'accorde à dire que *T. melanosporum* affectionne les sols profonds, bien aérés, bien draînés, avec une réserve en eau suffisante pour approvisionner les corps fructifères en période sèche. La texture doit favoriser l'aération et le draînage. Il est cependant inexact de dire que la texture doit être obligatoirement «équilibrée». Les sols sableux s'avèrent d'excellents sols à truffes et pourtant la texture n'est pas équilibrée*. T. uncinatum* et *T. brumale* supportent des sols beaucoup plus lourds que *T. melanosporum*. Une étude récente menée en Auvergne a montré que, à partir de 40% d'argile, *T. melanosporum* ne peut plus se développer mais est remplacé par *T. brumale* ou *T. uncinatum* (Marley, 2008). La valeur brute du taux d'argile est à moduler en fonction de la pierrosité des sols, qui a été trop souvent négligée (Ricard, 2003) et de leur teneur en calcaire. Une forte teneur en argile, à l'origine de la «lourdeur» du sol, peut être compensée par une forte pierrosité, une forte activité biologique, un taux élevé en calcaire actif.
Du point de vue chimique, la plupart des chercheurs s'accordent sur le fait que *T. melanosporum* est strictement calcaricole et ne se développe que dans des «terrains calcaires». Ce n'est pas tout-à-fait exact. Il est possible de récolter des truffes (*T. melanosporum*) dans des terrains non calcaires, mais à complexe absorbant saturé en calcium.
Les chercheurs espagnols de Madrid et Valencia (Garcia-Montero *et al.*, 2006, 2007) ont bien mis en évidence que la calcium joue un rôle-clé en trufficulture. L'analyse statistique a montré que l'influence collective des facteurs édaphiques conventionnels (granulométrie, pH, fractions calcaires) sur la production de truffes (*T. melanosporum*) est basse. Par contre, une haute concentration en calcaire actif dans le sol favorise la production de truffes et les dimensions des brûlés; or le calcaire actif constitue une réserve importante de calcium échangeable. De fait, il existe une corrélation positive entre calcaire actif et calcium échangeable. La présence de calcaire actif et de calcium échangeable sont donc très importants pour la production de truffes. Il existe également une corrélation significative entre la concentration en calcaire actif et la pierrosité en surface.
La teneur en calcaire total est donc de faible valeur pour l'interprétation d'une analyse de sol. Sourzat (2008) considère que la mesure du calcaire actif n'a pas lieu d'être en trufficulture. Il est préférable de mesurer le calcium échangeable. Selon Jaillard *et al.,* (2007), «les rares auteurs qui ont mesuré le calcaire actif des sols truffiers font état de teneurs très faibles, de l'ordre de quelques pour mille seulement. Les sols truffiers sont faiblement carbonatés à décarbonatés, leur pH est neutre à alcalin, leur complexe d'échange est légèrement désaturé».
Pour nous, il est clair que l'élément important pour la production de truffe est bien le calcium. Nous avons constaté qu'il existe une corrélation entre la texture du sol et la teneur en calcium. Plus le sol est sableux, moins la teneur en calcium est élevée.
Les mêmes chercheurs espagnols suggèrent que le calcaire actif favorise la fructification *de T. melanosporum*, par rapport à celle *de T. aestivum*, *T. mesentericum* et *T. rufum*. Pour lutter contre ces espèces, l'apport d'amendement calcaire pourrait être utile alors que, pour *T. brumale*, il n'a pas d'effet.
Le taux de matières organiques dans les sols producteurs de truffes est très variable. Il ne doit pas, cependant, être très élevé; il peut être très bas. *T. melanosporum* préfère des terres avec des matières organiques évoluant vers la stabilisation. Les sites producteurs de truffes contiennent moins de matières organiques que les sites non producteurs (la couleur de la terre dans le brûlé est plus claire). Le rapport C/N est inférieur. Les sites à truffes contiennent moins de matières organiques libres («fraiches») à minéralisation rapide. La biomasse microbienne est moins élevée dans les sites à *T. melanosporum*. L'activité microbienne est également faible. La minéralisation du carbone en gaz carbonique est faible (Callot *et al.*, 1999). La matière organique dans le substrat de culture de l'arbre n'est pas indispensable à la mycorhization des plants qui peut être importante dans des substrats exclusivement minéraux (Dupré *et al.,* 1982). L'alimentation carbonée du champignon est assurée exclusivement par

les glucides racinaires. Ce n' est que plus tard que la matière organique peut jouer un rôle dans la fructification. La truffe montre une grande capacité à s'adapter à des biotopes différents que l'analyse ne caractérise que partiellement au niveau de la «parcelle» (Olivier *et al.*, 2008).

Conséquences pour la trufficulture

L'analyse de terre classique est toujours nécessaire, mais il est indispensable à l'avenir de mieux cibler les éléments importants à analyser qui sont la texture (en relation avec la pierrosité) et la teneur en calcium échangeable, sachant qu'il existe une interaction entre le taux de calcium échangeable indispensable à la truffe et les propriétés physiques du sol, le taux de calcium devant être beaucoup plus important dans un sol argileux que dans un sol sableux.

Correction de la texture

La truffe (*T. melanosporum*) craint particulièrement les terres lourdes, compactes, non aérées, à mauvais drainage. Chatin est un précurseur (1869) quand il conseille: «Du sable pourrait aussi être utilement ajouté à une terre trop argileuse, et réciproquement». En fait, il existe bien d'autres matériaux que le sable. Le gravier calcaire («castine»), la pouzzolane ont donné de bons résultats.

Amendement des sols acides

L'idée d'amender les sols acides pour les rendre aptes à la production de truffes n' est pas nouvelle. Toujours en 1869, Chatin écrit «On pourra améliorer les truffières ou même les rendre possibles là où elles ne s'établiraient jamais naturellement en ajoutant au sol une quantité suffisante de calcaire». Les Américains (Garland, 2001), les NéoZélandais (Hall et coll.), les Australiens (Malajczuk & Amaranthus, 2007) ont réussi à cultiver la truffe (*T. melanosporum*) dans des sols a priori défavorables (acides) en apportant des quantités importantes d'amendement calcaire. La truffe de Bourgogne (*T. uncinatum*) a pu être cultivée en Limousin, dans des sols sur micaschistes, à pH initial 5 (Chevalier, 2003).

LE PLANT MYCORHIZE

Qualité des plants et performances

La supériorité des plants mycorhizés certifiés sur les plants traditionnels n'est maintenant plus contestée. Les premières truffes peuvent être récoltées 3 ans après plantation. La production est très variable car elle dépend de nombreux facteurs liés au climat, au sol, aux méthodes culturales. Les meilleurs plants mycorhizés ne produiront jamais s'ils sont plantés en sol acide. En conditions optimales, il est possible d'obtenir des rendements de 130 kg et plus à l'hectare, après 15 ans de plantation, pour *T. melanosporum*, et plus de 300 kg, pour *T. uncinatum*, mais ce sont des cas exceptionnels. Il est également possible d'obtenir 100% de plants producteurs (pourcentage obtenu en cumulant les plants en production et ceux qui ont cessé de produire). Le fait que la totalité des plants mycorhizés puisse un jour produire, bien qu' ils soient tous différents génétiquement, laisse penser que tout arbre est apte non seulement à mycorhizer mais à produire des truffes. Il n'y a pas d'individus «résistants» à la mycorhization, comme il existe des individus résistants à certaines maladies. Le mythe du «gland truffier» s'effondre. Chez les pépiniéristes producteurs de plants mycorhizés sérieux, le plant non mycorhizé par la truffe n'existe pas. Il n'y a plus, non plus, de problèmes de contaminations par les champignons ectomycorhiziens classiques des serres (*Sphaerosporella brunnea*, *Tomentella*, hébélomes).

Une amélioration constante

La production de plants mycorhizés par semis de glands ou de noisettes présente de nombreux inconvénients qui ont été abondamment cités (Guinberteau *et al.,* 1990).

Différentes étapes jalonnent l'amélioration du plant mycorhizé par multiplication végétative.

1978: G. Chevalier, J. Grente, J. Garbaye, I. Ferraby & C. Desmas-Rodary mycorhizent avec *T. melanosporum* des boutures racinées de chêne rouvre (*Quercus petraea*).

1987: Guinberteau *et al.,* à l'INRA de Bordeaux, mycorhizent avec *T. melanosporum*, des vitroplants de noisetiers clonés.

Les premiers corps fructifères de *T. melanosporum* produits par un chêne pubescent multiplié par bouturage ont été récoltés en décembre 1997 dans le sud-ouest (Belvès-en-Périgord), soit exactement 20 ans après l'obtention de la première truffe sous noisetier dans l'Yonne (plant inoculé en 1991 à l'INRA de Clermont-Ferrand, puis planté en mars 1993).
En janvier 2000, c'est la production de corps fructifères de *T. melanosporum* sous trois chênes pubescents du même clone multipliés par vitropropagation à l'Université de Nancy, inoculés à l'INRA de Clermont-Ferrand et plantés en mai 1990.
Les avis concernant le concept d'arbre «bon producteur» divergent. Dans le rapport de 2008 «Trufficulture et expérimentation en France», on peut lire, page 12, que «le concept d'arbre bon producteur ne peut être aujourd'hui validé. Aucune donnée de récolte ne donne un plus au profit de ce type de matériel sur la base de l'origine arbre bon producteur». Une expérimentation menée depuis 9 ans par l'INRA de Clermont-Ferrand dans le sud-est montre, au contraire, que l'origine géographique des truffes qui ont servi à inoculer les plants d'un même clone de chêne pubescent ont une influence très importante sur la production (tableau ci-dessous).

Production (en grammes) d'un clone de chêne pubescent (15 plants) , inoculé avec des truffes (*T. melanosporum*) d'origine géographique différente , comparée à celle de plants du commerce issus de semis (licence INRA)

5,5 ans de plantation)

Origine / Saison des truffes	2004-2005	2005-2006	2006-2007	2007-2008	Total
Sud-ouest (Dordogne)	350	1070	925	5790	8135
Sud-est (Hautes-Alpes)	100	/	210	2250	2560
Centre-est (Yonne)	/	/	250	2215	2465
Plant mycorhizé du commerce	/	800	1330	3390	5520
Total	450	1870	2715	13645	18680

Ces résultats confirment indirectement ceux de Murat *et al.*, (2004) mettant en évidence une hétérogénéité génétique chez *T. melanosporum* beaucoup plus forte qu'on ne le pensait (Gandeboeuf *et al.*, 1997; Callot *et al.*, 1999). Dans la recherche du partenaire le plus actif pour la production de corps fructifères dans le couple arbre-champignon, il est maintenant probable que ce soit la truffe.
Les nouvelles approches sur la sexualité de la truffe (Rubini *et al.,* 2007; Murat & Martin, 2008) peuvent-elles avoir une influence négative sur la qualité des plants mycorhizés? Non, car les plants sont inoculés avec des mélanges de spores et il est impossible qu'il n'y ait pas de rencontre et de fusion entre mycelia de signes opposés.

Le plant mycorhizé par *T. magnatum*

La maîtrise de la culture d'une espèce de truffe implique la plantation de plants mycorhizés fiables. Ce n'est pas le cas pour *T. magnatum*. De nombreux plants censés mycorhizés par cette espèce ont été plantés en Italie, quelques-uns en France, depuis le début des années 80. Les résultats positifs ont été rares (Giovannetti, 1990). Il en a résulté un doute sur la qualité des plants mycorhizés. Par l'analyse des isoenzymes, Bullini *et al.,* ont démontré, en 1994, qu'aucun des plants de *T. magnatum* du commerce testés n'était porteur de mycorhizes de cette espèce, mais que les profils enzymatiques des mycorhizes correspondaient à ceux de *T. borchii* et *T. maculatum*. En 2001, Mello *et al.,* et Rubini *et al.,* par analyse moléculaire,

démontrent que la morphologie des mycorhizes de *magnatum* ne correspond pas à celle présentée dans la littérature scientifique italienne depuis 1978.
Quelques descriptions de cultures de *T. magnatum* obtenues par bouturage de fragments de gléba ou à partir de mycorhizes ont été également faites, mais elles sont douteuses. En effet, la plupart des chercheurs européens spécialistes des cultures de mycélium de truffe qui ont essayé d'isoler des cultures de *T. magnatum* en utilisant les techniques classiques ont échoué.
En France, M. Buee, à l'INRA de Nancy, a obtenu, en janvier 2006, des cultures mycéliennes de *T. magnatum* avec des hairy roots de peuplier (*Populus alba*) et de ciste (*Cistus incanus*). Ce travail a fait l'objet d'un brevet I.N.R.A.
A l'I.N.R.A. de Clermont-Fd, la synthèse des mycorhizes de *T. magnatum* sur chêne pubescent est effectuée couramment depuis 2008 par D. Mabru, à partir de cultures mycéliennes sur hairy roots.

Spores de *T. magnatum* germées au contact d'une racine transformée de *Q. pubescens* (photo D. Mabru, I.N.R.A. Clermont-Ferrand)

Culture mycélienne de *T. magnatum* au contact d'une racine transformée de *Q. pubescens* (photo D. Mabru, I.N.R.A. Clermont-Ferrand).

La production de mycorhizes de *T. borchii* ou *T. maculatum* sur des plants inoculés avec des corps fructifères de *T. magnatum*, phénomène qui a induit en erreur quelques chercheurs, peut s'expliquer (Chevalier *et al.,* 2005). En effet, les corps fructifères de *T. magnatum* portent, à leur surface et à l'intérieur de la gléba, des propagules de champignons mycorhiziens étrangers (incluant diverses espèces de truffes) qui vivent dans l'environnement immédiat des truffes. Ces propagules sont transportées à l'intérieur des corps fructifères par la macrofaune du sol. Comme les spores de *T. magnatum* germent difficilement, cette espèce est désavantagée par rapport aux contaminants dont les spores germent beaucoup plus facilement et qui, de cette façon, prennent le dessus sur la truffe noble et colonisent le système racinaire des plants inoculés.

LES ESSENCES TRUFFIERES

Les truffes sont capables de mycorhizer toutes les essences à ectomycorhizes. Le choix d'une essence dépendra essentiellement de son adaptation aux conditions climatiques et édaphiques du futur site de plantation. Des facteurs importants dont il faudra tenir compte sont la résistance au froid en hiver, aux fortes températures en été, à la sécheresse, au calcaire.

Pour *T. melanosporum*, les 3 essences les plus utilisées en trufficulture sont le chêne vert (*Quercus ilex*), le chêne pubescent (*Quercus pubescens*) et le noisetier commun (*Corylus avellana*). Le chêne vert est aussi précoce que le noisetier (production possible 3 ans après plantation). C'est maintenant l'essence la plus plantée en France (60% dans le sud-ouest). Il est plus résistant au froid qu'on ne le pensait. Avec le réchauffement climatique, il va être de plus en plus utilisé et son aire de plantation est en train de se déplacer vers le nord de la France. Le noisetier est de moins en moins planté à cause des résultats de production irréguliers, de ses fortes exigences en eau et de sa tendance à produire *T. brumale*. D'autres essences sont utilisées à un degré moindre: chêne pédonculé (*Quercus robur*), chêne kermès *(Q.coccifera*), tilleuls (*Tilia sp.*), charme commun (*Carpinus betulus*), charme-houblon (*Ostrya carpinifolia*), noisetier de Byzance (*Corylus colurna*).

Pour *T. uncinatum*, les essences les plus utilisées sont les chênes: pédonculé, rouvre (*Q. petraea*) et pubescent, le noisetier commun, le charme commun, le pin noir d'Autriche (*Pinus nigra austriaca*). Le cèdre de l'Atlas (*Cedrus atlantica*), à cause de ses besoins en eau limités, a donné d'excellents résultats. Le charme-houblon, rare en France, s'est avéré aussi un excellent producteur. D'autres essences sont utilisées à un degré moindre: le hêtre (*Fagus sylvatica*), le noisetier de Byzance. En année sèche, les résineux donnent de meilleurs résultats que les feuillus. Les conséquences néfastes du réchauffement climatique devraient inciter à les employer davantage.

LE MAINTIEN DES CONDITIONS DE MILIEU

La truffe noire est particulièrement sensible aux variations de son environnement (sol, climat). «Elle peut être considérée comme un indicateur vulnérable de l'état du milieu dans le contexte spécifique de l'évolution de la pelouse calcicole vers les peuplements arborés» (Olivier *et al.,* 2008). Au cours des douze dernières années s'est développée en France une approche écologique qui a profondément changé la vision de la trufficulture. Cette approche d'écologie plus globale a permis de mieux connaître les facteurs actifs et les indicateurs pertinents de la dynamique des écosystèmes.

La flore végétale

La flore végétale des truffières a été particulièrement étudiée par P. Sourzat. Les truffières naturelles et plantées sont remarquables par les associations végétales traduisant des organisations phytosociologiques qui, malgré des variations locales, peuvent constituer des indicateurs. Ce travail d'écologie botanique s'est traduit dans une évolution de la conception des pratiques culturales. Sans exclure le travail total du sol, qui a donné, dans quelques cas, d'excellents résultats, les observations effectuées en France au cours des XIème et XIIème

Plans ont montré l'importance du peuplement floristique non seulement comme indicateur, mais aussi comme auxiliaire.

Conséquences pour la trufficulture
Les recommandations ont fortement évolué vers des modes de conduite dite «enherbée» recouvrant deux pratiques: laisser se constituer après plantation le cortège floristique naturel ou intervenir sur la couverture du sol par semis de graminées «légers» c'est-à-dire à densité suffisamment faible pour éviter «l'encroûtement végétal» de la surface du sol (Olivier *et al.,* 2008).

La flore fongique
Les champignons de la macroflore peuvent être retenus comme indicateurs écologiques.
A la suite d'observations en relation avec la problématique de *Tuber brumale*, P. Sourzat a défini, au début des années 2000, les notions de «dynamique du milieu» et de «cortège fongique». Ce dernier correspond à la succession et la cohabitation d'espèces sur un système racinaire au cours de son vieillissement. Il existe une véritable structuration des cortèges fongiques, dans le temps (jusqu'à la truffière fermée) et dans l'espace (du tronc à la périphérie du brûlé). Parmi les principales espèces mycorhiziennes fructifiant en truffières, les plus communes sont les sclérodermes, inocybes, russules, bolets, tricholomes, tous champignons mycorhiziens donc susceptibles d'interférer avec la truffe.
Dans une truffière plantée avec des arbres mycorhizés au départ, la dynamique de ce cortège est liée directement à l'expression de la virulence de *T. melanosporum* qui «ouvrira» ou «fermera» les portes aux autres espèces.

Conséquences pour la trufficulture
Le cortège fongique est fragile et sensible à des perturbations: le travail linéaire désorganise le système et peut être impliqué dans la prolifération de *Tuber brumale*, de même que les excès d'eau ou de matière organique.

La faune
Les travaux de G. Callot et J.C. Pargney ont mis en avant le rôle de la macrofaune et de la mésofaune comme contribuant à la vie du sol (aération, et structuration, décomposition de la matière organique, vection des spores). Il faut cependant relativiser: il est possible de récolter des truffes dans des sols sableux où les vers de terre sont absents.

Conséquences pour la trufficulture
L'aération du sol par la faune tellurique est surtout importante dans les sols lourds.
Il faut mettre en oeuvre des pratiques susceptibles d'aider la faune du sol, comme le paillage à l'aide de pierres, branches, pailles, mulchs artificiel) et éviter les pratiques pouvant la contrarier (tassement, certains pesticides). L'«inoculation» du sol avec des vers de terre serait à tester.

La truffe

La dynamique de la mycorhization
Le développement des mycorhizes et celui du système racinaire ne sont pas synchrones. La mycorhization par la truffe a toujours un certain retard par rapport au front de progression des racines. La vitesse de progression de la mycorhization par la truffe est connue. Une croissance trop rapide de l'arbre présente des inconvénients. En particulier, si le système racinaire de l'arbre se développe trop rapidement, la mycorhization n'arrive pas à suivre la périphérie du système racinaire qui alors «piège» des champignons indésirables, en particulier d'autres espèces de truffes, dont *T. brumale* et *T. aestivum* (Chevalier *et al.,* 1982).
La croissance de l'arbre doit cependant être suffisante. Elle influe sur la rapidité de l'entrée en production. De Bosredon écrivait déjà: «Plus l'arbre se développera rapidement, promptement et, par la suite, plus la truffière sera rapidement constituée»

Conséquences pour la trufficulture

Il est indispensable de contrôler la croissance racinaire de sorte que la mycorhization par la truffe suive la progression des racines. Ce contrôle peut être indirect, par une taille aérienne appropriée, ou direct, par une taille racinaire (par exemple, par passage à la limite du brûlé d'un outil à dents qui sectionne les extrémités racinaires). L'herbe correctement gérée dans les interangs peut aussi constituer un frein au développement des racines (Chevalier et Wehrlen, 2008).

Les capacités saprophytiques de la truffe

Le mycélium de *T. melanosporum* croît en présence de nombreuses sources simples de carbone organique différentes. Il est aussi capable de croître en présence de sources de carbone organique qui ne sont pas directement assimilables, c'est-à-dire de substances carbonées complexes: cellulose, amidon, lignine, chitine, tanins, acides humiques et leurs produits de dégradation (Barry, 1997). Le comportement saprophytique de type secondaire s' exerce aussi bien dans le milieu environnant que sur l'arbre-hôte au niveau du système racinaire.

Le mycélium de la truffe est capable de coloniser le parenchyme cortical des racines (Chevalier, 1973). Il est également capable de pénétrer dans les cellules externes de l'écorce racinaire particulièrement riches en tanins. les hyphes montrent une forte capacité de dégradation des matériaux du cortex racinaire. Elles démantèlent les constituants cellulaires (parois et tanins) en les fragmentant et les incorporant aux stromas, qui sont des boursouflures brunâtres, constituées d'hyphes agrégées. Les résidus tanifères intégrés au stroma sont progressivement fragmentés et la fragmentation assimilable est mobilisée par les hyphes. Les stromas constituent donc des structures végétatives privilégiées, dotées d'une certaine autonomie, vis-à-vis de leur nutrition organique.

Ce comportement saprophytique de type secondaire n'apparaît pas immédiatement sur le plant mycorhizé, puisque l'on peut obtenir une bonne mycorhization sur des substrats exclusivement minéraux où les sucres ne sont fournis que par les racines de l'arbre. Le comportement saprophytique est l'un des préalables à la fructification. Il se traduit par l'«usure» du système racinaire, que les Anciens observaient déjà au XIXème siècle (De Bosredon, 1887). Lorsque le comportement saprophytique devient trop important et que le champignon demande à l'arbre beaucoup plus qu'il ne lui apporte, il devient parasite. L'arbre en souffre, prend un aspect chétif et peut finir par mourir.

Conséquences pour la trufficulture

Les tanins des racines constituent une part importante de l'alimentation organique du champignon. Au fur et à mesure de l'extension du brûlé, le champignon «mange» le système racinaire. C'est la raison pour laquelle il est très difficile de trouver des mycorhizes de truffe à proximité des corps fructifères. Dans la partie ancienne du brûlé où la truffe n'a plus rien à consommer, l'herbe repousse et il se reconstitue un nouveau chevelu racinaire qui va héberger *T. brumale* ou des basidiomycètes mycorhiziens (hébélomes). En avant du brûlé, dans l'espace de conquête, il y a un chevelu abondant prêt à être consommé par la truffe. Lorsque les brûlés se sont rejoints, les truffes deviennent petites et la production diminue puis finit par disparaître. Avant qu'il ne soit trop tard, il faut «ouvrir» la truffière, en éliminant une partie du système aérien des arbres et, avec un outil adéquat, casser le système racinaire, pour provoquer la formation de radicelles qui vont servir d'aliment à la truffe.

Le brûlé

Bien des mystères entourent encore le mécanisme du brûlé; par exemple, pourquoi est-il possible de récolter des truffes (*T. melanosporum*) sous un tapis d'herbe verdoyant? Ce phénomène est cependant l'exception et le brûlé précède le plus souvent la truffe. Il joue un rôle essentiel dans la production de truffes en préparant sa venue.

S'il est établi que le brûlé est provoqué par l'action phytotoxique du mycélium de la truffe, les effets physiques et chimiques physiques provoqués par la truffe sur le sol commencent

seulement à être connus. Les conditions environnementales créées par le brûlé sont très favorables au développement végétatif de la truffe et à sa fructification. Il provoque une modification de la structure de la terre de surface. Il est bien connu qu'une terre lourde, hors brûlé, peut devenir légère, cendreuse, dans le brûlé. L'effet du calcium sur la structure du sol est bien connu. Il n'en est pas de même de l'effet sur la structure de la dégradation des minéraux par la truffe (Neel *et al.,* 2008).

Le brûlé se traduit également par une modification de la composition chimique du sol.

Garcia-Montero *et al.,* (2006, 2007) ont étudié pendant plusieurs années l'effet du brûlé sur le taux de calcaire actif dans le sol. Le développement de la truffe et les conditions écologiques des brûlés favorisent la formation de quantités importantes de calcaire actif et de calcium échangeable. La quantité de calcaire actif est significativement plus haute et celle de calcaire total plus basse dans les brûlés qu'à l'extérieur. Le mycélium de *T. melanosporum* peut acidifier son environnement immédiat et solubiliser différentes fractions carbonatées dans les brûlés. Par la suite, les conditions de milieu particulières dans les brûlés peuvent favoriser une précipitation de carbonate secondaire avec une nette augmentation du calcaire actif. Or, l'activité de *Tuber melanosporum*, la fructification et la taille des brûlés sont simultanément favorisés par une forte concentration en calcaire actif et en calcium échangeable. Il en résulterait un processus de «feedback» qui, en retour, favoriserait le développement du mycélium de la truffe. Ce phénomène expliquerait que le développement de *T. melanosporum* et la production de truffes augmenteraient avec la taille du brûlé, elle-même en relation avec la bonne croissance du champignon. Enfin le processus de feedback fournirait à la truffe un avantage supplémentaire sur ses compétiteurs (en particulier *T. aestivum* et *T. mesentericum*), quand le calcaire actif atteindrait une concentration élevée dans les brûlés à *T. melanosporum*. Le calcaire actif est un facteur essentiel dans la fructification, le développement des brûlés et l'agressivité de *T. melanosporum* à l'encontre des autres espèces de truffes.

Le champignon dégrade également la matière organique. La teneur en matière organique dans les brûlés est plus faible que dans les sites enherbés. Cette diminution explique la couleur plus claire du sol dans le brûlé. Sa nature varie également entre les zones brûlées et celles qui ne le sont pas. Le brûlé contient moins de matière organique libre, «fraîche», à évolution rapide, que de matière organique stable, qui correspond à la matière organique liée à la fraction argileuse et contenue dans la fraction inférieure à 50 microns. C'est une matière organique très évoluée, structurante, à évolution lente, fortement polymérisée. La biomasse et l'activité microbiennes sont plus faibles que dans la zone non brûlée. Il est très possible que l'agressivité de la truffe se manifeste, en plus de son action sur la flore et sur le système racinaire de l'arbre-hôte, également sur la vie microbienne du sol dont l'activité chute à l'endroit où sa manifestation est la plus évidente.

La truffe puise également des éléments nutritifs dans les racines en train de mourir (Plattner et Hall, 1995). Au laboratoire, les mycorhizes de la truffe sont capables de dégrader en quelques mois la tourbe blonde (Chevalier non publié).

La production de truffes peut être associée à la vigueur de l'arbre et la progression du brûlé. En 2003, P. Sourzat propose le concept de «virulence» du champignon, qui traduit simplement qu'un bon écosystème truffier perdure par sa propre agressivité vis-à-vis de son environnement. La virulence de *T. melanosporum* se traduit par des brûlés très agressifs qui progressent de 15 à 25 cm par an, détruisant la flore herbacée de l'espace de conquête. Cette agressivité, lorsqu'elle est optimum, peut conduire jusqu'à détruire un tapis dense de fétuque ovine semée. La vigne même ne résiste pas à l'agression de ce brûlé. Au fur et à mesure qu'il avance, le système racinaire de l'arbre est dégradé au même titre que la végétation de surface est détruite. La virulence peut être appréciée par la relation entre le rayon de la canopée de l'arbre, celui du brûlé et la progression annuelle du brûlé. Elle est bonne quand le rayon du brûlé est égal ou supérieur à 1,5 fois le rayon de la canopée de l'arbre.

Garcia-Montero *et al.,* ont démontré récemment que, chez quatre essences symbiotes de la truffe, dont *Quercus ilex ssp. ballota* en particulier, la taille des brûlés est un facteur très significatif dans la production de truffes. Elle dépend elle-même fortement de la concentration en calcaire actif du sol.

La notion de virulence entraîne une relation d'ajustement entre le développement de l'arbre (pas d'excès de vigueur, mais aussi pas de faiblesse, contrairement à ce qui était parfois affirmé) et la progression du champignon.
Enfin, une caractéristique du brûlé est qu'il assécherait le sol (Sourzat, 2008), avec pour conséquence la diminution de sa réserve en eau. Le phénomène de sécheresse constaté à l'intérieur du brûlé a évidemment pour corollaire une transformation de l'état organique et microbien du sol.

Conséquences pour la trufficulture
Par le brûlé, la truffe «prépare son nid» avant la fructification (Dessolas *et al.,* 2007, 2008). Elle dégrade les minéraux et la matière organique, elle allège le sol. Elle favorise la production d'une quantité importante de calcaire actif et de calcium échangeable. En conditions difficiles (sol lourd), les brûlés vont être peu accentués et la truffe va mettre 15 - 20 ans avant de fructifier. En sols pauvres en calcium, les brûlés vont régresser puis disparaître au bout de quelques années. Sauf en cas de sols sableux, par définition bien aérés et à bonne porosité, il faut «aider» la truffe à faire son brûlé par un travail du sol approprié pour l'aérer, recarbonater en surface, si nécessaire, éliminer les mauvaises herbes qui pompent l'eau et encroûtent la surface.
Il ne suffit pas de «laisser agir la nature», comme le préconisent de nombreux auteurs, anciens ou non. Si nécessaire, il sera utile d'apporter des amendements calcaires (pratique déjà préconisée par Chatin en 1869!).

LA M.R.T. (méthode raisonnée de trufficulture)
Les deux méthodes de trufficulture les plus pratiquées en France («méthodes «Pallier» et «Tanguy») ne donnent pas toujours des résultats satisfaisants.
La méthode Pallier présente l'avantage de permettre une production rapide, mais le grave inconvénient est, comme il l'a été signalé, sur certain sites infectés, de propager les contaminants, dont des espèces de *Tuber* indésirables, en particulier *T. brumale*. Cependant, dans certaines situations où il n'y a pas de contaminants et où les qualités physico-chimiques du sol sont optimales, il faut reconnaître qu'elle peut donner d'excellents résultats, des truffières âgées de 30 ans, mais correctement cultivées, continuant à produire abondamment.
La méthode Tanguy permet de limiter les contaminants mais elle a le grave inconvénient d'aboutir à une production tardive (plus de 10 ans après la plantation). La confrontation des tactiques de la truffe et de ses ennemis permet d'aboutir à une nouvelle méthode basée sur la **culture différentielle de la zone qui va produire ou a commencé à produire et de celle qui n'a pas encore produit ou qui a cessé de produire**. Les pratiques culturales de la M.R.T. visent à favoriser l'agressivité de la truffe (lui «donner un coup de main»), en intervenant sur:

- le sol (travail du sol, éventuellement apport de calcaire, de fertilisants, de matériaux pour alléger le sol)
- l'arbre (maintenir un équilibre entre sa vigueur et l'expression d'agressivité de la truffe, indiquée par l'étendue du brûlé: taille, élagage).
- le climat: arrosage, paillage, pose de branchages…

La M.R.T. préconise, pour les différentes phases de la vie de la truffière, des actions spécifiques:

1/ Implantation de la truffière
Deux éléments clefs sont à prendre en compte: le calcaire actif et la compacité du sol.
Dans l'analyse de sol, il est nécessaire dorénavant de considérer l'«indice de compacité». Si le sol est trop «lourd», l'alléger par apport de sable, de granulat, de pouzzolane.
En cas d'insuffisance en calcium, apporter un amendement calcaire de fond (calcaire pulvérulent pour action rapide + calcaire en grain pour action de longue durée).
L'importance de l'antécédent cultural est moindre que ce que l'on pensait. Diverses expériences récentes montrent qu'un plant bien mycorhizé par *T. melanosporum* planté dans un sol infesté

par *T. brumale*, à l'emplacement d'un plant producteur de cette espèce qui a été arraché, produit *T. melanosporum*

2/ Plantation et installation de l'arbre mycorhizé

Il faut installer l'écosystème truffier avec le cortège fongique et des conditions de milieu et de culture favorables, c'est-à-dire la pelouse calcicole. On utilisera donc la végétation herbacée spontanée, si elle est jugée comme favorable à la truffe. Des «trous» seront aménagés, dans l'herbe, par piochage ou à défaut désherbage chimique avec un défoliant, à l'emplacement des futurs plants mycorhizés.
Si la végétation herbacée préexistante est de mauvaise qualité ou si le sol est cultivé, il peut être utile de semer de l'herbe dans les inter-rangs, en utilisant des graminées rustiques et des légumineuses adaptées à la situation locale, mais à des densités plus faibles que pour la constitution de prairies.

3/ Phase de préfructification (développement du brûlé)

L'apparition du brûlé est une phase importante avant la fructification. La truffe «prépare son nid». Il faut donc l'«aider» à faire son brûlé en décompactant le sol et l'aérant, avec un outil adapté (bigot, grelinette, pioche à herser Becker, motoculteur, outil spécialisé, qu'il reste à inventer, etc.), ceci d'autant plus que les conditions physiques de sol s'éloignent de l'optimum (sol «lourd», compact, peu aéré). Les excellents résultats obtenus par E. Angelozzi, près d'Ascoli Piceno (Italie), qui pioche intensément ses brûlés, confirme ces recommandations.
L'apport de calcaire peut aussi aider le brûlé à se former. Il en est de même du désherbage chimique avec un désherbant non systémique.
La taille doit être initiée, soit taille méthode De Bosredon en cône inversé, soit «taille bonzaï» méthode Angelozzi.

Le mauvais exemple: truffière enherbée
(sud-ouest de la France)

Le bon exemple: truffière travaillée manuellement
(truffière Angelozzi, Roccafluvione)

4/ Phase de fructification

Le travail des brûlés doit se poursuivre, avec les mêmes outils. Il en est de même pour la taille des arbres. La gestion de l'herbe dans les inter-rangs est assurée par gyrobroyage (un seul passage dans l'année!).
Le trufficulteur avisé gèrera la progression de ses brûlés par l'enherbement et le travail du sol (éventuellement taille des extrémités racinaires avec un outil à lame), l'objectif étant d'étendre la zone de système racinaire mycorhizé au détriment de la zone enherbée.

5/ Phase de déclin

Quand les brûlés se rejoignent, la production de truffes est effective sur toute la surface de

la truffière. Il arrive cependant un moment où, même après éclaircissage des arbres, la taille des truffes diminue, puis la production finit par s' arrêter. Il ne faut pas attendre ce stade ultime pour réagir.
Alors que, dans les phases précédentes, on utilisait un outil qui aère le sol mais ne coupe pas les racines (outil qui «pioche» ou «bêche»), il s'agit, cette fois, de régénérer le système racinaire en provoquant la formation de chevelu. Les outils appropriés devront être, cette fois, des outils à lames qui coupent les racines, sans cependant endommager gravement les grosses racines.

Sous-soleur Multifonctions avec décapeur Becker

Pioche-herse Becker

LES VOIES DE L'AVENIR

La trufficulture dépend fortement des évolutions du climat au cours du prochain siècle. Si les modifications actuelles persistent, les zones de production de truffes vont se déplacer vers le nord. Dans cette hypothèse, le sol n'est pas le facteur limitant. De nombreux sols français de Bourgogne et Lorraine sont aussi favorables à la truffe que ceux du Quercy, du Périgord ou de Provence, en particulier parce qu'ils ont la même origine géologique. Le potentiel truffier français est énorme, d'autant plus qu'il est possible de cultiver la truffe dans des sols au départ très acides, en apportant une dose massive d'amendements calcaires. La truffe est maintenant cultivée dans le Limousin (Chevalier, 2003). La culture en Bretagne est dans un avenir proche.
Le plant mycorhizé va encore être amélioré par une sélection plus poussée et l'emploi de la multiplication végétative. La sélection va porter à la fois sur l'arbre et sur le champignon. Sur l'arbre, les critères pris en compte seront, entre autre, le port aérien et racinaire, la vitesse de développement du système racinaire, la résistance au calcaire, aux maladies, à la sécheresse, au froid, voir l'«appétabilité des racines» pour la truffe *T. melanosporum*, dont la variabilité génétique est plus importante que prévue et favorisée par la reproduction sexuée (hétérothallisme) sera sélectionnée pour son adaptation aux caractéristiques physico-chimiques des sols et au climat. Par exemple, pour contrer les effets néfastes du réchauffement climatique, on pourra introduire en France des truffes du sud de l'Espagne (Andalousie). L'objectif est de sélectionner les meilleurs «couples plante-champignon», en fonction des conditions pédoclimatiques des zones de trufficulture. Le couple arbre-champignon champion, le meilleur partout, est une utopie.

Enfin, de nouvelles techniques de trufficulture, adaptées à chaque espèce, basées sur le travail différentiel des zones en production, des zones qui ne le sont pas, des zones qui ne le sont plus, la fertilisation organique des truffières, l'irrigation raisonnée, le paillage vont être progressivement mises en pratique. Face au réchauffement climatique, les trufficulteurs doivent garder le moral. Pour la conduite des truffières et les moyens connus pour pallier, au moins en partie, aux aléas du climat, l'état des lieux décrit par L. Genola (2008) est réconfortant: «Le constat reste optimiste, concluant sur l'absence de fatalité, mais la nécessité d'investissement en travail».

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ESTABLISHMENT OF FINNISH TRUFFLE ORCHARDS

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Abstract
Truffle is an expensive fungus that forms ectomycorrhizal association with forest trees or with annual plants. It forms edible fruit bodies below ground. Truffles do not belong to the traditional Finnish kitchen nor are they a common topic in the media. In 2006, 10 truffle orchards were established in three small cities in Finland with a total of 200 oak seedlings inoculated with *Tuber eastivum*. The oak seedlings survived the harsh long Finnish winters and the low temperature. Also, the *T. eastivum* mycorrhiza was present in all orchards. As a result of this project 1200 commercial oak, hazel and lime seedlings were planted during 2006-2008 and the number of orchards increased to be 20 around Finland.
Key words: truffle cultivation, *Tuber aestivum*, *Quercus robur, Quercus pubescens*, *Corylus avellana*, mycorrhizal growth.

Introduction

Truffles are the most expensive edible fungi in the world. Truffles are hypogeous gourmet fungi, belonging to the family *Tuberaceae*, the order *Tuberales* and phylum *Ascomycotina*, which grow in symbiosis with certain trees such as oak (*Quercus robur*), hazel (*Corylus avellana*) and birch (Chevalier & Frochot, 1979; Harley & Smith, 1983; Pomerico *et al.,* 2006). The life cycle involves a first phase of growth as filamentous mycelium, a second phase of symbiotic association of the fungal hyphae with the host root (ectomycorrhiza) and finally the organization of a hypogeous fruit body with asci and ascospores (Peterson & Bonfante, 1994). There is a growing international market for truffles, while annual world production of wild truffle over the past century has dropped dramatically from ca. 1000 to 200 tons (Hall *et al.,* 2003). This decline has led to the establishment of truffle orchards, while truffle production begins after 5-7 years following orchard establishment (Olivier *et al.,* 2002). Currently, more than half of the harvested truffles are produced in cultivated orchards. Truffles are cultivated e.g. in France, Italy, Spain and other parts of the world. The most Northern cultivation of truffles thus far has been conducted in Gotland Sweden (Wedén *et al.,* 2004a). *T. aestivum* grows also naturally in Gotland in oaks and hazel (Wedén *et al.,* 2004b). *T. melanosporum* (Périgord black truffle) is economically the most valuable black truffle. Perigord black truffles mature during the winter. In contrast, *T. aestivum* (Burgundy black truffle) produces fruits during the late autumn. For this reason *T. aestivum* was chosen for the cultivation experiments in Finland. Truffles do not belong to the traditional Finnish kitchen nor are they a common topic in the media. The establishment of truffle orchards in Finland was done in order to study seedlings development, mycorrhizal survival in long winter and low temperature and different orchards managements.

Materials and Methods

Establishment of truffle orchards

Finnish truffle orchards were established in 2006 in Savo area with a total of more than 200 seedlings. Each orchard consists of 20 *Quercus pubescens* inoculated with *Tuber aestivum*. The inoculated seedlings were obtained from the French nursery Ropin pépiniéres. The seedlings and the *T. aestivum* inoculums used to produce the mycorrhiza originated from Northern France to most likely endure the climate in Finland as good as possible. The seedlings were planted in rows with 4 meters between the seedlings within the rows, to enable fast colonization of the

soil by the truffle and root system and future shade from the canopies. The distance between the rows was 4-6 meters depending on the size of the soil harrowing machine. Chipped bark or plastic weave were used as weed control around each seedling. The soil between the rows was plowed, harrowed or weeded mechanically, and covered in some orchards by chipped bark or plastic weave. The orchards were irrigated by using hose or bucket during the summer period, especially during the first months after planting the seedlings. The seedlings were cultivated with or without a protecting tube (around the lower part of the tree). Spruce branches and plastic clothes were used in truffle orchards to protect the seedlings from frost during the winter.

Monitoring of the truffle orchards

The amount of wet in the orchard soil was followed and the orchards were irrigated when necessary. The growth of the seedlings and the number of the new leaves was followed immediately after the seedlings were planted and during the growth seasons. The frost protection systems were removed immediately after melting the snow in the spring to prevent the growth of molds.

Results

Orchards used in field trials

The first truffle orchards in Finland were established in 2006 in Juva, Rantasalmi and Mikkeli. Ten truffle orchards were established in 2006 and additional four orchards were established in 2007, and six orchards in 2008. 200 oak seedlings inoculated with *Tuber aestivum* were planted in 2006. Altogether, more than 1200 tree seedlings (oak, haze and lime; 400 were Finnish oaks) inoculated with *T. aestivum* (obtained from Robin pépiniéres nursery, France) were planted during 2006-2008 in 20 orchards in different areas in Finland in lands owned by the local farmers. All the orchard lands were used earlier as crop fields, and they had not been in use on average during the last 3-5 years. The orchard places were chosen to be close to water resources. The farmers were interested in joining the experiment as a new source for income.

Environmental conditions in the orchards

The calcium fertilizer was added in summer 2006 in order to reach the optimal pH for the growing of *T. aestivum.* The soil pH increased due to liming by about one unit in one year in the orchards planted in 2006. Because of the demanding climate conditions in Finland for southern tree types, we measured regularly the soil temperature in the orchards. The temperature in the ground (10 cm depth) was below zero in the coldest times of the winter. It was observed that the ground temperature has to be above -5^{o}C to guarantee the survival of the seedlings. The material covering the ground appears to affect the survival due to its effect on the ground temperature. Fig. 1 shows the design of the winter protection. This system was used during the winter 2006-2007. After that saw dust, wood chips or straw was used in most orchards.

Fig. 1. Winter protection of oak seedlings in truffle orchard. Spruce branches with plastic cover and protection tubes were used to protect the seedling during the winter.

Discussion

The climate in Finland is challenging for both truffles and tree seedlings coming from France. Oak and hazel grow in the hemiboreal zone in the southern coast of Finland, whereas downy oak is a Southern European tree. We established truffle orchards in the southern boreal forest region, mostly in South-Eastern Finland in places (Juva, Rantasalmi and Mikkeli) that are about 100 kilometers to the north from the coastal oak-growing zone. Some seedlings were planted also to the hemiboreal zone (Espoo) and to more northern area (Iisalmi) as a control. After establishing the truffle orchards the winter 2006-2007 came late, but in February there were cold days with lowest night temperatures being below -30°C. Instead, the winter 2007-2008 was warmest in 100 years, about 5°C higher than average. Although the climate in Finland can be too cold for the seedlings originating from Southern Europe, the field trials show that the temperature conditions can be managed by proper protection. Soil and the environmental conditions appear to be favorable to the association of the used trees and truffle mycorrhizae. Neutral soil pH was obtained in one year due to liming. *T. aestivum* mycorrhiza had survived in orchards having soil parameters even outside of the range of the natural truffle sites in Gotland (Wedén *et al.,* 2004b). Due to the use of coverings and also removal of weed in some orchards weeds have not caused any problem in our orchards. The cold Finnish climate appears to require stronger protection system. Since the growth period is short it is possible that the development of the fruit bodies takes more time in Finland than in other parts of Europe, such as France and Italy.

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LONGTERM REGIONAL LAND PLANNING TO PROMOTE TRUFFLE CULTIVATION BASED ON SOIL AND WEATHER MODELS IN CATALONIA, NE SPAIN

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Abstract

Black truffle (*Tuber melanosporum* Vittad.) cultivation through the establishment of orchards with truffle-inoculated trees continues to be a promising economic alternative in areas with suitable truffle habitat. The continuing increases in demand for black truffles and their high market value provide real incentives in Spain, where presently there are about 7,500 ha dedicated to truffle orchards. To provide a basis for long term planning for the Autonomous Community of Catalonia, in northeastern Spain, we have identified areas with suitable truffle habitat based on present climate conditions and for conditions predicted for 2040. The parameters chosen to determine suitable habitat are the following: mean annual precipitation, summer precipitation, mean annual temperature, mean temperature of the coldest month, mean temperature of the hottest month, soil pH and texture. Our estimated variation of temperature and rainfall for 2040 is based the Intergovernmental Panel on Climate Change (IPCC) report for the Iberian Peninsula. We have categorized geographical regions within Catalonia according to their suitability for black truffle cultivation according to The Digital Climate Atlas for Catalonia, an Earth Digital Model and soil analyses. Our results indicate a total of 1,582,662 ha are presently suitable for truffle cultivation, of which 506,804 ha would require additional irrigation or applications of lime to maintain adequate pH. Within this total area defined, 383,758 ha are presently dedicated to dryland farming activities that require subsidies from the European Union CAP in order to remain competitive, or lands which have suffered forest fires. The estimated climate variation expected by 2040 will reduce suitable truffle lands by 14%, from the present 49% to 35% of the total land surface of Catalonia.

Key words: Land planning, GIS, truffle cultivation habitat.

ACTIVIDAD TRUFÍCOLA NAVARRA. CUESTIONES SIN RESPUESTA

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Abstract: Truffle growing activity in Navarra. Questions without an answer.
In Navarra, first plantations were carried out in 1989. The ignorance about truffle and trufficulture was total. Almost everything about truffle was unknown, almost even its existence, but as a consequence of the permanent search for new crops that contribute to diversify and improve agricultural productions of the region, it was decided with great interest to carry out the first plantations, and find information, mainly in France and Italy.
Twenty years have passed. Technical and scientific advances have taken place but there are still many doubts. The expectations raised at first have not been reached in many cases. In spite of that, trufficulture is a reality, that covers important areas in those zones suitable for truffle growing, both in Navarra and in Spain, and it is still increasing.
In Navarra, with the experience acquired, a few points can be made:

- Mycorrhized plant production has evolve positively in Spain, which guarantees one of the most important factors in the reasonable trufficulture, together with land selection, climate or cultivation techniques.
- The soil: lands selected for new plantations suit truffle needs. They are bigger plots, which is favourable to the correct management of plantation.
- Irrigation: in new trufficulture the irrigation studies and practice have become very necessary. The experimentation in this subject is necessary to cover crop needs.
- Spreading and training: promoting trufficulture as a diversification alternative in rural areas has been the aim reached through courses, trips, conferences, festivals, commercial exhibitions, competitions…, which has culminated with the creation of the first Truffle Museum in Spain.

Key words: truffle growing, cultivation techniques, truffle aptitude.

Introducción

El tema de la Truficultura en Navarra se está desarrollando desde hace casi 20 años en equipo, entre el ITGA, la Universidad de Navarra (Botánica) y los truficultores. La relación agrícola-técnica y científica (Truficultores-ITGA-Universidad) ha sido una de las claves para el avance de la Truficultura en nuestra comunidad.
En el presente trabajo se recogen a) aspectos históricos de la truficultura en Navarra, vividos desde la perspectiva de nuestra actividad. Se partieron de unos objetivos técnicos y científicos a desarrollar a corto y largo plazo que han sido satisfechos y que han permitido que la truficultura constituya una actividad agrícola de diversificación en nuestra comunidad; b) aspectos técnicos desarrollados y éxitos alcanzados; c) las nuevas plantaciones, avances y mejoras, basados en la evolución de las plantaciones; d) cuestiones sin respuesta y e) algunas consideraciones finales. Las pautas de implantación están de alguna manera dominadas y el manejo ajustado, pero la *producción sigue siendo incierta*.

Comienzos de la truficultura en Navarra

Se partía de un desconocimiento total del tema en la región y quizá por ello, estas plantaciones se realizaron de forma precipitada, ante la influencia comercial sobre el "sector agrícola" adecuado.
La instalación de las primeras plantaciones se hizo con material vegetal de origen dudoso y micorrización incierta; se introdujeron tres simbiontes, con el agravante de 50% avellano y densidades elevadas.

Las deficiencias de partida (en el material, en las condiciones) han sido en parte la causa de que los resultados en estas plantaciones no hayan sido muy satisfactorios.
No por ello el tema se ha visto abandonado, más aún, con el paso de los años y la adquisición de experiencia, se puede decir que ahora las cosas se intentan hacer mejor con criterios más racionales.
La inquietud en los pioneros en animarse a poner plantaciones, llevó a buscar información allí donde la hubiera, como al Périgord. Con los viajes de los agricultores así como de los técnicos, se inician las relaciones nacionales e internacionales buscando apoyo científico.
Uno de los primeros temas desarrollados fue el conocimiento del área potencial de desarrollo de trufa en Navarra, para impulsar su cultivo en las zonas favorables. Y pronto se iniciaron los trabajos en campo, para el seguimiento de las plantaciones.
En nuestro trabajo ha sido decisivo el fortalecimiento de las relaciones nacionales con centros de Investigación y Universidades como CITA de Aragón, CIFV Soria, IRTA de Barcelona y Universidad de Murcia**,** Alcalá, Madrid y Lleida entre otras.
Por otra parte, los cursos de formación y otras actividades han sido el foro y un motor que ha dinamizado y contribuido enormemente a la fluidez entre los interesados e implicados en la truficultura. Estas actividades además de permitir el intercambio científico y técnico, y el derivado enriquecimiento mutuo, permiten fortalecer la relación entre todos.
Al mismo tiempo, las plantaciones iban evolucionando y también han sido objeto de visitas realizadas por técnicos y agricultores de distintas procedencias.
De forma paralela y a nivel nacional, se ha realizado un esfuerzo en dinamizar el sector. La investigación llevada a cabo en España, integrada con la investigación que se realiza en Europa, ha permitido el avance en los conocimientos y en el desarrollo de la truficultura en nuestro país.

Establecimiento de las primeras plantaciones.
A partir de 1989 se establecen numerosas plantaciones que se realizan sin los criterios técnicos requeridos, al no existir experiencias previas en España.
Se eligieron parcelas no apropiadas y simbiontes que con el paso del tiempo se han revelado no adecuados en nuestras condiciones, como es el caso del avellano. Se utilizó material vegetal de dudosa calidad en cuanto a su estado de micorrización. La densidad de plantación fue muy elevada obligando al arranque de árboles prácticamente en todas las plantaciones.

Fotos 1 y 2 Plantación de *T. melanosporum*. Arranque de avellanos y robles

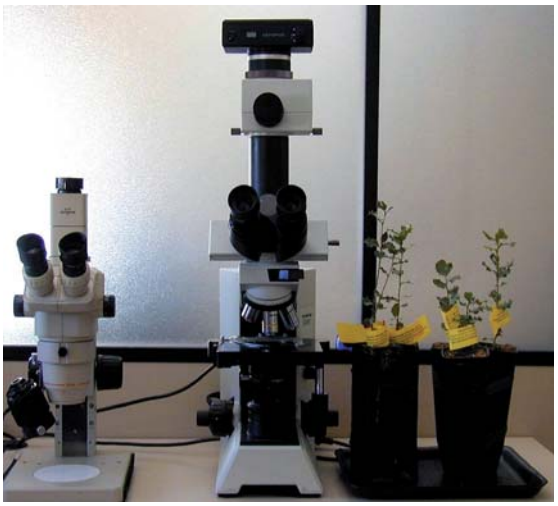
En el inicio se emplearon encina, roble y avellano y más avellanos que encina. Se pusieron muchas expectativas en el avellano que ha desaparecido prácticamente de las plantaciones actuales. En parte, por los resultados obtenidos en el seguimiento de la micorrización, por el tema de competencia micorrícica y en parte por el tema de producción, en la mayoría de las plantaciones se han retirado los avellanos.

Seguimiento de la micorrización

Desde el inicio de nuestra actividad, hemos trabajado ininterrumpidamente en el seguimiento del estado de micorrización de los árboles de las plantaciones para disponer de información sobre el desarrollo de la micorriza de la trufa y la evolución de aquellas. En este sentido y en línea con Europa, se tomaron como modelo trabajos precedentes realizados en Italia y Francia por Baciarelli Falini, Bencivenga, Chevalier, Granetti, Sourzat y Zambonelli entre otros (Baciarelli Falini, Granetti, 1998; Bencivenga *et al.,* 1995; Chevalier, Frochot 1997; Giraud, 1988; Granetti, Baciarelli Falini, 1997; Reyna, 2007; Sáez, de Miguel, 2008; Sourzat, 1994; Sourzat *et al.,* 1993; Zambonelli *et al.,* 1998) y se acomodaron a Navarra. Se han muestreado anualmente en primavera y otoño numerosas parcelas. Las muestras de raíces son llevadas al laboratorio y allí se constata la presencia de la micorriza de la trufa en las raíces de los árboles o por el contrario su desplazamiento por otras especies competidoras.

Estos estudios han permitido conocer la evolución de la diversidad micorrícica en los árboles truferos y las micorrizas presentes en concreto en los árboles productores. Son más de 40 los tipos de micorrizas caracterizados, entre los que dominan especies competitivas, con rizomorfos y/o abundantes hifas que emanan. Según el trabajo de Agerer 2001, muchos de ellos se corresponden a tipos exploratorios de larga distancia (*Scleroderma, Hebeloma, Pisolithus*). Resulta relevante la presencia casi generalizada del tipo AD de Giraud 1988, recientemente denominado *Quercirhiza quadratum* por Aguéda *et al.,* 2008, así como la abundancia de tipos teleforoides.

Hemos estudiado también la diversidad de fructificaciones que acompañan a la trufa (otras trufas y hongos hipogeos). En conjunto, especies fúngicas que conviven con la trufa en un equilibrio dinámico, pero del que se sabe muy poco todavía.



Fotos 3 y 4 Seguimiento de la micorrización. Observación e identificación.

Nuevas plantaciones

Puesto que la entrada en producción en las situaciones comentadas con anterioridad fue lenta e irregular, no ha existido un desarrollo espectacular de nuevas plantaciones. Pese a ello, el interés por la truficultura no ha descendido y se sigue plantando. En los dos últimos años se ha duplicado la superficie en Navarra. Las nuevas plantaciones se establecen sólo con encina, por ser la especie autóctona del entorno y por los mejores resultados obtenidos en la práctica hasta el momento con este simbionte, tanto en estado de micorrización como en producción.

Por otra parte, como consecuencia de las ayudas a forestación de tierras agrarias, importantes superficies con escaso valor agrícola, terrenos pedregosos, poco profundos, que en su momento eran terrenos forestales y que fueron roturados para cultivos, han vuelto a su vocación inicial al ser destinados a la truficultura:

Foto 5 Vista general de una nueva plantación

- Son terrenos enclavados dentro de las áreas de producción de trufa natural,
- Las labores de preparación del terreno son las adecuadas para conseguir una implantación en las mejores condiciones,
- Se buscan densidades de plantación de 250 árboles por hectárea,
- Se dan labores superficiales para combatir la presencia de flora adventicia,
- Las plantaciones con posibilidad han instalado sistemas de riego,

Uno de los aspectos que se ha podido mejorar considerablemente en las nuevas plantaciones ha sido la selección del material de partida. Desde nuestra experiencia, tras la irrupción de numerosos viveristas en España, se ha constatado, salvo excepciones, la mejora de la calidad de la planta micorrizada. En muchas ocasiones, nuestro interés nos lleva a realizar un control de la calidad de las plantas adquiridas por los truficultores antes de realizar la plantación.
Supuestamente las cosas se han hecho mejor y se tiene la esperanza de que con las nuevas plantaciones mejore el rendimiento.

Cuestiones sin respuesta

La falta de producción ocasiona en muchos casos la decepción del truficultor. Se recoge trufa negra, pero no de forma regular ni las cantidades estimadas inicialmente.
¿Qué factores pueden estar implicados en este hecho?
De siempre se ha referido que la producción está muy ligada a la climatología. Meses de verano con lluvias y tormentas son un buen síntoma de una buena producción en invierno. Pero no se trata sólo de regar. El manejo del agua de riego no está resuelto. No hemos realizado experiencias con rigor sobre la repercusión del riego en la producción. Hay respuesta rápida al riego pero se desconocen consecuencias a largo plazo.
La complejidad del ecosistema trufero hace necesario el estudio en profundidad de las características del suelo. En este momento, vamos a centrar nuestra atención en este tema, participando en el proyecto CTP titulado *Tipología de las Estaciones de las Trufas de las Regiones Pirenaicas: Trufa Pirenaica",* con Benoît Jaillard del INRA de Montpellier, como director del proyecto.

Consideraciones finales

Como se ha reflejado al principio, las pautas de implantación están de alguna manera dominadas y el manejo ajustado, pero la producción sigue siendo incierta.

La formación es la vía para canalizar estos avances científicos y técnicos hacia los truficultores. En nuestra opinión, es positivo el contacto directo, constante y continuo de todas las partes implicadas. Seguir adelante aunque se mantengan dudas e interrogantes; programar viajes, como aliciente, para tener la posibilidad de intercambiar impresiones entre truficultores de otras regiones o países; organizar cursos, ferias, fiestas, mercados, jornadas en los que se da a conocer al público en general las excelencias del producto.
La labor conjunta que se viene realizando alrededor de la trufa y de la truficultura en Navarra, con el empeño e ilusión de todos, ha hecho posible la creación del Museo de la Trufa-Centro de Interpretación de Metauten, www.museodelatrufa.com o info@museodelatrufa.com, primer Museo de la Trufa en España. Su función es convertirse en un lugar de encuentro de los truficultores, un lugar de venta y de divulgación de la trufa y contribuir a la diversificación y desarrollo de una zona con pocas alternativas. Es deseo de todos que llegue a ser un referente.

Foto 6 Exterior del Museo de la Trufa - Centro de interpretación de Metauten.
www.museodelatrufa.com e info@museodelatrufa.com

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AN INNOVATIVE TECHNIQUE FOR SOIL INOCULUM DETERMINATION APPLIED TO TRUFFLE CULTIVATION

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Abstract
The recent advances in Genetics and Molecular Biology have allowed emergence of new tools that are both reliable and low cost and can be applied to several domains. For example in our case, PCR permitted the specific detection of the *Tuber* species under different biological forms: spores, mycelia, fruiting-body and mycorrhiza. However, these new tools are essentially used by Research Laboratories.
For the past two years, the Alcina company has developed a series of tests for the detection in the soil of inoculum (spore and mycorrhiza) suitable for truffle cultivation and experimentation.
This battery of tests is genuinely innovative, one of the most original aspects of this series consists in a technique to isolate specifically *Tuber* spores from agricultural or forest soils. The sample of spore obtained is pure and ready for PCR analysis.
After sample collection (spore, mycorrhiza), DNA is extracted, amplified by PCR multiplex. The migration of DNA fragments in agarose gel is evaluated by visually monitoring migration.
We developed this battery of tests for three species: *Tuber melanosporum*, *T. aestivum* and *T. brumale* and offers truffle producers a double interest:

- Soil assessment is of key importance before plantation of a truffle orchard: spore analysis of the soil determines the presence or not of a natural inoculum and provides refined evaluation of the production potential.
- In the specific context of ruffle orchards management, mycorrhiza analysis enables accurate evaluation of the fungus dynamics (whether the fungus is stable, has disappeared or suffers from species competition) and consequently permits to better adapt culture practices to truffle cultivation.

These tests are also adequate in the context of experimental programs as they permit precise monitoring of fungus dynamics before and after orchard plantation or actions in natural production areas (clearing).
A short-term evolution of these techniques will be mycelia analysis and the development of the tests to other *Tuber* species and competitors.
Key words: spore extraction from soil, mycorrhiza, Polymerase Chain Reaction, *Tuber*, truffle cultivation, experimentation.

TRUFFLE CULTIVATION IN FRANCE AND ITALY

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Abstract
The truffle cultivation for the production of *Tuber melanosporum* in France and Italy presents some common points and differences in relationship with the agricultural history and environment evolution. With the example of the truffle cultivation practiced or used by Angellozzi brothers (Roccafluvione, Marches), we have tried to understand what are the elements which can contribute to improve the methods applied in the two countries. After to have defined the actual conceptions of the truffle cultivation in France and Italy, we have studied the conditions, the technical characteristics and the way of thinking which do the worth at this example. Conditions concerns agricultural history; technical characteristics aims early working of the soil, pruning the 2nd or 3rd years, irrigation in the phase of production; way of thinking regards the balance between environment and cultivation techniques. It results of the comparison of French and Italian models with Angellozzi example that the management of the host tree, in its aerial parts and subterranean one in deep soil, has two objectives: 1) maintain the root system in juvenile stage, 2) favour a lasting truffles production with born and growth of many fruit buddies. In getting out of the classical models of cultivation, Angellozzi's method is characterized by 2 steps: 1) the first one corresponds at the planting of perfectly mycorrhized trees settled in apparently very good conditions of environment with a management limited to hoeing and pruning; 2) the second one consists mainly to deeply hoe the soil, irrigate frequently in small quantity, control the growth of trees by a hard pruning. Angellozzi's method can improve French and Italian truffle cultivation, especially on the way of thinking the control of the host tree growth regarding the lasting of the truffle production.
Key words: *Tuber melanosporum* Vittad., hoeing, irrigation, pruning.

Problématique et objectif
Ce travail résulte de la comparaison des différentes approches de la culture de la truffe *Tuber melanosporum* en France et en Italie. Après une présentation générale de la trufficulture sous ses différentes formes en France, il a pour but de rechercher quels bénéfices apportent la pratique de la famille Angellozzi (Ascoli Piceno, Italie) à l'amélioration de la trufficulture, en général, et à la définition récente d'un «modèle intégré» de trufficulture en Italie, en particulier.

Caractéristiques de la trufficulture du *Tuber melanosporum* en France
On peut distinguer trois types de trufficulture qui connaissent des variantes et des résultats différents selon les environnements et au cours du temps. Les trufficulteurs ne sont pas tous d'accord sur la méthode à employer d'autant que chacun apporte ses nuances à l'approche qu'il a choisi de privilégier par rapport à son type de sol, son climat et surtout l'existence ou non d'une tradition familiale en trufficulture.

Ces principales méthodes en France sont:

1. la méthode traditionnelle pratiquée avant l'apparition du plant mycorhizé et dont les adeptes sont de moins en moins nombreux,
2. la méthode d'arboriculture truffière (ou «Pallier») conçue sur des principes simples et modernes dès le début de la commercialisation des plants mycorhizés en 1974,
3. la méthode de la pelouse calcicole (ou «Tanguy») à partir des années 1990 et qui se calque sur le fonctionnement des truffières naturelles.

La méthode traditionnelle

La méthode traditionnelle, inventée en France au début du 19ème siècle grâce au semis de glands de trufficulteurs observateurs de la nature, recouvre différentes pratiques qui, dans l'ensemble, présentent une certaine homogénéité. Si de nos jours ses performances rendent discutables son intérêt, il n'empêche qu'elle a été à l'origine de formidables quantités de truffes à la fin du 19ème et au début du 20ème siècle.

Caractéristiques de la méthode traditionnelle:

- fabrication de plants à partir de semences (glands) sélectionnées sur de bons truffiers (chênes truffiers),
- plantation dans une vieille vigne (Sud-Ouest), une lavanderaie (Sud-Est) ou une parcelle de terre labourée sur laquelle il est difficile de réaliser une culture de rapport économique,
- travail du sol ininterrompu mais adapté à l'âge des plants depuis la plantation des arbres jusqu'à leur production; toutefois, certains trufficulteurs sarclent à la main les jeunes arbres et même les adultes en production,
- pas d'irrigation mais parfois pose de branchages sur les bons brûlés avant les fortes chaleurs pour limiter l'évaporation de l'eau,
- peu ou pas de taille des arbres.

Photo 1 plantation traditionnelle à Escamps (Lot, France)

Résultats actuels de cette «culture d'arbres à vocation truffière»:

- une production de plus en plus tardive (15 à 25 ans) car il faut attendre la mycorhization aléatoire et naturelle par le *Tuber melanosporum* sur les arbres plantés voire semés,
- des productions très inégales avec une proportion d'arbres producteurs pouvant varier de zéro jusqu'à plus de la moitié sur une même parcelle; dans les vieux bassins de production où le *Tuber melanosporum* est encore puissamment implanté (Richerenches, Aups), les rendements avec cette méthode rivalisent avec ceux des autres méthodes; là où le *Tuber brumale* a gagné du terrain, les résultats sont médiocres ou nuls,
- généralement un fort pourcentage d'arbres stériles parce que ceux-ci sont mycorhizés par d'autres champignons que la truffe.

La méthode traditionnelle est à la base de la trufficulture du 19ème siècle. Toutefois, aujourd'hui le contexte a changé au plan écologique (le milieu s'est fermé) et socio-économique (moins de main d'œuvre à la campagne et une agriculture industrielle par opposition à vivrière). Les plantations selon cette méthode s'observent encore dans les anciennes régions de production mais de moins en moins de gens la pratiquent dans la mesure où l'usage du plant mycorhizé se généralise. Il faut toutefois noter que le plant mycorhizé a été inventé pour pallier un déficit de résultat de la méthode traditionnelle; autrement dit, un manque d'inoculum dans le sol ou une perte en parallèle de la virulence du *Tuber melanosporum*. Il est probable que si cette situation négative ne s'était pas manifestée dans les années 1950 et 1960, la méthode traditionnelle aurait toujours autant de succès, à l'exception bien évidemment des régions nouvelles où l'introduction du plant mycorhizé serait nécessaire pour amener l'inoculum.

L'arboriculture truffière (méthode «Pallier»)

C'est au début des années 1970, avec la mise sur le marché du plant mycorhizé sous licence INRA-ANVAR (hiver 1974-75) que la méthode s'est développée. Jean Rebière[1] avait commencé dans le Périgord, dès les années 1960, à esquisser la méthode sans les plants mycorhizés. Raymond Pallier a expérimenté et vulgarisé ce type de trufficulture pendant près de 30 années à Sainte-Foy de Longas dans le Périgord. Cette méthode est pratiquée dans toutes les régions de France, en Europe et même au-delà.

Photo 2 plantation en arboriculture truffière sur le canton de Lalbenque (Lot, France)

Caractéristiques de la méthode:

- plantation d'arbres contrôlés mycorhizés par la truffe *Tuber melanosporum* sur un sol calcaire favorable,
- travail du sol (cultivateur, vibroculteur, « griffon ») ininterrompu mais adapté à l'âge des plants depuis la plantation des arbres jusqu'à l'arrêt de la production,
- irrigation des arbres truffiers principalement au mois d'août et surtout pendant la période de production de truffes,
- taille des arbres d'autant plus vigoureuse que leur croissance est renforcée par l'irrigation de la plantation et que la densité est importante (400 et 800 plants par hectare),

1 Jean Rebière a publié en 1967 la première édition de «La truffe du Périgord» aux éditions Fanlac à Périgueux.

- apports d'amendements calcaires pour maîtriser la compétition du *Tuber brumale* et essais de fertilisants organiques tels que Fructitruf,
- utilisation de produits chimiques pour désherber (glyphosate), supprimer les drageons, se débarrasser de parasites (limaces, liodès, etc).

Résultats de la méthode:

- une production précoce, en particulier avec le noisetier où la production débute à la quatrième année,
- des rendements qui peuvent raisonnablement varier de 15 à 30 kg à l'hectare entre 15 et 20 ans,
- des problèmes de contamination par notamment le *Tuber brumale* dans le Sud-Ouest et de plus en plus dans le Sud-Est,
- des temps de travaux importants, surtout pour la taille des arbres relevant parfois de l'élagage.

La trufficulture en pelouse calcicole (méthode «Tanguy[2]»)

C'est à la suite de la saison truffière 1993-94 que la méthode a pu être identifiée et codifiée, soit presque 20 ans après les débuts de la méthode «Pallier», à partir de plantations d'arbres mycorhizés réalisées au début des années 1980 et entretenues sommairement.

Les plantations truffières sur la méthode «Tanguy» qui ont frappé les esprits à partir de 1994 se trouvaient dans le Vaucluse (Apt), le Lot (Miers), le Périgord (Sainte-Alvère, Pézuls, Saint-Pantaly d'Excideuil) et bien entendu dans le Tarn et Garonne (Puygaillard). Ces plantations à haut niveau de production sont souvent et curieusement sur des surfaces inférieures à un hectare (entre 10 et 60 ares pour celles citées). Il est très probable que les personnes qui ont des plantations aussi performantes préfèrent rester dans l'ombre même si de nouveaux trufficulteurs (exemples à Beauvais sur Matha en Charente Maritime ou à Masquières en Lot et Garonne) acceptent aujourd'hui de parler de leur méthode et de ses résultats.

Caractéristiques de la méthode:

- plantation d'arbres garantis mycorhizés par la truffe *Tuber melanosporum* sur un sol calcaire labouré ou recouvert d'une pelouse,
- travail du sol ou désherbage autour des jeunes arbres les deux premières années pour favoriser la reprise du plant truffier,
- abandon du travail du sol et du désherbage à partir de la deuxième ou troisième année pour favoriser l'installation du champignon tout en ralentissant (ou pénalisant) la croissance des arbres mycorhizés,
- entretien de la plantation par tonte de la végétation à base de graminées spontanées (parfois semées), rarement un travail du sol au cultivateur sinon lorsque la production s'est installée depuis plusieurs années (ce travail servant probablement à favoriser la pousse de nouvelles racines fines susceptibles de porter des mycorhizes),
- arrosage des arbres truffiers les deux premières années pour favoriser leur reprise puis, lorsque la production a démarré, reprise de l'arrosage par micro-aspersion des «bons brûlés»,
- taille éventuelle des arbres au départ pour une forme érigée, abandon ensuite, puis reprise lorsque l'espace truffier commence à se former,
- pas d'apport d'amendement ni de fertilisant particulier ni de désherbage chimique.

2 Marcel Tanguy a acheté en même temps que sa maison une plantation de noisetiers mycorhizés abandonnée depuis plusieurs années, à Puygaillard (Tarn et Garonne). A la suite de la découverte des premières truffes sous des arbres âgés de 10 ans, il a entrepris d'entretenir sa parcelle par la tonte de l'herbe et l'irrigation des brûlés.

Photo 3 plantation en pelouse calcicole à Castelnau-Montmiral (Tarn, France)

Les résultats de la méthode:

- une production qui débute plus tardivement qu'avec la méthode «Pallier», soit vers 10 à 12 ans, en raison de la compétition des plantes du milieu sur la croissance des arbres,
- pas ou très peu de contamination par d'autres champignons ou espèces de *Tuber*,
- des rendements par arbre de 1 kg et parfois plus,
- une récolte de surface sensible au gel et aux déprédateurs,
- des temps de travaux inférieurs à la méthode «Pallier»,
- une pérennité de la production mal connue car ce modèle de trufficulture est d'un emploi relativement récent.

Caractéristiques de la trufficulture pratiquée par la famille Angellozzi à la base du «modèle intégré» de trufficulture en Italie

L'enseignement des truffières naturelles dans la définition du «modèle intégré»

L'originalité de la trufficulture pratiquée par la famille Angellozzi est d'avoir été définie à partir de l'expérience et de l'observation des truffières naturelles. La mise en pratique de l'enseignement du milieu naturel a fait l'objet de nombreux essais qui ont abouti à une trufficulture empirique et intelligente, rejoignant par bien des aspects celle définie en France dès la fin du 19ème siècle. Tous les essais n'ont pas donné les résultats escomptés: ils ont toutefois conduit à la définition de pratiques adaptées aux conditions pédo-climatiques locales, montrant que l'expérience permet parfois de trouver des solutions avant d'avoir les explications approfondies de certains fonctionnements de la nature.

Les arbres plantés sont des chênes pubescents à plus de 95%, comme dans le milieu naturel; les chênes verts constituent le reste des essences utilisées. Il s'agit d'arbres mycorhizés fabriqués par des pépiniéristes italiens, notamment Giusto Giovannetti (Turin). Les écartements pratiqués sont de 4,5 m x 4,5 m dans la plantation de 1994, soit en théorie 494 arbres / ha. On comprend l'importance de la taille avec une telle densité.

Le travail du sol est fondé sur les observations conduites pendant la récolte avec le chien mais également à partir du piochage systématique. Plusieurs horizons[3] ont été remarqués dans ces conditions:

1. les premiers centimètres en surface forment un écran contre le desséchement du sol,
2. la couche de naissance et de croissance des truffes se situe entre 5 et 20 centimètres, parfois jusqu'à 30 cm de profondeur,
3. l'horizon de racines mycorhizées est placé sous la couche de naissance des truffes.

Ce travail du sol est réalisé de façon manuelle sur des truffières en pente, enherbées entre les brûlés, en respectant ainsi la sensibilité naturelle du milieu à l'érosion. La progression du brûlé est prise en compte[4] dans les interventions manuelles (voir plus bas).

L'arrosage des truffières est conduit avec la perception de ces couches du sol. Emidio Angellozzi considère qu'il faut humecter l'écran de surface de façon à éviter le desséchement de la couche à truffes.

La taille a été établie à partir de deux éléments distincts:

1. le comportement des truffières naturelles dont les branches de chêne étaient coupées pour nourrir les troupeaux de brebis,
2. la motivation de retarder la fermeture du milieu entraînant la disparition[5] des sites naturels.

Les pratiques culturales dans leurs détails

Si le contexte de la définition des pratiques culturales est important à connaître, le détail de celles-ci apporte un complément d'information.

Photo 4 travail du sol à la houe contrairement au «bigos» équipé de 2 ou plusieurs dents en France

3 Ces observations rejoignent celles réalisées en France (Aujols, Cours, Lot) sur de vieilles truffières naturelles montrant que les racines mycorhizées sont parfois présentes entre les lits de pierres du Jurassique (Kimméridgien) et rares dans la partie de récolte de truffes où la virulence de la truffe (le brûlé) semble les avoir dégradées, décharnées, détruites.

4 On peut dire que lors du travail du sol (zappatura), l'organisation verticale et horizontale du brûlé est prise en compte.

5 «Si l'on ne taillait pas les arbres en plantation, la production disparaîtrait aussi rapidement que dans le milieu naturel» affirme Emidio Angellozzi.

Le travail du sol (la zappatura) est effectué dès la fin de la récolte, en février, mars et avril. En truffières naturelles, où les truffes étaient récoltées à la pioche pour gagner du temps en raison de la compétition entre les chercheurs, le piochage était effectué dès l'automne ou le début de l'hiver. Ce travail consiste à fracturer le sol avec une houe (pas un outil à dents) facile d'utilisation en sol léger. Il est effectué jusqu'à la couche de racines mycorhizées situées entre 15 et 20 cm de profondeur, voire jusqu'à 30 cm. En fait, dès que la couche de racines est atteinte, précise Emidio Angellozzi, le piochage en profondeur trouve sa limite. Si de grosses racines sont rencontrées dans l'horizon à truffes, elles sont coupées et enlevées, réalisant de la sorte une taille du système racinaire.

Le brûlé est travaillé sur toute sa surface sans toutefois piocher la zone de progression de l'année précédente. Cette particularité est en accord avec les préconisations de De Bosredon dans son Manuel de trufficulture (1887) ainsi qu'avec les recommandations de ne pas perturber les équilibres se mettant en place (cortèges fongiques) lors de la formation des brûlés et jusqu'au déclenchement de la production truffière.

L'arrosage, dont l'objectif est de maintenir humide les deux à trois premiers centimètres de la surface du sol, est effectué au besoin tous les trois jours, avec des apports de quelques millimètres en micro-aspersion. Les canalisations fournissant l'eau aux micro-asperseurs sont enterrées. Emidio Angellozzi considère, d'une part, que l'eau en juillet n'est pas déterminante pour la truffe, d'autre part que si l'on donne beaucoup d'eau, «alors, les problèmes commencent».
La taille est pratiquée à partir notamment du constat d'Emidio estimant que la croissance de l'arbre devient exponentielle[6] à partir de 6, 7 à 8 ans. Pour éviter la transformation en bois des truffières plantées, il souligne l'importance d'anticiper la fermeture plutôt que d'intervenir afin de corriger la situation. Il attache de l'importance à la conservation des branches basses pour faire de l'ombre sur le sol. Quant aux branches taillées, il les utilise en fagots pour pailler ou couvrir le sol.
La taille, pratiquée toute l'année, est réalisée sur le principe (défini par Emidio Angellozzi) consistant à renouveler la plante (l'arbre) tout en évitant son vieillissement. Elle est focalisée, non pas sur les dégâts à l'arbre, mais sur les repousses, comme le montrent les schémas ci-dessous. Cette méthode aboutit à maintenir les arbres à hauteur d'homme, donnant l'impression de bonzaïs avec des troncs et des branches latérales principales proportionnellement de gros diamètres par rapport à la dimension de l'arbre.

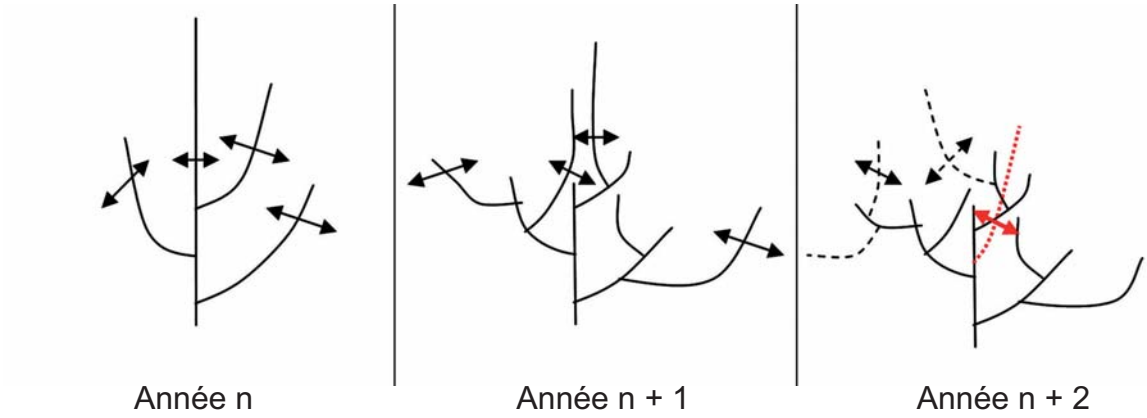

Schéma 1 stratégie de la taille

L'année n, la tige principale est rabattue en laissant toutefois un tire-sève. L'année n+1, la tige démarrée sur le tire-sève sera à son tour rabattue ainsi que les tiges à croissance verticale; en

6 Ce constat est caractéristique des sols profonds par opposition aux sols superficiels. Il a fait l'objet de graphiques explicatifs dans les comptes rendus des études régionales Midi-Pyrénées en 2007.

revanche, les tiges à croissance horizontale seront pincées ou laissées indemnes. L'année n+2, on procèdera de même, de sorte que l'arbre aura tendance à conserver une forme ramassée, voire étalée (recherchée pour ombrer le brûlé), avec une croissance apicale parfaitement maîtrisée.

Photo 5 taille sur jeune chêne pubescent en 3ème feuille (août 2007)

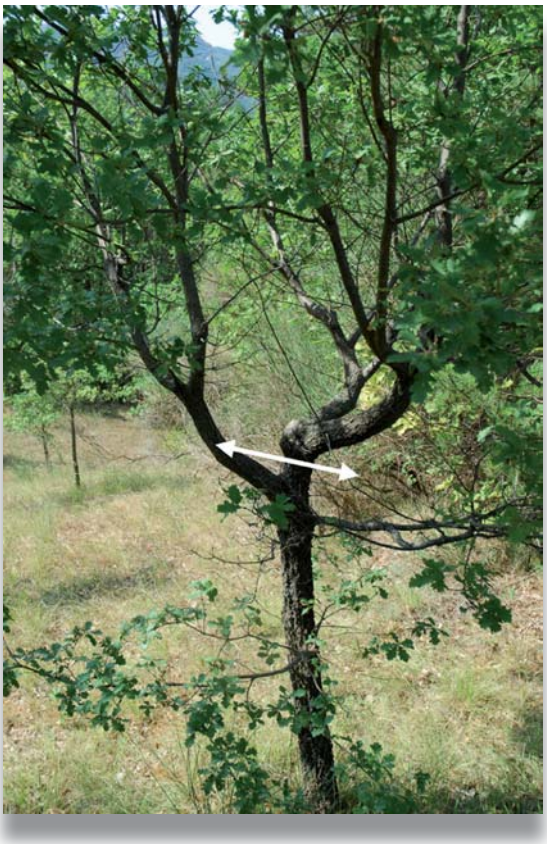

Photo 6 chêne âgé «chez le cousin» dont la taille n'a pas été continuée en coupant notamment à la flèche.

On peut tailler l'arbre pour un redémarrage sur une tige basse (voir dessin et photos des arbres jeunes et adultes avec pointillés et flèche). Le maintien de l'arbre dans ce type de morphologie suppose des interventions fréquentes car les repousses à croissance verticale sont nombreuses. En fait, Emidio Angellozzi précise qu'il intervient plusieurs fois par an, aussi bien en hiver (de novembre à mars), qu'au printemps (en pleine pousse), et été (au besoin plusieurs fois). Dans les jeunes plantations, il opère de la même façon comme le montre la photo verticale ci-dessus.

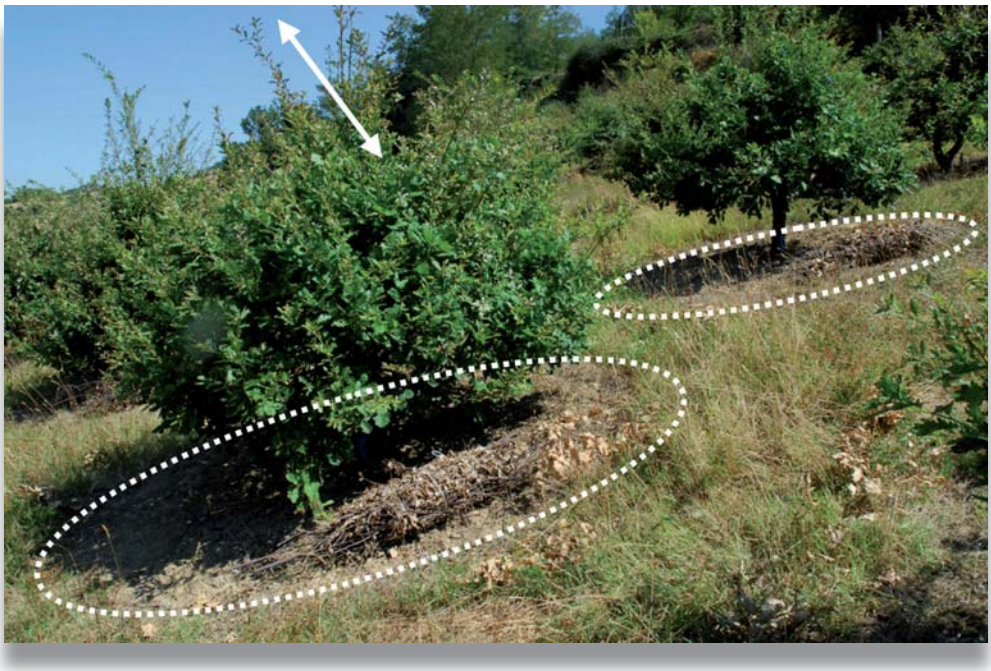

Photo 7 chênes pubescents avec brûlés débordants (virulents) et fagots de branches issus de la taille utilisés en couverture du sol. Noter la vigueur des repousses, certaines mesurant jusqu'à 1 mètre de long soulignée par la flèche.

Si le grand principe de la taille, selon Emidio Angellozzi, consiste à éviter la fermeture du milieu, il n'en demeure pas moins que celle-ci a aussi pour but de conserver un bon équilibre entre la vigueur de l'arbre et la virulence de la truffe qui se traduit par des brûlés débordants (Rb ≥1,Rf) comme le montre la photo ci-dessus. Etant donné la profondeur du sol (fertilité, réserve en eau) de la plantation, on comprend que pour maintenir cet équilibre entre la puissance de l'arbre et celle du champignon, la taille soit pratiquée presque toute l'année. La vigueur (diamètre et longueur) des repousses après la taille témoigne de la problématique du sujet à laquelle on est confronté dans ce type de sol où, de plus, l'arrosage est régulièrement effectué.

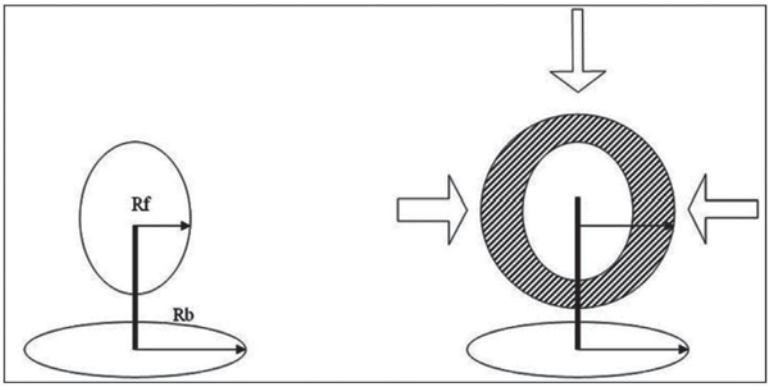

Schéma 2 montrant à gauche un arbre sur sol superficiel naturellement équilibré dans sa relation Rb ≥1,Rf (Rb = rayon du brûlé et Rf = Rayon de la frondaison). A droite, sur sol profond comme à Roccafluvione, cet équilibre est rétabli par une taille drastique.

La taille régulière et soigneuse appliquée par la famille Angellozzi requiert des temps de travaux importants. Il est évident que les très bons résultats des plantations justifient cet investissement en main d'œuvre. Des résultats moins bons en France ou ailleurs ne motivent pas une telle implication: ils incitent à des méthodes moins sophistiquées (taille simplifiée ou élagage) ou mécanisée (lamier) qui ont aussi leur valeur.

Photo 8 vue des arbres taillés en hiver

Le désherbage, dans les plantations en production, est pratiqué entre les rangs au girobroyeur et sur le rang avec la débroussailleuse thermique équipée d'une lame. Les jeunes plantations sont entretenues principalement par un binage traditionnel à la main autour des arbres et le passage de la débroussailleuse sur le rang complété par le girobroyage entre les rangs.

Résultats de la méthode Angellozzi

Près de 100% des arbres présentent un brûlé. Malgré une taille qui favorise les pousses latérales de l'arbre, les brûlés sont débordants (Rb = 1,2 à 1,5 Rf) soulignant la virulence de la truffe sur ce site. Cette virulence est également marquée par le dessèchement du chiendent[7] à l'intérieur du brûlé (*Cynodon dactylon* Pers.). Le chiendent redémarre après le travail du sol au printemps mais meurt ensuite au début de l'été (comme s'il était traité au glyphosate). Emidio Angellozzi précise que lorsque les racines du chiendent atteignent l'horizon où sont présentes les racines mycorhizées avec une forte densité mycélienne, la plante meurt. Entre les brûlés, la flore est surtout composée de graminées: *Cynodon dactylon, Dactylis glomerata, Brachypodium pinnatum* (la palène) ainsi que de dicotylédones telles que *Sanguisorba minor*, *Daucus carota*. A partir de la limite de travail du sol à la main, la destruction de cette flore provoquée par la progression du brûlé est nettement visible.

Les premières truffes sont récoltées dès la troisième année de plantation. Le niveau de production est exceptionnel puisque 93% des arbres plantés sur plusieurs ha sont producteurs avec une moyenne de 600 à 700 g par arbre: de nombreux sujets produisent 3 kg. La récolte sur les 35 ha actuellement en production compense la chute de celle des truffières naturelles à leur apogée en 1985.

7 Ce chiendent semble un bon indicateur de l'agressivité ou de la virulence du *Tuber melanosporum*. En France, son desséchement dans le brûlé a été observé avec plus ou moins de netteté à la même époque (Lebreil, Lot).

Photo 9 arbre taillé en été en période d'arrosage.

La méthode d'arrosage consistant à ne pas intervenir dans le processus de croissance des truffes implique chez les corps fructifères une capacité à l'économie d'eau. Emidio Angellozzi prétend que la truffe sait attendre[8] pour grossir jusqu'à novembre et même décembre. Il a observé la formation de marques très tardives. Il souligne que la truffe «explose en 4 jours» après une bonne pluie mais, «lorsque la phase de croissance est lancée», s'il advient un coup de chaleur et de sécheresse, beaucoup de truffes meurent.
L'absence de toute récolte de *Tuber brumale* est importante à noter, aussi bien en plantation que dans le milieu naturel. En conditions naturelles ou sauvages, la production se répartit comme suit: 95% *Tuber melanosporum* et 5% *Tuber aestivum*.
Des apports d'inoculum du *Tuber melanosporum* sont réalisés dans les plantations. Les truffes invendues sont broyées et mélangées avec de la terre pour former un substrat d'inoculation.

Discussion
Les résultats de la comparaison entre les méthodes de trufficulture moderne en France (arboriculture truffière et pelouse calcicole) et celle pratiquée par la famille Angellozzi mettent en évidence deux particularités spécifiques à la gestion de l'arbre-hôte par la famille Angellozzi. La gestion des parties aériennes et de celles souterraines est caractérisée par la poursuite de deux objectifs:

- le maintien d'un système racinaire à un stade juvénile,
- la recherche d'une production pérenne en favorisant les naissances et la croissance de nombreux corps fructifères.

La premier objectif répond parfaitement aux exigences d'un système racinaire jeune (Questions d'écologie appliquées à la trufficulture), avec des racines fines (La truffe, la terre, la vie), pour la production du *Tuber melanosporum* dans la logique de la dynamique du cortège fongique. En fait, un travail du sol profond suppose l'établissement d'un cortège fongique, non pas seulement horizontal mais étagé verticalement. La structure verticale de l'horizon humique du sol comportera une couche de racines fines mycorhizées maintenues à 20 cm de profondeur et une couche supérieure dédiée à la naissance et à la croissance des corps fructifères du

8 Gian-Luigi Grégori a illustré cette attente en prenant l'exemple de la diapause du fœtus chez le chevreuil.

Tuber melanosporum. La couche de racines fines est régénérée régulièrement par l'action contondante de la houe, considérée comme de la taille racinaire.
Le deuxième objectif découle du premier dans la mesure où la strate à mycorhizes à 20 cm de profondeur entretient une biomasse mycélienne dans la couche supérieure aérée (notamment, par le travail du sol). Cette biomasse mycélienne, en présence de très peu de racines fines (coupées par le travail du sol), favorise un espace réservé à la fructification et à la croissance des corps fructifères (non concurrencée dans leur besoin hydrique par l'arbre hôte) à une profondeur plus importante que celle habituellement observée dans les truffières cultivées mécaniquement en France. En fait, comme on l'a vu plus haut, on cherche à maintenir un écran d'humidité très en surface par des arrosages fréquents et peu importants dans la mesure où les excès d'eau sont redoutés.
En tenant compte des modèles classiques de trufficulture, on retiendra également que la méthode Angellozzi présente un itinéraire technique singulier, à rapprocher de celui de la méthode en pelouse calcicole en France, adapté sous le terme d'itinéraire technique de précaution. Cet itinéraire technique comporte deux étapes:

1. l'installation d'arbres parfaitement mycorhizés installés à l'évidence dans de très bonnes conditions d'environnement avec un entretien limité au binage du sol et à la taille des arbres,
2. une conduite en phase de production caractérisée par un travail du sol manuel profond, le maintien d'une humidité de surface faisant écran au dessèchement de la couche de fructification, le contrôle de la croissance des arbres par une taille vigoureuse et fréquente.

Cet itinéraire technique est intéressant à comparer avec notamment la méthode de trufficulture traditionnelle dans sa version ancestrale, à savoir du temps où la mécanisation n'avait pas encore été introduite ni dans l'agriculture ni en trufficulture. Le travail du sol manuel, tel qu'il est pratiqué depuis le début de la plantation puis en phase de production, permet d'éviter les inconvénients d'une désorganisation du cortège fongique en phase d'installation des brûlés (et de l'écosystème) et de créer des conditions adaptées à la naissance et à la croissance des corps fructifères avec le souci d'éviter les excès d'eau préjudiciables à un champignon réputé xéro-thermi-calcicole.
Concernant la taille, il est clair que celle-ci répond particulièrement à la recherche de cet équilibre formulé dans la relation de virulence (Rb ≥ 1,5 Rf), soulignant l'importance de ne pas donner trop de vigueur à l'arbre et de conserver une truffe agressive à partir d'un brûlé très présent et relativement conquérant. En fait, en raison d'une forte densité de plantation, la taille aérienne vigoureuse, et aussi racinaire de l'arbre, contribue à régénérer le système racinaire (par émission de racines fines mycorhizées et en capacité d'accueillir des mycorhizes) tout en économisant de l'espace de conquête. Le brûlé est considéré relativement conquérant parce que la taille vigoureuse (dite parfois «bonzaï») ralentit à la fois la progression du système racinaire et celle du brûlé.
On notera enfin dans cette discussion d'autres particularités intéressantes de l'environnement truffier. En effet, on retrouve curieusement dans les plantations de Roccafluvione, des éléments floristiques communs[9] avec les plantations de Sarrion (Doñate Manolo, Terruel, Espagne), tels l'importance du chiendent (*Cynodon dactylon*) et la présence de la pimprenelle sanguisorbe (*Sanguisorba minor*). De même à Sarrion, dans les espaces boisés susceptibles de produire la truffe, seules les espèces *Tuber melanosporum* et *Tuber aestivum* sont présentes; *Tuber brumale* est naturellement absent.
On soulignera pour terminer que les plantations de Roccafluvione constituent un terrain excellent pour rechercher les facteurs bénéfiques dans l'écosytème truffier qui semblent fonctionner comme dans les meilleures années de la production française au 19ème siècle ou dans la première moitié du 20ème siècle. On peut envisager ce travail par la recherche des facteurs négatifs, ou des facteurs positifs par défaut, lesquels pénalisent la production truffière

9 Gian-Luigi Grégori souligne que les exsudats racinaires de certaines plantes peuvent favoriser l'allélopathie du mycélium. Des travaux ont été effectués sur le sujet en Italie par Letizi H.

dans de nombreux endroits où l'on a essayé de relancer (presque vainement) la production en plantant des dizaines voire des centaines hectares d'arbres mycorhizés. A Roccafluvione, les plantations sont réalisées aussi bien au sommet des collines que dans les vallées.

Conclusion

La méthode de trufficulture mise au point par la famille Angellozzi à Roccafluvione peut contribuer à reconsidérer certains aspects des méthodes employées en France et en Italie pour la production du *Tuber melanosporum*. Elle est en tout cas très cohérente avec les pratiques modernes et mêmes anciennes à condition bien évidemment d'ajouter l'utilisation du plant mycorhizé. Emidio et Zénobio Angellozzi ont su avec perspicacité profiter de leur expérience du milieu naturel, lorsque celui-ci était à l'optimum de la production avec ses truffières naturelles, pour transposer celle-ci au niveau du contrôle de la croissance de l'arbre hôte et de la pérennité de la production truffière. Ils ont opportunément opéré la transition des truffières naturelles vers la trufficulture. Enfin, tout semble indiquer qu'ils n'ont pas été soumis à la pression de contamination d'un environnement boisé avec en particulier un *Tuber brumale* agressif.

Remerciements

La famille Angellozzi, en particulier Emidio et Zénobio ainsi que leur épouse respective, doit être remerciée pour son hospitalité, l'accueil très chaleureux qu'elle nous a toujours réservé sur ses terrains de production truffière, ainsi que pour les données qu'elle a accepté de livrer lors de nos différentes visites.

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CAN BURNT FOREST LANDS BE USED FOR BLACK TRUFFLE CULTIVATION?

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Abstract

Forest fires stop the flow of carbohydrates from the host plants to the ectomycorrhizal (ECM) fungi colonizing their roots, causing potential decrease or loss of the fungi in the soil community. The viability of reforestation with *Tuber melanosporum*-inoculated seedlings in burnt forestlands where soil and climatic characteristics are suitable for truffle cultivation depends partly on the competition of native ECM in the soil after fire. We evaluated the biotic aptitude of burnt forestlands for *T. melanosporum* by examining the survival and competitiveness of this fungus compared with the native ECM community when out planted with *Quercus ilex* (holm oaks) seedling. We also evaluated the role of the ECM host plants, which resprout after a forest fire, in the maintenance and dynamic processes of the ECM fungal community. The study followed a factorial design with two levels: 1) *T. melanosporum*-inoculated and non-inoculated *Q. ilex* seedlings and 2) presence and absence of ECM host plants capable of resprouting after the fire. We established 10 experimental plots where 360 holm oaks were planted. Four and a half years after plantation, the truffle-inoculated holm oaks maintained 36% of their root tips colonized with *T. melanosporum*, and 8 years after plantations (2008), 45% of the inoculated holm oaks displayed a burn area. Additionally, the inoculated oaks presented greater survival rates and greater root and aerial development, compared to the non-inoculated oaks. A greater ECM morphotype richness was associated with seedlings planted in plots with the presence of ECM host plants, whereas the *T. melanosporum* mycorrhizal ratio was constant. These results suggest that reforestation with *T. melanosporum*-inoculated seedlings could be successful following forest fires and highlight the competitiveness of this fungus within the ECM community in these soils.

Key words: forest fire, *Tuber melanosporum*, reforestation, ECM competitiveness.

RISULTATI DELLE RICERCHE NELLE TARTUFAIE DELLA REGIONE UMBRIA

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Abstract: Research results in truffle beds of the Umbria Region

In 1980-1981 two Regional laws were issued by the Region Umbria, with the aim of regulating the truffle picking and promoting its cultivation. Based on these laws, a Regional Truffle Plan began for the years 1983-1986, to let farmers know about this new type of agricolture, and to test cultivation techniques applicable to truffle cultivation. Thanks to this Plan, 59 experimental truffle plantations were completed, comprising 115 hectares spread across the Region, characterized by different pedoclimatic properties. In the year 1997, the Umbria Region signed an agreement with the Department of Plant Biology, now Dept. of Applied Biology, of the University of Perugia, to investigate the state of art of its truffle plantations and to test some cultivation techniques. The research work carried out over the years 1997-2000 (in 14 year-old truffle plantations) investigated the truffle plantation conditions and their degree of mycorrhization. Among the 59 truffle plantations, only 7 appeared to have completely failed; in the remaining 52, the analysis of the mycorrhization level showed that mycorrhizae of the cultivated truffle were maintained, expecially in species of the genus *Quercus*. In the years 2001-2004 (in 18 year-old truffle plantations) the research work focused on the cultivation techniques applicable to the truffle-producing plants, to favour fruit body formation and their growth. The experimental work involved 12 truffle plantations: soil tilling, watering, plant pruning, truffle spore inoculum soil supply and soil corrections were mainly tested. During the year 2008, the first results have been recorded. In particular, spore inoculum soil supply, $CaCO_3$ soil supply and watering showed to be beneficial; while pruning and soil tilling had a limited effect, maybe due to the truffle bed age. The analyses have also shown that all the investigated Umbrian truffle plantations were productive. Truffle production level was variable due to different pedoclimatic conditions, and in some instances the truffles produced were different from those cultivated. *Tuber magnatum* Pico truffle plantations were found productive (a limited production of 2-4 kg per hectare) and economically interesting. Unfortunately, in Italy the truffle does not belong to the land owner, and it is often therefore subject to a deleterious picking. It follows that truffle production levels recorded in the Regional plantations are underestimated and not realistic.

Key words: Cultivation, *Tuber*, mycorrhization.

Introduzione

La tartuficoltura umbra ha avuto origini antiche, già nel 1564 Ceccarello da Bevagna descriveva la coltivazione di *Tuber melanosporum* Vittad. seminando i corpi fruttiferi in terreni adatti e irrorandoli con un decotto di tartufo. Francolini (1931) e poi Mannozzi Torini (1956), hanno effettuato prove sperimentali di coltivazione che fornirono le prime produzioni di tartufi. Successivamente, con la scoperta della pianta tartufigena (Fontana, 1967), la messa a punto di un metodo di produzione delle piante tartufigene in vivaio (Bencivenga, 1982) e vari lavori scientifici per la produzione delle piante micorrizate con le specie pregiate di tartufo, emerse tra gli agricoltori un forte interesse verso la coltivazione di questi pregiati funghi ipogei.

La Regione dell'Umbria ha sempre mostrato propensione verso i tartufi e la tartuficoltura ed è stata la prima Regione a legiferare sul tartufo e ad investire denaro pubblico per la realizzazione di tartufaie coltivate con fini sperimentali e dimostrativi (Rizza, 1990).

Nel 1982 in Umbria venne nominata una commissione per sviluppare il Programma Tartufigeno Regionale che consentì di impiantare 59 tartufaie su una superficie di circa 115 ha in terreni

demaniali o di proprietà pubblica. Il piano tartufigeno regionale (Monaldi *et al*., 1990) fu attivato in base ai risultati scientifici ottenuti sulla operatività di due leggi emanate dalla Regione Umbria inerenti la raccolta del tartufo (L.R. 38/80) e le concessioni di finanziamenti a soggetti privati che intendevano realizzare le tartufaie (L.R. 55/81). Questo fervore consentì, tra il 1982 ed il 1993, di realizzare in Umbria circa 1500 tartufaie su una superficie di oltre 1000 ha.
Gli entusiasmi iniziali vennero, con il passare degli anni, a diminuire perché le attese buone produzioni spesso tardavano a venire e in alcuni casi le tartufaie rimanevano improduttive.
Molti degli insuccessi furono causati soprattutto dalla limitata conoscenza dell'ecologia delle specie pregiate di tartufo e, ancor meno, delle tecniche di coltivazione delle tartufaie.
Nel 1997 iniziò una collaborazione tra il Dipartimento di Biologia Vegetale dell'Università degli Studi di Perugia e la Regione Umbria per monitorare le tartufaie del piano tartufigeno. Successivamente furono stipulate altre convenzioni di collaborazione, fino al 2009, che hanno permesso di mettere in atto una serie di prove sperimentali relative all'irrigazione, alla sarchiatura, alla potatura, agli ammendamenti ed alla distribuzione di inoculo sporale.
In questo lavoro vengono riportati i risultati preliminari dello studio sulle tartufaie coltivate della Regione dell'Umbria riguardante l'ecologia e la sperimentazione di tecniche di coltivazione.

Materiali e Metodi

La Regione dell'Umbria nel 1983 avviò il Programma Tartufigeno Regionale, realizzando 59 tartufaie con finalità sperimentali e dimostrative distribuite in tutto il territorio regionale su una superficie complessiva di circa 115 ettari. In totale vennero messe in coltivazione specie pregiate di tartufo, quali: *Tuber magnatum* Pico, *Tuber melanosporum* Vittad., *Tuber aestivum* Vittad., *Tuber brumale* Vittad. f. *moschatum* Ferry, *Tuber borchii* Vittad. e *Tuber mesentericum* Vittad. in simbiosi con varie piante simbionti (*Quercus pubescens* Willd., *Quercus cerris* L., *Quercus ilex* L., *Quercus robur* L., *Ostrya carpinifolia* Scop., *Corylus avellana* L., *Populus* spp., *Salix* spp.).
Le tartufaie furono impiantate su terreni pubblici, in ambienti pedoclimatici differenti, per il tramite delle Comunità Montane dell'Umbria che avevano il compito di gestire le piantagioni (tab. 1).

Tab. 1 Tartufaie sperimentali del piano tartufigeno della Regione dell'Umbria.

Tartufaie di *Tuber melanosporum* Vittad.	
1 - Capralina	ex Com. Mont. Alto Chiascio
2 - Costa Sparagara	ex Com. Mont. Alto Chiascio
3 - Castagneto	ex Com. Mont. Alto Chiascio
4 - Banditelle 1	ex Com. Mont. Monte Subasio
5 - Cancelli	ex Com. Mont. Monte Subasio
6 - Banditelle 2	ex Com. Mont. Monte Subasio
7 - Assisi	ex Com. Mont. Monte Subasio
8 - Pratarelle	ex Com. Mont. Valle del Nera M. S. Pancr.
9 - Vigne della Campella	ex Com. Mont. Valle del Nera M. S. Pancr.
10 - Vigne Parenzio 1	ex Com. Mont. Valle del Nera M. S. Pancr.
11 - Vigne Parenzio 2	ex Com. Mont. Valle del Nera M. S. Pancr.
12 - Castiglioni	ex Com. Mont. Valnerina
13 - Acquedove	ex Com. Mont. Valnerina
14 - Pianalati	ex Com. Mont. Valnerina
15 - Costa del Pero	ex Com. Mont. Valnerina
16 - Valcasana	ex Com. Mont. Valnerina
17 - Valle Versara	ex Com. Mont. Valnerina
18 - Acerone - Cortigno	ex Com. Mont. Valnerina
19 - Orsano 1	ex Com. Mont. Valnerina

21 - Casale Manenti (Lagovecchio)	ex Com. Mont. Valnerina
20 - Orsano 2	ex Com. Mont. Valnerina
22 - Sellano	ex Com. Mont. Valnerina
23 - Valle L'Aia	ex Com. Mont. Monte Peglia - Selva di M.
24 - Montenero	ex Com. Mont. Monte Peglia - Selva di M.
25 - Colle del Cornio	ex Com. Mont. M.ti Martani e del Serano
26 - Milano di Monte Martano	ex Com. Mont. M.ti Martani e del Serano
27 - Coste di Monte Martano	ex Com. Mont. M.ti Martani e del Serano
28 - Costa della Madonna	ex Com. Mont. M.ti Martani e del Serano
29 - Casa Cantoniera	ex Com. Mont. M.ti Martani e del Serano
30 - Piscino di Panicale	ex Com. Mont. M.ti del Trasimeno
31 - Castellaro di Paciano	ex Com. Mont. M.ti del Trasimeno

Tartufaie di *Tuber magnatum* Pico

1 - S. Patrignano	ex Com. Mont. Alto Tevere Umbro
2 - M. Maggiore 1	ex Com. Mont. Alto Tevere Umbro
3 - M. Maggiore 2	ex Com. Mont. Alto Tevere Umbro
4 - Cai Firenze - Bocca Serriola	ex Com. Mont. Alto Tevere Umbro
5 - Il Toppo	ex Com. Mont. Alto Tevere Umbro
6 - Pieve dei Saddi - Funati	ex Com. Mont. Alto Tevere Umbro
7 - Pieve dei Saddi - Olmo	ex Com. Mont. Alto Tevere Umbro
8 - Il Campo	ex Com. Mont. Alto Tevere Umbro
9 - Sesse	ex Com. Mont. Alto Chiascio
10 - Salia	ex Com. Mont. Alto Chiascio
11 - Le Lame	ex Com. Mont. Alto Chiascio
12 - Carestello	ex Com. Mont. Alto Chiascio
13 - Caldea	ex Com. Mont. Alto Chiascio
14 - Colbassano	ex Com. Mont. Alto Chiascio
15 - Pettinara	ex Com. Mont. Alto Chiascio
16 - S. Pietro	ex Com. Mont. Monte Subasio
17 - Molinello	ex Com. Mont. Monte Peglia - Selva di M.
18 - Il Monte	ex Com. Mont. M.ti del Trasimeno
19 - Ponticelli	ex Com. Mont. M.ti del Trasimeno

Campi Catalogo coltivati con più specie di tartufo

1 - Campeglia	ex Com. Mont. Monte Peglia - Selva di M.
2 - Monte Pincio	ex Com. Mont. M.ti Martani e del Serano
3 - Mercatale di Giano	ex Com. Mont. M.ti Martani e del Serano
4 - Colle Picone	ex Com. Mont. M.ti Martani e del Serano
5 - Forte Cesare	ex Com. Mont. Amerino e Croce di Serra
6 - San Girolamo	ex Com. Mont. M.ti del Trasimeno
7 - Petrignano del Lago	ex Com. Mont. M.ti del Trasimeno
8 – Collestrada	ex Com. Mont. M.ti del Trasimeno

Tartufaie di *Tuber brumale* Vittad. forma *moschatum* Ferry

1 - Acquaviva	ex Com. Mont. Monte Peglia - Selva di M.

In particolare le tartufaie erano state realizzate allo scopo di rendere nota una nuova forma di agricoltura agli operatori agricoli e di effettuare un'ampia sperimentazione sulle varie tecniche di coltivazione delle specie pregiate di tartufo nel territorio umbro.

Dopo 12-14 anni dalla realizzazione delle tartufaie, nel 1997, iniziò lo studio delle 59 piantagioni; le attività di ricerca scientifica si sono prolungate fino al 2009, toccando vari aspetti, per conoscere meglio la coltivazione del tartufo e l'ecologia delle specie simbionti.
Il lavoro è stato suddiviso in tre fasi:

1) monitoraggio delle 59 tartufaie regionali

Tra il 1997 e il 1999, lo studio è iniziato con la ricognizione di tutte le tartufaie regionali allo scopo di localizzarle, rilevare la dimensione media delle piante simbionti, le loro eventuali malattie parassitarie, la percentuale delle fallanze e la presenza di pianelli. Inoltre, dall'1% di ciascuna specie simbionte presente in ogni tartufaia, sono stati prelevati campioni di radici per valutare il loro grado di micorrizazione; inoltre, in ogni piantagione sono stati prelevati campioni di terreno da analizzare dal punto di vista chimico e fisico.

2) Studio del grado di micorrizazione delle tartufaie

Tra il 2000 e il 2002, sono stati prelevati campioni di radici nel 10% di ciascuna specie simbionte presente in tutte le tartufaie, con lo scopo di avere dati attendibili sull'effettivo stato di micorrizazione.

Da questa seconda fase di lavoro risultò evidente che in molte tartufaie bisognava intervenire con pratiche agronomiche atte a creare le condizioni favorevoli alla produzione di tartufi e che gli interventi dovevano essere individuati caso per caso in base alla specie di tartufo, alla pianta simbionte e alle condizioni pedoclimatiche del sito. Furono allora scelte 9 tartufaie dove poter testare varie tecniche colturali.

Tutte le analisi della micorrizazione sono state fatte su base morfologica, avvalendosi di numerose pubblicazioni (Agerer, 1987-2002; Giraud, 1988; Zambonelli *et al.*, 1993; Donnini e Bencivenga, 2005; Granetti, 1995).

Inoltre, per avere informazioni sulla effettiva produzione delle tartufaie regionali sono stati richiesti i dati produttivi alla Regione dell'Umbria e alle Comunità Montane di pertinenza.

3) Sperimentazione di tecniche colturali

In tab. 2 sono riportati i caratteri delle 9 tartufaie dove sono state condotte le sperimentazioni.

Tab. 2 Caratteri delle 9 tartufaie sottoposte a prove sperimentali.

Località	Anno d'imp.	Densità d'imp. m	Sup. m^2	Altit. media m s.l.m.	Incl. gradi	Espos.	Tartufo coltivato	Piante simbionti
Valle l'Aia	1985	da 5x4 a 5x8	2.800	580	4	S/SE	*T. melanosporum*	*Quercus pubescens Corylus avellana*
Castagneto	1985	2 m a filari	1.500	830	0	N/NE	*T. melanosporum*	*Quercus pubescens*
Vigne Parenzio I	1984	5x4	2.025	540	15	N/NW	*T. melanosporum*	*Quercus ilex Corylus avellana Ostrya carpinifolia*
Valcasana	1984	4x4 e 8x8	3.100	380	0	N	*T. melanosporum*	*Quercus pubescens Corylus avellana*
Castellaro di Paciano	1984	8x5	4.000	480	5	SW	*T. melanosporum*	*Quercus pubescens*

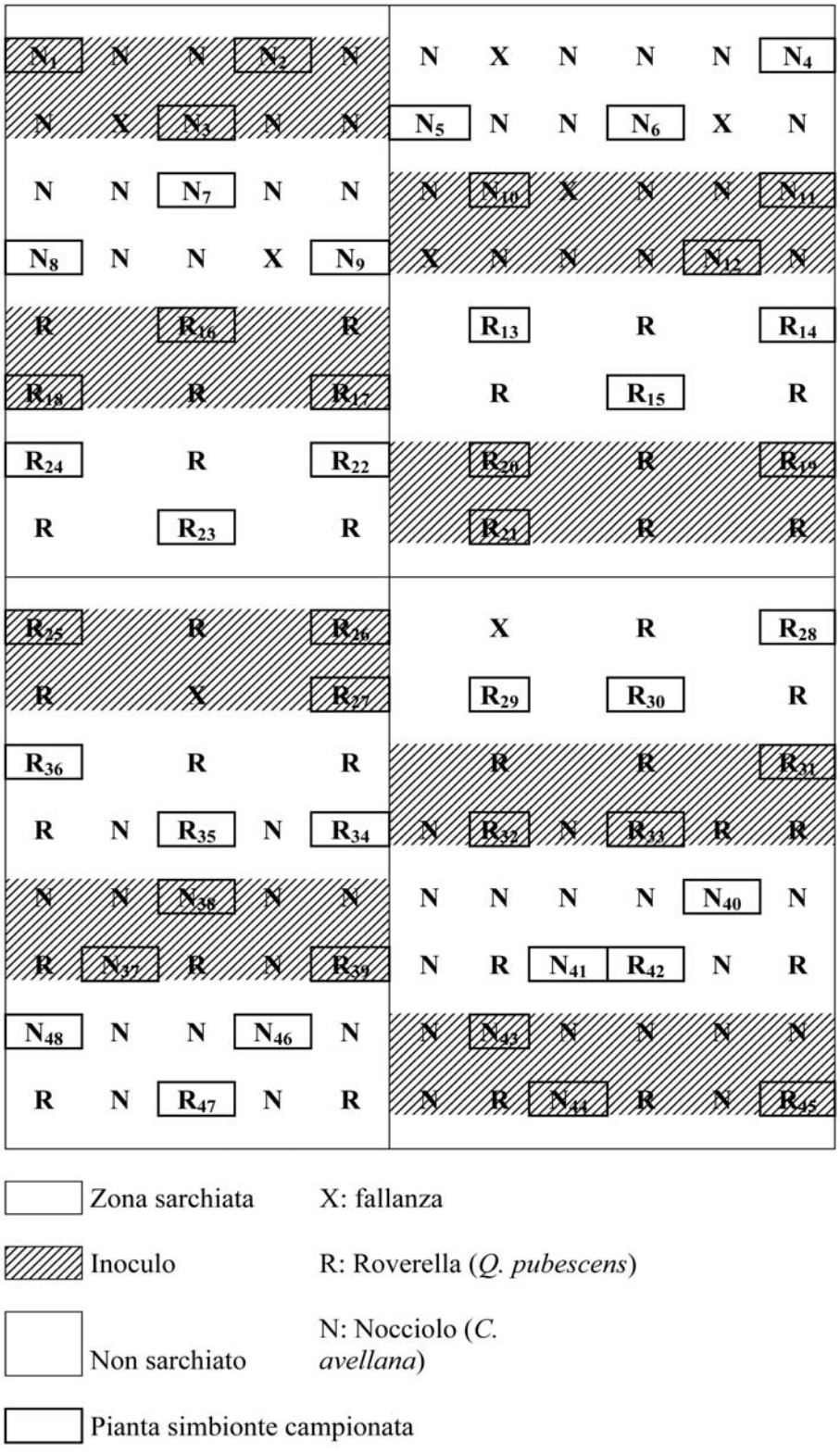
Montenero	1992	7x3	3.000	390	16	S	*T. melanosporum*	*Quercus ilex*
Banditelle I	1984	5x4 e 5x8	2.000	810	11	E/SE	*T. melanosporum*	*Quercus pubescens Corylus avellana*
S. Pietro	1984	5x4 e 5x8	1.500	400	15	N/E	*T. magnatum*	*Quercus pubescens Corylus avellana*
S. Patrignano	1984	5x4 e 5x8	2.500	260	-	-	*T. magnatum*	*Quercus pubescens Corylus avellana*

Le tartufaie sono state scelte in base ai seguenti criteri:
- regolare sviluppo vegetativo delle piante simbionti;
- elevata presenza di micorrize di tartufo;
- accessibilità per le macchine operatrici;
- vicinanza di fonti per l'approvvigionamento idrico;
- caratteristiche pedoclimatiche.

All'interno delle piantagioni, le prove sperimentali furono realizzate individuando delle parcelle omogenee, sia dal punto di vista orografico, che delle piante simbionti.
Le tecniche di coltivazione sperimentate furono:
- Sarchiatura del terreno **-** Durante le indagini preliminari era stato osservato che in quasi tutte le situazioni il terreno era troppo compatto o tendeva a compattarsi. Si è deciso quindi di effettuare prove di sarchiatura del terreno per renderlo più soffice, più areato, più permeabile all'acqua, in definitiva più idoneo alla "fruttificazione" del tartufo.
- Irrigazione – Sono state previste varie tesi di irrigazione, quali periodi e apporti idrici diversi. Le tartufaie dove sono state realizzate le prove di irrigazione sono: Castagneto, Valcasana e Castellaro di Paciano.
- Ammendanti e correttivi – Dal confronto dei dati riportati in lavori scientifici effettuati in Umbria nelle tartufaie naturali (Bencivenga *et al.,* 1990) è emerso che le tartufaie di Montenero e di Castellaro di Paciano presentavano terreni poveri di materia organica e di carbonato di calcio e di conseguenza il pH risultava troppo basso. Perciò a Montenero sono state realizzate prove di apporto di materia organica e carbonato di calcio e a Castellaro di Paciano di carbonato di calcio. Le prove sono state associate o no alla sarchiatura del terreno.
- Apporto di spore – Le analisi della micorrizazione hanno mostrato che in alcune tartufaie di *T. melanosporum* scelte per le prove sperimentali si era ridotta notevolmente la percentuale di micorrizazione con il tartufo coltivato a causa della competizione operata da altre specie di funghi e di tartufi.
L'aggiunta di spore è stata fatta manualmente preparando una miscela di tartufo macinato e acqua di fonte, successivamente distribuita intorno alle piante per un raggio di circa 2m; ogni pianta ha ricevuto circa 5 litri di acqua e 10 grammi di tartufo. L'aggiunta di spore è stata testata in combinazione con la sarchiatura e l'irrigazione. Questa sperimentazione è stata effettuata nelle tartufaie di Vigne Parenzio 1, Valle L'Aia, Banditelle I, S. Pietro e S. Patrignano.
- Potature delle piante simbionti – Dai sopralluoghi sulle tartufaie è emerso che in alcuni casi l'impianto risultava troppo denso e le piante simbionti determinavano un ombreggiamento eccessivo del terreno. Questo fenomeno è stato osservato nelle tartufaie di Montenero e di Banditelle I, dove sono state realizzate prove di potatura a diversa intensità al fine di verificare, al variare dei fattori luce, umidità e sostanza organica nel suolo, l'influenza di tale intervento sulla sopravvivenza delle micorrize di tartufo e sulla competizione operata dagli altri funghi

micorrizici. Prima di avviare la sperimentazione, all'interno di ogni parcella è stato effettuato un approfondimento delle analisi della micorrizazione delle piante simbionti per avere un quadro generale sulla situazione micorrizica delle piante simbionti. In fig. 1 è riportata la planimetria della prova sperimentale condotta a Valle L'Aia dove sono evidenziate le piante campionate.

Fig. 1 Planimetria della prova sperimentale della tartufaia di Valle L'Aia e piante campionate.



Dopo 5 anni circa di prove sperimentali, sono stati ripetuti i campionamenti di radici per valutare la dinamica delle micorrize. Le analisi sono state eseguite utilizzando anche le tecniche più

recenti: Agerer, 2008, Baciarelli Falini *et al*. 2006; Granetti *et al*., 2005; Rubini *et al*., 2010.
Nei risultati verranno riportati i dati ottenuti dalla sperimentazione effettuata sulle tartufaie coltivate a *T. melanosporum* di Valle L'Aia, di Castagneto e di Banditelle I.

Risultati

1) Monitoraggio delle 59 tartufaie regionali
Dal monitoraggio delle 59 tartufaie regionali è emerso che:
- 52 tartufaie presentano piante generalmente sviluppate e più o meno vigorose a causa delle condizioni climatiche e delle cure colturali apportate nel corso degli anni;
- 6 tartufaie di *T. melanosporum*, realizzate in condizioni pedoclimatiche estreme (pendenza del terreno elevata, roccia affiorante, ecc.) e non sottoposte a periodiche cure colturali. Queste piantagioni sono: Cancelli, Colle del Cornio, Costa Sparagara, Pratarelle, Acerone-Cortigno e Costa della Madonna. Inoltre, la tartufaia di Collestrada, coltivata a più specie di tartufo, a causa dell'ampliamento e degli svincoli della strada E45, è stata espropriata e/o distrutta per oltre la metà della superficie.
Dalle analisi della micorrizazione dell'1% delle piante presenti in ogni piantagione è risultato che:
- 25 tartufaie di *Tuber melanosporum* versano in buone condizioni, infatti, in quasi tutte le piante sono presenti le micorrize di questa specie. In alcuni campioni è stata riscontrata la sostituzione delle micorrize di *T. melanosporum* con quelle di altri tartufi, come il *T. aestivum* e il *T. brumale* f. *moschatum* e di altri funghi come *Coenococcum geophilum* Fr., *Hymenogaster citrinus* Vittad., *Pisolithus arhizus* (Scop.: Pers.) Rauschert, *Scleroderma verrucosum* (Bull.) Pers., *Trichophaea woolhopeia* (Cooke & W. Phillips), ecc.;
- in 4 tartufaie di *T. magnatum* (Salia, Colbassano, Il Toppo, Pieve de' Saddi, Funati) sono state rilevate piante provviste delle micorrize di tartufo bianco, anche se con percentuali non elevate (5 - 40%). Tuttavia, in queste tartufaie, nella maggiore parte delle piante, si è verificata la sostituzione delle micorrize di tartufo bianco con quelle di altri tartufi e altri funghi.
- nelle tartufaie coltivate con più specie di tartufo (campi catalogo) è stato riscontrato che ha preso sempre il sopravvento la specie di tartufo favorita dalle condizioni ambientali. Ad esempio, nella tartufaia di Forte Cesare, coltivata con più specie simbionti micorrizate con *Tuber melanosporum*, *T. aestivum*, *T. brumale*, *T. uncinatum* e *T. borchii* sono state trovate in quasi in tutte le piante le micorrize di *T. aestivum*.
Le analisi del terreno, prelevato in tutte le tartufaie, hanno evidenziato che non tutte le piantagioni erano state realizzate in ambienti vocati alla specie di tartufo coltivata (Bencivenga *et al*., 1990; Lulli, 1995; Olivier *et al*., 2002), come ad esempio Casa Cantoniera, Casale Manenti, Valle L'Aia, Montenero, Piscino di Panicale, Castellaro di Paciano, coltivate a *T. melanosporum,* ed Il Campo, Sesse, Le Lame, Caldea, S. Pietro e Pettinara, coltivate a *T. magnatum*.

2) Studio del grado di micorrizazione delle tartufaie
In questa seconda fase sono state campionate complessivamente 1748 piante simbionti in 52 piantagioni. In generale è risultato che il 52,4% di esse presentava le micorrize di tartufo:
- n. 24 tartufaie ben micorrizate con il tartufo coltivato e impiantate in terreni vocati (Colbassano, Salia, Valcasana, Milano, Costa del Pero, Vigna della Campella, Acquaviva, ecc.);
- n. 10 tartufaie micorrizate con il tartufo coltivato insieme ad altri tartufi, impiantate in siti poco idonei al tartufo messo a dimora (Valle l'Aia, Campeglia, Vigne Parenzio 1, Montenero, ecc.);
- n. 16 tartufaie micorrizate solo con tartufi diversi da quello coltivato: Casa Cantoniera, Pieve de' Saddi, Caldea, S. Patrignano, ecc.;
- n. 1 tartufaia coltivata con più specie di tartufo (Petrignano del Lago) su un terreno sub-acido (pH di 5,1), presentava solo una piccola percentuale di micorrize di *T. borchii*.
- n. 1 tartufaia, Piscino di Panicale, coltivata a *T. melanosporum* su un terreno con pH di 6,8, è priva delle micorrize di *T. melanosporum*, confermando ulteriormente la necessità che ha questa specie di svilupparsi in ambienti calcarei (Donnini *et al*., 1997).
Dallo studio della micorrizazione, inoltre, è emersa anche la capacità che hanno le varie

specie simbionti di conservare le micorrize di *T. melanosporum* nelle diverse condizioni pedoclimatiche (Baciarelli *et al*., 2000) e di consentirne la "fruttificazione". Infatti, *Q. pubescens* è la specie simbionte che ha dato i migliori risultati in tutte le piantagioni, sia in ambienti idonei che meno idonei allo sviluppo di *T. melanosporum*. E' sicuramente la specie simbionte più importante per l'Italia centrale, dove è responsabile della maggior parte delle tartufaie naturali (Bencivenga *et al.*, 1990). *C. avellana*, propagandato per la coltivazione dei tartufi perché ritenuto capace di fornire una produzione abbastanza precoce, non si può ritenere un'ottima pianta simbionte, in quanto le micorrize del tartufo coltivato, in quasi tutti gli ambienti, sono state sostituite da quelle di altri funghi e tartufi. *O. carpinifolia*, ha un comportamento diverso a seconda delle condizioni ambientali di coltivazione ed inoltre acquista con facilità le micorrize prodotte da altri funghi e tartufi. Questa specie sembra che conservi abbastanza bene le micorrize di tartufo nero negli ambienti poco favorevoli allo sviluppo vegetativo della pianta. *Q. ilex* è un'ottima specie simbionte, ma il suo impiego è limitato date le sue esigenze termiche. Dove è possibile la sua utilizzazione, è capace di mantenere a lungo le micorrize di tartufo e di fornire una buona produzione.

3) Sperimentazione di tecniche colturali

Durante l'autunno 2008 e la primavera 2009, dopo circa 5 anni di sperimentazione, sono stati effettuati i campionamenti di radici in tutte le parcelle delle 9 tartufaie sottoposte a sperimentazione; i campioni sono stati prelevati dalle stesse piante e nel medesimo punto del campionamento iniziale. Nella tartufaia di Valle l'Aia, sono state effettuate prove di inoculazione di spore di tartufo in combinazione con la sarchiatura. La prova sperimentale ha una superficie di 2.800 m^2, con 133 piante simbionti delle seguenti specie: *Q. pubescens* e *C. avellana*. La prova prevede due tesi principali (inoculato/non inoculato), due tesi secondarie (sarchiato/non sarchiato) e quattro ripetizioni. In questa prova sono state campionate 48 piante prima e dopo i 5 anni di sperimentazione.

Dalla tab. 3, si nota una riduzione della percentuale di micorrizazione nella tesi sarchiatura, effettuata su piante mai sottoposte a interventi colturali, che ha provocato il danneggiamento delle radici superficiali e probabilmente delle micorrize.

Tab. 3 Risultati della prova sperimentale effettuata nella tartufaia di Valle L'Aia.

	No sarchiatura + inoculo		**Sarchiatura + no inoculo**		**Sarchiatura + inoculo**		**Controllo**	
	Prima della prova	Dopo la prova	Prima della prova	Dopo la prova	Prima della prova	Dopo la prova	Prima della prova	Dopo la prova
piante micorrizate con *T. melanosporum*	50,0%	58,3%	75,0%	58,3%	33,3%	41,6%	41,6%	66,6%
piante micorrizate con altri funghi	50,0%	83,3%	50,0%	50,0%	66,6%	91,6%	66,6%	66,6%

L'inoculazione con spore di tartufo è risultata positiva in tutte le varie tesi sperimentali: va rilevato, però, che all'aumento della micorrizazione da parte del tartufo si è verificato un analogo aumento dei funghi competitori. Sembrerebbe che l'apporto di spore abbia stimolato la pianta a migliorare la sua micorrizazione sia con il tartufo che con gli altri funghi.

Nella tartufaia di Castagneto la sarchiatura è stata combinata a varie tecniche di irrigazione. La tartufaia è stata realizzata su un terreno sistemato a gradoni con una fila di *Q. pubescens* in ognuno di essi. La prova sperimentale è stata realizzata su una superficie complessiva di circa 1500 m^2, sulla quale sono presenti 152 piante di roverella. La prova prevede tre tesi: non irrigato; irrigato ogni quindici giorni a partire dal 1° giugno fino al 15 di settembre con apporti

idrici di 40l di acqua a pianta; irrigato ogni primo del mese, a partire da luglio fino a settembre con 80l di acqua per pianta e due sottotesi: sarchiato/non sarchiato, con 4 ripetizioni.
In questa prova sono state campionate 48 piante.
Dalla tab. 4, si conferma l'effetto negativo della sarchiatura effettuata in tartufaie mai sottoposte ad interventi di coltivazione. L'irrigazione senza sarchiatura provoca un incremento dei funghi concorrenti, mentre l'irrigazione su terreno sarchiato non ha provocato effetti positivi.

Tab. 4 Risultato della prova sperimentale di sarchiatura ed irrigazione effettuata nella tartufaia di Castagneto

	No sarchiato + irrigazione ogni 15 giorni		**No sarchiato + irrigazione ogni 30 giorni**		**Sarchiato + no irrigato**		**Sarchiato + irrigazione ogni 15 giorni**		**Sarchiato + irrigazione ogni 30 giorni**		**Controllo**	
	Prima della prova	Dopo la prova	Prima della prova	Dopo la prova	Prima della prova	Dopo la prova	Prima della prova	Dopo la prova	Prima della prova	Dopo la prova	Prima della prova	Dopo la prova
piante micorrizate con *T. melanosporum*	75,0%	87,5%	75,0%	50,0%	75,0%	50,0%	75,0%	75,0%	62,5%	62,5%	62,5%	75,0%
piante micorrizate con altri funghi	75,0%	75,0%	62,5%	100,0%	37,5%	87,5%	62,5%	100,0%	62,5%	100,0%	75,0%	100,0%

Nella tartufaia di Banditelle I, è stata effettuata una prova di inoculazione sporale di *T. melanosporum*.
La prova prevede l'arricchimento del terreno con spore di *T. melanosporum* combinato con la sarchiatura su una superficie di circa 2000m^2 comprendente 64 roverelle. La prova prevede quattro tesi principali (inoculo sarchiato e non sarchiato – non inoculo sarchiato e non sarchiato) e due tesi secondarie (potatura energica e no potatura) e 2 ripetizioni. In questa prova sono state campionate 18 piante.
Dalla tab. 5 si evidenzia una situazione abbastanza diversificata e non facilmente interpretabile. In particolare, l'effetto della sarchiatura è positivo nella maggioranza dei casi e analogo andamento riguarda anche la potatura energica. In questo caso la distribuzione dell'inoculo sporale ha determinato risultati diversi in piante diverse.

Tab. 5 Risultato della prova sperimentale di sarchiatura, inoculo sporale e potatura effettuata nella tartufaia di Banditelle I

	No sarchiato + no inoculo + potatura energica		No sarchiato + inoculo + no potatura		No sarchiato + inoculo + potatura energica		Sarchiato + no inoculo + no potatura		Sarchiato + no inoculo + potatura energica		Sarchiato + inoculo + no potatura		Sarchiato + inoculo + potatura energica		Controllo	
	Prima della prova	Dopo la prova	Prima della prova	Dopo la prova	Prima della prova	Dopo la prova	Prima della prova	Dopo la prova	Prima della prova	Dopo la prova	Prima della prova	Dopo la prova	Prima della prova	Dopo la prova	Prima della prova	Dopo la prova
piante micorrizate con *T. melanosporum*	–	50,0%	100,0%	50,0%	50,0%	50,0%	33,3%	66,6%	33,3%	66,6%	–	100,0%	50,0%	–	–	–
piante micorrizate con altri funghi	100,0%	50,0%	–	100,0%	50,0%	50,0%	66,6%	66,6%	100,0%	100,0%	100,0%	50,0%	100,0%	100,0%	100,0%	100,0%

Produzioni

Allo scopo di verificare le produzioni delle tartufaie oggetto di studio la Regione Umbria, dopo pochi anni dalla messa a dimora delle piante tartufigene, aveva incaricato due dipendenti regionali per la ricerca dei tartufi in tutte le proprie tartufaie. Purtroppo non sempre gli incaricati sono riusciti a fare sopralluoghi periodici, spesso si sono verificati prelevamenti da parte di animali selvatici e da cavatori di frodo, per cui non è stato possibile conoscere la reale produzione delle piantagioni.

Tuttavia, pur con dati falsati in negativo è stato verificato che 32 tartufaie (il 61% delle 52 attualmente in coltivazione) producono discreti quantitativi di tartufo (tab. 6).

Tab. 6 Tartufaie della Regione Umbria produttive.

Tartufaia	Specie di tartufo coltivato	Specie di tartufo prodotto
Capralina	*T. melanosporum*	*T. aestivum*
Castagneto	*T. melanosporum*	*T. melanosporum*
Banditelle I	*T. melanosporum*	*T. melanosporum* + *T. aestivum*
Assisi	*T. melanosporum*	*T. melanosporum* + *T. aestivum*
Vigne della Campella	*T. melanosporum*	*T. melanosporum*
Vigne Parenzio I	*T. melanosporum*	*T. aestivum* + *T. brumale f. moschatum*
Vigne Parenzio II	*T. melanosporum*	*T. aestivum* + *T. brumale f. moschatum*
Costa del Pero	*T. melanosporum*	*T. melanosporum*
Valcasana	*T. melanosporum*	*T. melanosporum*
Orsano I	*T. melanosporum*	*T. melanosporum*

Orsano II	*T. melanosporum*	*T. melanosporum*
Valle L'Aia	*T. melanosporum*	*T. melanosporum + T. aestivum + T. brumale f. moschatum + T borchii*
Montenero	*T. melanosporum*	*T. melanosporum + T. aestivum*
Milano di Monte Martano	*T. melanosporum*	*T. melanosporum*
Casa Cantoniera	*T. melanosporum*	*T. aestivum*
Castellaro di Paciano	*T. melanosporum*	*T. melanosporum*
S. Patrignano	*T. magnatum*	*T. aestivum + T. brumale f. moschatum + T. borchii*
Pieve dei Saddi - Funati	*T. magnatum*	*T. aestivum*
Pieve dei Saddi - Olmo	*T. magnatum*	*T. aestivum*
Sesse	*T. magnatum*	*T. aestivum + T. brumale f. moschatum + T. borchii*
Salia	*T. magnatum*	*T.magnatum + T. aestivum + T. brumale f. moschatum + T. borchii*
Carestello	*T. magnatum*	*T. aestivum*
Caldea	*T. magnatum*	*T. aestivum + T. brumale f. moschatum + T. borchii*
Colbassano	*T. magnatum*	*T. magnatum*
Pettinara	*T. magnatum*	*T. aestivum + T. brumale f. moschatum + T. borchii*
Molinello	*T. magnatum*	*T. borchii+ T. aestivum*
Il Monte	*T. magnatum*	*T. borchii*
Campeglia	*T. melanosporum+ T. borchii + T aestivum + T. uncinatum + T. mesentericum*	*T. aestivum*
Monte Pincio	*T. melanosporum+ T. borchii + T aestivum + T. brumale + T. magantum*	*T. aestivum*
Mercatale di Giano	*T. melanosporum+ T. magantum+ T aestivum*	*T. aestivum*
Forte Cesare	*T. melanosporum+ T. borchii + T aestivum + T. uncinatum + T. brumale*	*T. aestivum + T. borchii*
Acquaviva	*T. brumale* f. *moschatum*	*T. brumale f. moschatum + T. aestivum*

Dalla tab. 6 si evidenzia che nelle tartufaie viene prodotta la specie di tartufo coltivato, ma anche specie diverse favorite dal microambiente della piantagione o dalla carenza di tecniche colturali idonee.
In particolare, le produzioni riguardano (tab. 6): *T. magnatum* nelle tartufaie di Salia e di Colbassano (la tartufaia di Colbassano è stata parzialmente distrutta per il passaggio della superstrada Perugia-Ancona), *T. melanosporum* in 12 tartufaie, *T. aestivum* in 21, *T. borchii* in 9 e *T. brumale* f. *moschatum* in 9. Inoltre, in 18 piantagioni si è verificata la produzione spontanea di *T. aestivum* e in 8 di *T. brumale* f. *moschatum*. Le produzioni rilevate non sono entusiasmanti, tuttavia sono positive considerando la carenza di cure colturali praticate alle piantagioni. Va ricordato che il Programma Tartufigeno Regionale si era prefisso scopi dimostrativo-sperimentali e in virtù di questi, oltre a non dimenticare le scarse conoscenze ecologiche di cui si disponeva al momento della scelta dei siti, i risultati sono confortanti.

Conclusioni

Considerando i fini dimostrativi e sperimentali del Programma Tartufigeno Regionale, la presenza delle micorrize di tartufo in oltre la metà delle piante dopo 14-17 anni dalla loro messa a dimora si deve considerare un successo, alla luce delle conoscenze scientifiche che si avevano negli anni delle piantagioni.

Tuttavia i dati sulla micorrizazione non possono essere completamente significativi, in quanto tale analisi viene effettuata su piccole porzioni di apparato radicale che non possono ritenersi rappresentative dell'intera pianta. Tali dati hanno confermato che le migliori piante simbionti sono *Quercus pubescens* e *Q. ilex,* vista la loro capacità di conservare le micorrize del tartufo con cui erano state unite in simbiosi.

Per quanto attiene la sperimentazione delle tecniche colturali, un argomento poco affrontato dai ricercatori per la complessità attuativa e di interpretazione dei risultati, si possono trarre solo conclusioni parziali. Positivo è l'inoculo sporale, che può essere consigliato ai tartuficoltori soprattutto nelle piantagioni poco produttive. La sarchiatura si deve ritenere inopportuna in tartufaie rimaste indisturbate per molti anni, perché provoca il danneggiamento di molte radici superficiali favorendo la micorrizazione con altri funghi; è un intervento utile se effettuato fin dall'inizio dell'impianto, perché costringe le radici a colonizzare gli strati meno superficiali di terreno, dove gli eventuali corpi fruttiferi sono più protetti dai fattori ambientali e dai parassiti. L'irrigazione non ha mostrato risultati apprezzabili. La potatura energica ha evidenziato, in alcuni casi, un incremento della percentuale di micorrizazione, suggerendo quindi di approfondire la ricerca.

In conclusione, gli interventi colturali è bene che siano programmati dal momento dell'impianto della tartufaia ed eseguiti tutti gli anni utilizzando la stessa tecnica.

I dati produttivi costituiscono, senza dubbio, il risultato più importante: oltre il 60% delle tartufaie produce tartufi della specie coltivata o di altre specie. Purtroppo, come già evidenziato, i dati reperiti non sono completi, in particolare manca il dato quantitativo che avrebbe consentito di valutare in modo efficace i risultati della sperimentazione.

Il fallimento di alcune piantagioni va imputato a diversi motivi:

- la utilizzazione di ambienti non idonei a causa delle scarse conoscenze ecologiche del momento;
- la scelta obbligata di alcuni terreni che dovevano essere di proprietà pubblica;
- la credenza di allora che i tartufi vivono solo in zone marginali praticamente impossibili da coltivare;
- la mancanza di conoscenze sulle tecniche di coltivazione da adottare nelle tartufaie. Le conoscenze attuali indicano di realizzare le piantagioni nei buoni terreni agrari, dove è possibile effettuare le cure colturali che consentano la sopravvivenza delle micorrize, lo sviluppo delle piante simbionti e la formazione degli sporocarpi.

Le produzioni che si ottengono in tartufaie impiantate da privati in ambienti idonei e sottoposte a cure colturali razionali sono di stimolo per la diffusione della tartuficoltura nel territorio umbro che, per la sua particolare orografia e la natura dei terreni, può considerarsi una grande tartufaia naturale. Attualmente i proventi netti derivanti dalla raccolta dei tartufi spontanei e coltivati vengono stimati in oltre 12 milioni di Euro, proventi che non vengono forniti da nessuna attività agricola. Inoltre, la presenza di tartufi e l'indotto che ne deriva costituiscono anche un forte veicolo attrattivo nei confronti del turismo ed un beneficio socio-economico e ambientale per il territorio.

In conclusione si auspica che la ricerca scientifica applicata alla tartuficoltura, poco seguita dai ricercatori perché lunga, difficile, laboriosa e povera di risultati, venga stimolata con ogni mezzo per rendere sempre più certa e redditizia questa forma di agricoltura particolarmente importante per la Regione Umbria.

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AUXOMETRIA DEL CARPOFORO DI *TUBER AESTIVUM* VITTAD. (ALIAS *TUBER UNCINATUM* CHATIN)*

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Abstract

The *Tuber aestivum* (alias *Tuber uncinatum*) is a type of truffle that is being used more and more in truffle cultivation, especially as a substitute for *Tuber malanosporum*, because of its greater adaptability to the most varied growing environments. Despite its widespread use there are few studies on the development and macroscopic growth of the carpophore. Such studies would be very useful, in particular in order to define the handling of cultures and the timing of intervention as a function of the growth requirements of the carpophores. The Authors monitored the growth of several *Tuber aestivum* (alias *Tuber uncinatum*) carpophores in a truffle cultivation consisting of 28 European hophornbeams (*Ostrya carpinifolia*), mycorrhized with *Tuber aestivum* (alias *Tuber uncinatum*), planted in 1992 on land located in the province of Vicenza (Altavilla Vicentina). Maximum measurements in two perpendicular directions were taken to estimate the surface area of the carpophores and the way they develop. Such measurements of the carpophores were taken as they came up from the ground every week for two consecutive years (2007 and 2008) starting in the month of January through the month of June. Hence the growth curve was studied as a function of the date of the measurement and several climatic variables such as maximum, average and minimum temperatures, precipitation and wind. Once each carpophore reached maturity, it was collected and weighed. Using a multiple regression model with the stepwise elimination of the predictive variables the degree of dependence of the dependent variable (carophore growth) on the climatic predictive variables was quantified. In addition, the growth curve was parametrized using appropriate sigmoidal functions. This study, though preliminary, sheds light on the last stages of growth of the carpophores and allows us cultures more rationally.

Key words: auxometry, carpophores, growth curve, ecology, *Tuber aestivum /T. uncinatum*, truffle cultivation.

*Nota: * Si è ritenuto opportuno utilizzare la doppia nomenclatura Tuber aestivum Vittad, alias Tuber uncinatum Chatin anche perché, nel caso oggetto di studio, la produzione dei carpofori è avvenuta, un anno (2007) sia in estate che in autunno (il che farebbe pensare a Tuber uncinatum Chatin), ed un anno (2008) esclusivamente in estate (il che farebbe pensare a Tuber aestivum).*

Introduzione e scopo

La coltivazione dei tartufi intesa come attività agroforestale è molto diffusa non solo in Italia (Mannozzi-Torini, 1984; Bencivenga *et al.,*1989; Gregori,1991; Zambonelli *et al.,*1992; Granetti, 2005) ma anche in molti paesi europei (Francia, Spagna, Ungheria etc.) (Chevalier, 2001; Reyna, 2007; Bratek, 2007) ed extraeuropei (Cile, Australia, Nuova Zelanda etc.) (Hall *et al.,* 2007). Questo interesse ha fatto sì che esistano numerosi lavori e numerose ricerche sulle esigenze ecologiche dei tartufi e sui sistemi più appropriati di coltivazione, mentre pochi sono gli studi condotti sulle modalità d'accrescimento dei carpofori ed ancora meno quelli inerenti ricerche auxometriche.

Infatti gli studi esistenti in materia di accrescimento e sviluppo dei carpofori riguardano, per lo più, l'influenza dei fattori esterni che determinano la creazione e la formazione dei carpofori (Montant *et al.,*1990) o che agiscono sul loro sviluppo (Kulifaj, 1994).
Esistono anche ricerche che mirano a definire la struttura e la funzione delle ife esterne di carpofori adulti (Barry, 1992) o tendono a precisare le modalità di assorbimento dell'acqua e dei nutrienti da parte del carpoforo (Barry *et al.,*1994).
Tuttavia queste interessanti ricerche, volte ad individuare e definire, con la maggiore precisione possibile, lo specifico periodo di formazione del carpoforo, non si occupano mai della modalità del suo accrescimento né tanto meno riportano la misurazione di tale fenomeno nel tempo. Al contrario questi studi (auxometrici) sono di grande utilità pratica soprattutto per definire, in qualunque tipo di tartufaia (controllata e/o coltivata) i tempi di intervento per l'esecuzione delle cure colturali, le quali devono avvenire in funzione delle modalità di crescita e delle differenti esigenze che un carpoforo può manifestare nelle varie fasi del proprio ciclo biologico.

Probabilmente le ricerche di tipo auxometrico sugli ascomi dei tartufi, non sono così numerose per tutta una serie di oggettive difficoltà intrinseche.
Infatti per poter effettuare correttamente studi quantitativi dei fenomeni relativi allo sviluppo dei carpofori di tartufo (in vero anche per altre specie fungine) è necessario soddisfare ad alcuni importanti prerequisiti:

1) che si possano eseguire misurazioni dell'ascoma (tartufo) senza interferire in alcuna maniera con il suo naturale sviluppo;
2) che vi sia la possibilità di seguire, singolarmente, ciascun ascoma nel tempo;
3) che si possa disporre di un elevato numero di ascomi da misurare;
4) che ci sia grande omogeneità per quanto concerne le condizioni stazionali (pedologiche, climatiche etc.) del sito dove si trova la tartufaia;
5) parimenti che anche la stessa tartufaia presenti requisiti di omogeneità sia nei confronti delle piante simbionti (medesima specie forestale, stessa età, identica provenienza delle piante messe a dimora, stesso sesto, etc.) che del trattamento agro-colturale;
6) che vi sia la possibilità di poter disporre di dati climatici attendibili e specifici della tartufaia oggetto di studio.

Quindi, poiché ancora non sono sufficientemente note né le modalità di crescita dell'ascoma del tartufo né il numero ed il ruolo dei molti fattori endogeni ed esogeni coinvolti, lo scopo di questa ricerca è quello di cercare di capire in che modo e con che velocità avvengono le fasi di accrescimento del carpoforo a partire dal momento del suo affioramento dal terreno.
La comprensione di tale fenomeno dovrebbe essere il presupposto per intervenire culturalmente in maniera più razionale e precisa attraverso appropriati itinerari tecnici di gestione della tartufaia al fine di renderla più produttiva.

Materiali e Metodi

a) Principali caratteristiche della tartufaia coltivata

Per condurre le misurazioni auxometriche sugli ascomi è stata utilizzata una piccola tartufaia coltivata, (Fig.1) costituita da 28 piante di carpino nero (*Ostrya carpinifolia* Scop.), micorrizate con *Tuber aestivum* Vittad. (*alias Tuber uncinatum* Chatin), impiantata nel 1992 in un terreno ubicato in provincia di Vicenza (Altavilla Vicentina), e divenuta, a partire dal 2005, massicciamente produttiva (in media circa 20-25 Kg. a stagione).

Fig. 1 Panoramica della tartufaia, durante le misurazioni dei carpofori emersi, costituita da 28 piante di carpino nero (*Ostrya carpinifolia* Scop.) micorrizate con *Tuber aestivum* Vittad. (alias *Tuber uncinatum* Chatin) messe a dimora nel 1992.

La scelta del *Tuber aestivum* Vittad. (alias *Tuber uncinatum* Chatin), non è casuale. Infatti, tale specie si presta, molto più di altre, a questo tipo di studio, in quanto moltissimi dei suoi i carpofori sono semipogei; il che non solo rende possibile le misurazioni dei carpofori senza muoverli dal sito, ma permette anche di vederli. Inoltre tale specie viene utilizzata sempre di più in tartuficoltura in sostituzione del *Tuber melanosporum* (Gregori *et al.,*1995; Gregori, 1999; Chevalier *et al*., 1999; Belloli *et al.,* 2001; Vinay e Pirazzi, 2001; Tanfulli *et al.,* 2004; Weden *et al.,* 2004) per la sua maggiore adattabilità ai più svariati ambienti di coltivazione e la sua maggiore resistenza ad un clima che sembra evolvere verso un costante riscaldamento globale.

Le principali caratteristiche ecologiche della tartufaia sono così riassumibili:

- *Il suolo*, derivante dal disfacimento di rocce a base calcarea del secondario, si presenta poco profondo e, benché nelle zone boschive limitrofe sono evidenti numerosi massi affioranti, risulta privo di scheletro, (1%), con tessitura prevalentemente di tipo Franco-Sabbioso-Argilloso (FSA), a reazione leggermente alcalina (pH da 7,8 a 8,0), mediamente calcareo ($CaCO_3$ da 10,3% a 20,6%), ma con elevato calcare attivo (da 3,1 a 4,6%), e con poca (1,0%) o tanta (5,0) Sostanza Organica.
  Si riportano, in Tab. 1 i dati completi relativi alle analisi chimico fisiche del suolo della tartufaia.

Tab. 1 Risultati dell'analisi chimico fisica del suolo della tartufaia oggetto di studio.

Variabile		**Campione 1**	**Campione 2**	**Campione 3**
Scheletro	*%*	1,0	10,0	1,0
Sabbia grossa (>200 e <2000 um)	*g/Kg*	44	51	63
Sabbia fine (>100 e <200 um)	*g/Kg*	203	166	94
Sabbia molto fine (>50 e <100 um)	*g/Kg*	322	278	112
Limo (>2 e <50 um)	*g/Kg*	214	257	411
Argilla (<2 um)	*g/Kg*	217	248	320
Tessitura		FSA	FSA	FA
pH		8,0 *leggermente alcalino*	7,8 *leggermente alcalino*	7,9 *leggermente alcalino*
Calcare totale	*g/Kg*	179 *mediamente calcareo*	206 *mediamente calcareo*	103 *mediamente calcareo*
Sostanza organica	*g/Kg*	10,5 *basso*	20,1 *medio*	50,0 *elevato*
Carbonio organico	*(C) g/Kg*	6,09	11,66	29,00
Azoto totale	*(N) g/Kg*	0,70 *scarso*	1,30 *medio*	2,90 *ricco*
Fosforo ass.	*(P) mg/Kg*	8 *basso*	20 *elevato*	12 *medio*
Calcio scamb.	*(Ca) mg/Kg*	4450 *molto elevato*	5440 *molto elevato*	8860 *molto elevato*
Calcio scamb.	*meq/100g*	22,3	27,2	44,3
Magnesio scamb.	*(Mg) mg/Kg*	105 *medio*	131 *medio*	216 *elevato*
Magnesio scamb.	*meq/100g*	0,86	1,07	1,77
Potassio scamb.	*(K) mg/Kg*	138 *medio*	174 *medio*	189 *medio*
Potassio scamb.	*meq/100g*	0,35	0,45	0,48
Sodio scamb.	*(Na) mg/Kg*	9 *molto basso*	26 *molto basso*	40 *basso*
Sodio scamb.	*meq/100g*	0,04	0,11	0,17
Scambio cationico	*meq/100g*	15,9 *medio*	25,1 *elevato*	40,5 *elevato*
C/N		8,7 *tendente alla mineralizzazione*	9,0 *tendente alla mineralizzazione*	10,0 *equilibrato*
Mg/K		2,4	2,4	3,7
Calcare attivo	*g/Kg*	33 *medio*	46 *elevato*	31 *medio*
Ferro ass.	*(Fe) mg/Kg*	10,00 *basso*	13,00 *medio*	18,00 *medio*
Manganese ass.	*(Mn) mg/Kg*	5,80 *basso*	7,90 *basso*	13,40 *medio*
Rame ass.	*(Cu) mg/Kg*	2,20 *medio*	5,30 *alto*	6,10 *alto*
Zinco ass.	*(Zn) mg/Kg*	1,40 *basso*	2,60 *medio*	6,60 *medio*
Anidride fosforica	*(P2O5)*	19	45	27

- *Il clima* della zona è quello del "*Distretto climatico mediterraneo*" (Del Favero *et al.,* 1990) tipico, in generale, della pianura veneta. In particolare il clima della provincia vicentina viene definito (De Marchi, 1935) "*temperato caldo, sempre umido*", e la temperatura media annua risulta pari a 13°C, mentre la temperatura media mensile fluttua fra l'1.6°C

di gennaio, che è il mese più freddo, ed i 23,9° C di luglio che è il mese più caldo.
Le temperature medie invernali sono sensibilmente maggiori di 0° C, mentre quelle medie estive sono comprese tra i 21° C ed i 23° C.
Il regime pluviometrico della provincia di Vicenza, con una precipitazione media annua di 995 mm è stato chiamato da De Marchi “regime sub-litoraneo” per essere intermedio fra l’oceanico ed il continentale (Gregori, 1991). Tutti i dati climatici relativi alla tartufaia oggetto di studio, per gli anni 2007 e 2008, sono stati gentilmente forniti dai proprietari della stazione meteo posta al confine con la tartufaia medesima.
In appositi grafici (Fig. 2: a, b, c) vengono riportati alcuni parametri climatici (piovosità, temperatura media, umidità) relativi a: marzo, aprile, maggio 2007 e 2008, periodo di ingrossamento del carpoforo.

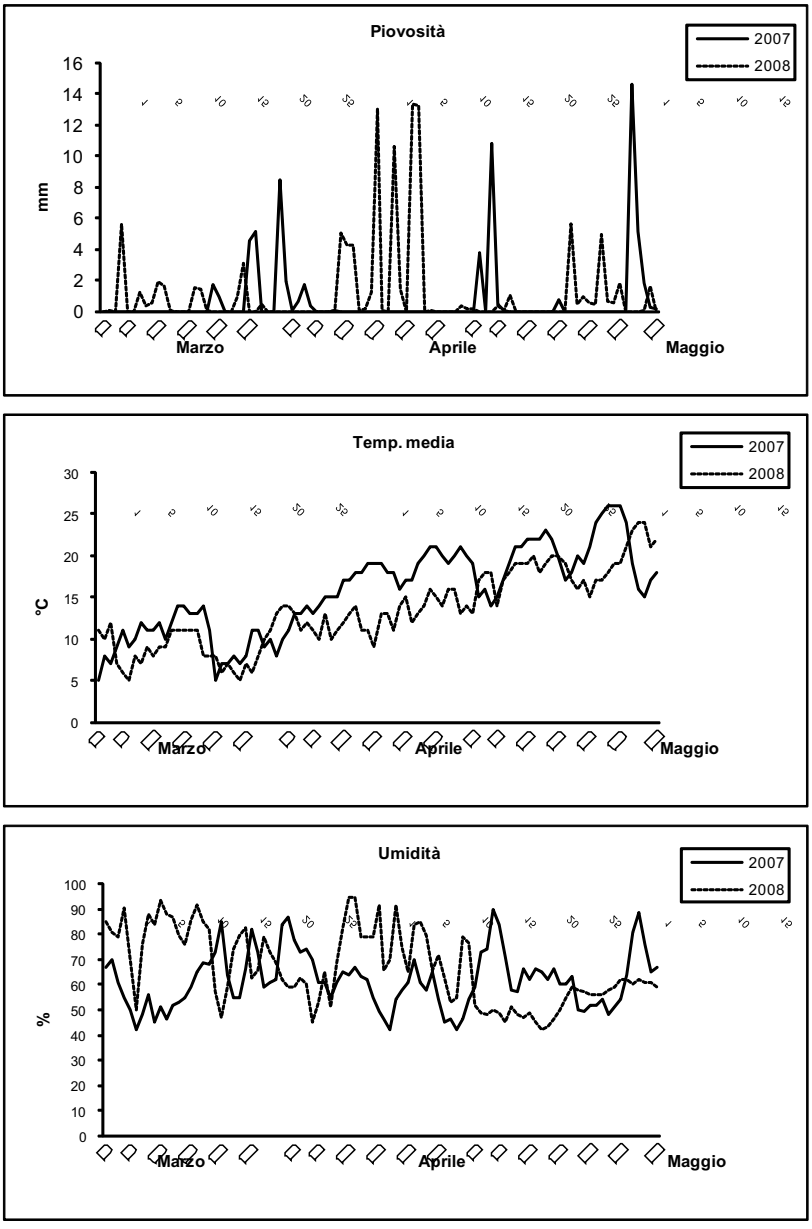


Fig. 2 Andamento della piovosità (2a), della temperatura media (2b) e dell’umidità(2c) nei mesi di accrescimento del carpoforo (marzo, aprile, maggio).

Per ogni tartufo “emerso” veniva segnalata (con un piccolo paletto) la sua posizione e riportata su un apposito foglio di carta millimetrata. Questo ha permesso di osservare che la fuoriuscita dei carpofori all’interno della tartufaia, non avviene in maniera del tutto casuale ed uniforme né

all'interno dello stesso pianello né rispetto alla superficie complessiva della tartufaia ma secondo dei gradienti di produzione. Infatti l'inizio dell'affioramento di uno o più tartufi in un determinato punto del pianello o della tartufaia, comportava, poi nel tempo, la comparsa di altri carpofori non in maniera diffusa ma, piuttosto, sempre nella medesima zona ed in prossimità del nucleo originario di emersione. Questo significa che, nella tartufaia, la produzione e l'ingrossamento degli ascomi (denunciati dalla emersione dei corpi fruttiferi) avviene non in maniera casuale e variabile ma con distribuzione aggregata, cioè a chiazze (Fig. 3), rappresentate da zone ricche di tartufi emersi.

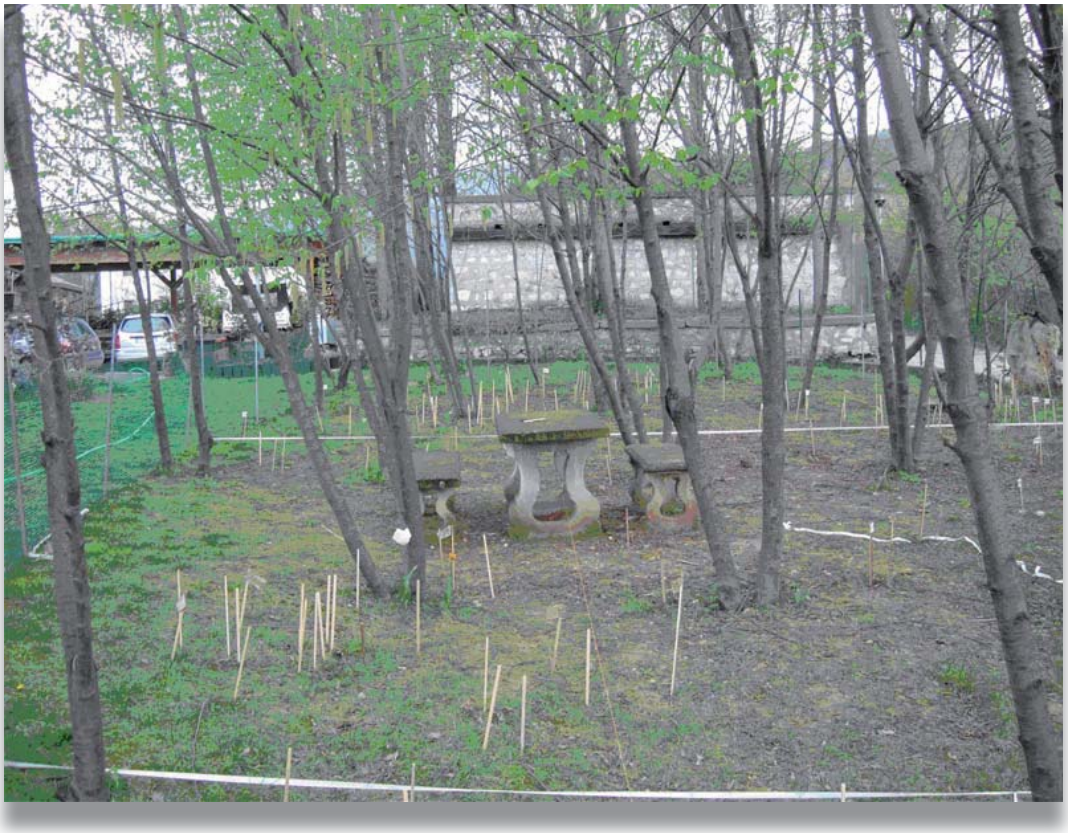


Fig. 3 L'emersione dei corpi fruttiferi non avviene in maniera casuale ma con distribuzione aggregata, vale a dire "a chiazze".

Infine, per entrambe le annate, partire da giugno e fin quando è durata la produzione annuale, man mano che i carpofori giungevano a maturità, sono stati raccolti e di ciascuno è stato registrato anche il singolo peso.

c) Analisi statistica

E' stata studiata la curva di accrescimento in funzione della data di misurazione e di alcune variabili climatiche quali temperatura massima, media e minima, precipitazioni, vento.
La curva di accrescimento è stata quantificata con regressione non-lineare.
Per modellizzare la crescita degli ascomi (area, asse y) in funzione del tempo (settimane, asse x) è stato scelto, come modello di regressione non-lineare, la seguente funzione logistica (altrimenti detta di tipo sigmoidale) (con 4 gradi di libertà) in quanto è quella che minimizza la distanza dei dati sperimentali dalla curva di regressione.

$$y = \frac{a}{\left(1 + \exp(\frac{c - x}{b})\right)} + d$$

(dove y=*a*+*d* è il valore massimo a cui tende la curva di crescita logistica; y=*d* è il valore

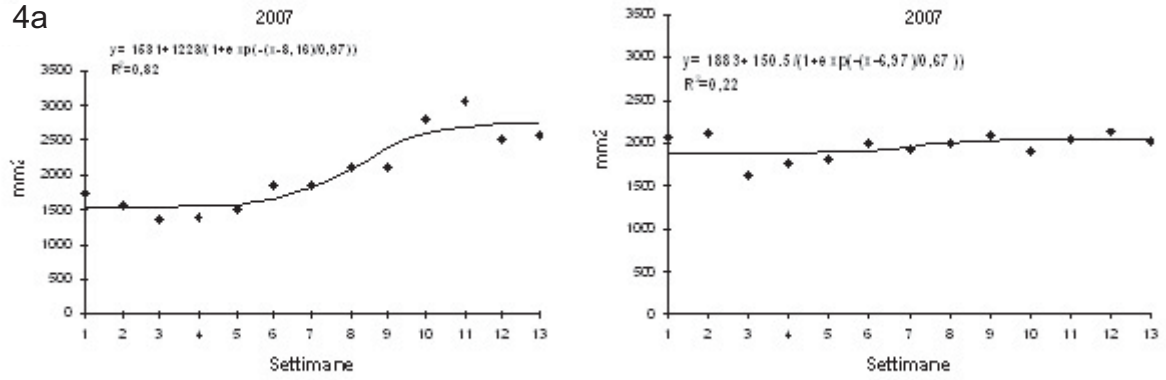
minimo a cui tende la curva logistica; x=*c* è il valore dove si ha la massima variazione di crescita, mentre *a/4b* quantifica la velocità di crescita nel punto di flesso).
La bontà di approssimazione ai dati sperimentali del modello scelto è quantificata dal coefficiente di determinazione multiplo corretto, altrimenti detto R^2, compreso fra 0 ed 1.
Quando R^2 → 0, significa che la distribuzione dei dati non è assolutamente schematizzabile da una curva logistica, mentre quando R^2 → 1, significa che la distribuzione dei dati è ottimamente descritta dalla curva logistica.
Inoltre, tramite un modello di regressione lineare multipla con eliminazione stepwise delle variabili predittive, si è quantificato il grado di dipendenza della variabile dipendente (crescita carpoforo, area superficie emersa) dalle variabili predittive climatiche.
Il metodo della regressione multipla considera la variabilità della risposta della y in relazione a molteplici variabili indipendenti x. Se la variabile di risposta è quantitativa continua, si utilizza la regressione lineare multipla.
L'equazione relativa alla regressione lineare multipla è:

$$\hat{y} = b_0 + b_1 x_1 + b_2 x_2 + \ldots + b_n x_n$$

Dove $x_1 \ldots x_n$ sono le n-esime variabili indipendenti predittive, mentre $b_1 \ldots b_n$ sono i coefficienti della regressione. Nei modelli con eliminazione stepwise, vengono progressivamente eliminate tutte quelle variabili predittive che non cambiano la predittività del modello considerato; in questo modo si semplifica il modello, pur non diminuendo significativamente la bontà di approssimazione (Shaeskin, 2000).

Risultati
Nell'arco dell'intero periodo delle osservazioni, sono stati misurati 296 e 342 carpofori, afferenti rispettivamente agli anni 2007 - 2008.
In entrambe le annate la durata della emersione dei corpi fruttiferi (che avviene in maniera irregolarmente intermittente) è stata costante (circa 120 gg.: da gennaio a maggio) così come costante è stato il rapporto fra il numero di tartufi emersi (circa 1/3) e quello dei tartufi completamente ipogei (circa 2/3 del totale). Differente, invece, è stata la durata del periodo di raccolta: fino al 24 di ottobre nel 2007 e fino al 6 agosto nel 2008.
Alla fine delle rilevazioni, nell'analisi delle aree stimate degli ascomi si è effettuata, una suddivisione in due gruppi: quello degli ascomi dall'area stimata più grande, (cioè superiore all'area mediana) e quello degli ascomi dall'area stimata più piccola (cioè inferiore all'area mediana).
L'elaborazione statistica dei dati è stata espressa con i seguenti grafici (Fig. 4a,b):

4a



4b

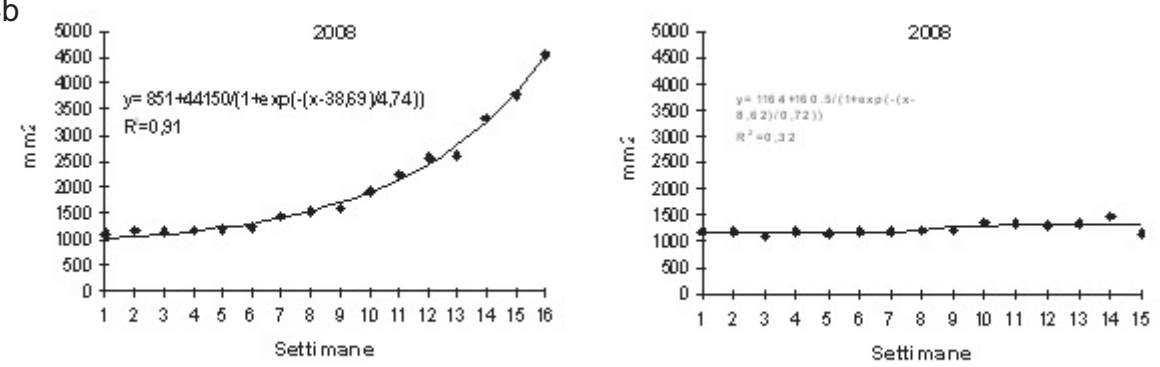


Fig. 4 Grafici della regressione lineare usando la funzione logistica a quattro gradi di libertà; si riportano le crescite (aree medie dei carpofori emersi) in funzione del tempo (settimane) relativi al 2007 (4a) e al 2008 (4b).

Appare evidente che, indipendentemente dall'area stimata al momento di inizio della registrazione delle misurazioni, ed indipendentemente dalle condizioni stagionali annuali, alcuni ascomi si accrescono notevolmente (gruppo dei grossi: parte sx dei grafici) altri ascomi rimangono pressoché identici (gruppo dei piccoli: parte dx dei grafici), le variazioni di accrescimento dipendono sia di fattori endogeni che ambientali.
I grafici della regressione non-lineare relativi agli ascomi piccoli mostrano che gli ascomi che rimangono piccoli, anche alla fine del periodo di crescita, non sono partiti da dimensioni inferiori rispetto agli ascomi grossi, ma da dimensioni pressoché identiche. E questo succede sia nel 2007 anno poco piovoso sia nel 2008 anno molto piovoso. I grafici della regressione non-lineare eseguiti sugli gli ascomi grossi, apparentemente fra loro differenti, sono invece spiegabili se osservati in rapporto all'andamento climatico. Infatti nel 2007, anno molto siccitoso, specie in primavera, e con poche precipitazioni, la variazione di crescita si verifica solo in concomitanza delle piogge e in ogni caso l'area stimata, a fine crescita, risulta mediamente inferiore (2500 mm) a quella del 2008 (4000 mm) anno in cui, invece, si sono verificate abbondanti e continue precipitazioni.
Le osservazioni hanno messo in evidenza anche che gli ascomi grossi non solo sono mediamente cresciuti maggiormente rispetto quelli della stessa categoria dell'anno precedente, ma hanno il punto di flesso della curva logistica che si colloca fuori dal grafico redatto. Ciò significa che la fase di crescita dipende fortemente dalle condizione climatiche, e che lo sviluppo del carpoforo avviene per 2-3 mesi; probabilmente la maturazione fisiologica, con sviluppo di spore mature ed aroma, avviene in funzione, oltre che di fattori endogeni, anche del microhabitat in cui il carpoforo si differenzia, nonché dell'andamento climatico complessivo.

Questa ultima ipotesi è stata attentamente valutata: infatti tramite un modello di regressione multipla con eliminazione stepwise delle variabili predittive indipendenti (climatiche) si è valutato il grado di dipendenza della variabile dipendente (accrescimento e variazione di crescita dell'ascoma) dalle suddette variabili predittive climatiche.
Le variabili climatiche esaminate sono state:
-**temperatura** (**T°**) media, min e max; -**umidità**, (**U**) media, min e max;-**punto di rugiada** (**R**) medio, min e max;-**ventosità (V)** media e a raffiche; -**pluviometria (P);**
per un totale di 12 variabili indipendenti confrontate con l'accrescimento generale degli ascomi espresso come variazione percentuale di crescita (Tab. 2).

Tab. 2 - Risultati della regressione lineare multipla stepwise; in prima colonna sono riportati i fattori predittivi:Temperatura massima (T hight) e media (T agv); Umidità media (U agv); Ventosità massima (W hight) e Pluviometria (Pluv), in penultima il valore della statistica t associata (t score) , in ultima la significatività (Sign.) della statistica t.

	VARIABILI	Beta	Beta stand.	S.E.	t score	Sign.
	T high	.032	.013	.777	2.467	.014
	T avg	.068	.031	1.533	2.203	.028
	U avg	.019	.008	.762	2.286	.022
	W high	-.022	-.006	.271	-3.949	.000
	Pluv	2.352	1.059	.109	2.221	.027

Delle 12 variabili climatiche considerate solo 4 risultano avere un ruolo nell'accrescimento dei carpofori: ***la crescita è positivamente correlata***, con la Pluviometria (Pluv) e con la Temperatura (T avg) media e la T massima (T high) e l'umidità media (U avg); più alti sono questi valori più alto è il valore medio dell'area degli ascomi.
La ***crescita è negativamente correlata*** con la velocità massima del vento (W high), cioè all'aumentare della velocità massima del vento, in media si accresce in meno l'ascoma.

Discussione e Conclusioni

Poco si sa sugli eventi che scatenano la fruttificazione e portano alla formazione del primordio dell'ascoma. Sicuramente, come riportato da numerosissimi studi, esso dipende dal metabolismo del fungo, da quello della pianta nonché dalle condizioni pedologiche e climatiche del sito.
I fattori pedologici sono condizione necessaria per il mantenimento e lo sviluppo del micelio e delle micorrize da cui deriva la potenziale capacità di fruttificare. L'induzione alla fruttificazione (formazione degli ascomi) dipende ed è messa in atto dai fattori climatici (precipitazioni, temperature etc). Nel successivo sviluppo ed ingrossamento dei carpofori, fino alla loro completa maturazione, entrano probabilmente in gioco anche dei fattori endogeni, questo spiegherebbe perché nelle stesse condizioni ambientali e pedoclimatiche alcuni di essi si atrofizzano e marciscono mentre altri completano il loro sviluppo; e fra quest'ultima parte alcuni raggiungono dimensioni maggiori ed altri restano a, al contrario, piuttosto piccoli. E' possibile ipotizzare che la formazione dei primordi del carpoforo sia un evento dipendente soprattutto dal partner fungino, mentre la successiva fase di crescita ed accumulo di sostanze dipenda dalla fisiologia della pianta, la quale rifornisce il fungo di zuccheri aventi funzione energetica.
In altri termini se è il fungo il responsabile della prima fase di formazione dei carpofori, è la pianta, con il suo metabolismo, che determina il loro ingrossamento. Ovviamente questa ipotesi deve essere sottoposta ad ulteriori e più stringenti prove sperimentali per avvalorarla o confutarla.
Tuttavia, dal presente studio emergono in maniera chiara e netta alcuni dati interessanti.
Innanzi tutto appare evidente che tra i numerosissimi carpofori che emergono all'interno di una tartufaia, non tutti arrivano a completa maturazione dal momento che alcuni vanno incontro a marcescenza, altri restano pressoché di dimensioni stabili, altri ancora si ingrossano abbondantemente. Va sottolineato inoltre che sia il periodo di affioramento dal terreno che quello di maturazione dei carpofori è indipendente dall'epoca della loro iniziale formazione potendo avvenire per alcuni in tempi brevi e per altri molto più lentamente senza condizionare il loro sviluppo.
Le osservazioni eseguite nella tartufaia hanno messo, infatti, in evidenza che lo sviluppo che porta all'affioramento dei carpofori, non avviene per tutti nello stesso momento ed in maniera simultanea, ma piuttosto richiede un certo arco di tempo valutato in: circa 40 giorni per il nel 2007 e circa 35 giorni per il 2008
E' anche interessante notare come il tempo impiegato per l'emersione non condizioni il successivo sviluppo dei carpofori.

Il monitoraggio dell'accrescimento dei carpofori senza voler interferire sul loro sviluppo esclude la misurazione di quelli ipogei e la determinazione del volume dei carpofori; malgrado questo lo studio ha evidenziato che l'auxometria degli ascomi di *Tuber aestivum* Vittad. (*alias Tuber uncinatum* Chatin) dipende sia da fattori endogeni che ambientali.
Infatti, indipendentemente dall'area che essi presentano al momento della loro affioramento alcuni si accrescono mentre altri rimangono pressoché identici (Fig.5).
Resta inoltre dimostrato che tale tipo di accrescimento può essere ben rappresentato e parametrizzato mediante una curva logistica sigmoidale, in cui la variazione percentuale risente positivamente solo di alcuni fattori climatici importanti quali le precipitazioni, la temperatura e

Fig. 5 Dopo la formazione alcuni carpofori ingrossano abbondantemente altri restano di dimensioni pressoché stabili.

l'umidità, mentre è negativamente correlata con la ventosità (velocità massima del vento).
Inoltre questa ricerca, sia pur preliminare, consentendo di comprendere l'andamento delle ultime fasi di accrescimento dell'ascoma di *Tuber aestivum* Vittad. (alias *Tuber uncinatum* Chatin) può tornare molto utile dal punto di vista pratico, per intervenire in maniera più razionale, (e cioè secondo l'andamento e le esigenze delle differenti fasi del proprio ciclo biologico) nella coltivazione di questa specie di *Tuber*, molto utilizzata in tartuficoltura.

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A QUARTER CENTURY OF TRUFFLE CULTIVATION IN NEW ZEALAND – SUCCESSES AND PROBLEMS

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Abstract
At the 1988 Spoleto congress Ian Hall briefly outlined his plans for cultivating truffles in New Zealand to cater for out-of-season Northern Hemisphere markets. The first *Tuber melanosporum* truffles were found in 1993 on a truffière owned by Alan Hall on the East Coast of the North Island at 38° 35′ S. However, it was not until 1997 when Alessandra Zambonelli unearthed a 250 g truffle in the same truffière that the first commercial harvest in the Southern Hemisphere began. Truffles were later harvested in New Zealand truffières between 38°S and 44°S. Truffle cultivation subsequently spread first to Australia in 1992, followed by South America and South Africa.
Here we outline the scientific, technical and other problems that were encountered in New Zealand's truffle research programme, how they were overcome, and the successful commercial cultivation of *Tuber melanosporum*, *Tuber borchii* and other edible ectomycorrhizal mushrooms.
Key words: *Tuber melanosporum*, *Tuber borchii*, cultivation, Southern Hemisphere, New Zealand.

Introduction

The germ of the idea to cultivate truffles in New Zealand and the Southern Hemisphere came during a conference in Colorado in 1979. During a lull in conversation with the late Jim Gerdemann, Ian Hall understood a fragment of conversation in French about the first production of truffles in France using new methods (Chevalier & Dupré, 1990). Some months later Ian Hall decided that if it could be done in France and Italy then surely it would be possible to do the same in New Zealand and produce truffles counter season to the Northern Hemisphere.
At the time the very word "mycorrhiza" did not engender enthusiasm in the minds of managers of the New Zealand Ministry of Agriculture and Fisheries. They had more or less closed down research on mycorrhizas in the early 1980s when it was realised that claims arbuscular mycorrhizas would replace phosphatic fertilisers were excessive and unlikely to become a reality (Hall, 1988). Consequently, considerable patience was required before Jock Allison, a more sympathetic and far sighted director, was placed in charge of Invermay Agricultural Centre near Dunedin, New Zealand. During this five year delay Ian Hall's requests for information from Europe on what the new techniques to cultivate truffles entailed, largely fell on deaf ears. However, sufficient snippets appeared in the literature to suggest that the methods were not very different from those used for arbuscular mycorrhizas, a topic Ian Hall was more familiar with (Hall, 1978, 1988). So working from basic principles, techniques were developed during 1985 and 1986 to produce Périgord black truffle infected plants using spores. This research was funded with a $1000 grant from the Miss E.L. Hellaby Trust matched by a similar sum from the new director of Invermay.

Climate of New Zealand

The three main islands of New Zealand span the latitudes 34° 15' S to 47° 20' S where mean

daily January (summer) air temperatures in habitable areas range from 20.5°C to 13.3°C and from 13.2°C to -1.0°C in July (winter). Because New Zealand is long and narrow and surrounded by ocean, temperatures tend to be cooler in summer and warmer in winter than for equivalent latitudes in France, Italy and Spain. There is also a wide ranging rainfall from about 11,000 mm in the wettest parts of the west coast of the South Island, down to about 340 mm in the rain shadow to the east of the Southern Alps. In Table 1 various climatic statistics are tabulated for areas where Périgord black and bianchetto truffles have been cultivated in New Zealand. A more extensive comparison of climates in various parts of the world where the Périgord black (*Tuber melanosporum*), bianchetto (*Tuber borchii*), Burgundy (*Tuber aestivum*) and Italian white (*Tuber magnatum*) truffles are found growing naturally or have been cultivated can be downloaded from Truffles & Mushrooms Ltd's web site (Hall *et al*., 2008).

Tab. 1 A rough comparison of the climatic conditions in the Périgord black truffle-growing areas of France and Italy and at New Zealand Meteorological Service stations (New Zealand Meteorological Service, 1983) adjacent to productive and non-productive Périgord black truffle truffières. (Also see Hall *et al.,* 2008.)

	Range in France, Italy and Spain	Productive areas in New Zealand						
		Opotiki	Gisborne	Taumarunui	Paraparaumu	Nelson	Waipara	Ashburton
Latitude (°)	41 to 47	38	39	39	41	41	43	44
Elevation (m above sea level)	200 to 1300	6	9	171	7	10	64	101
Annual rainfall (mm)	600 to 1200	1400	4058	1443	1054	986	729	757
Accumulated degree days (>10°C)	900 to 1900	1493	1430	1292	1167	1038	1049	896
Mean daily temperature in summer (July in Northern Hemisphere, January in Southern Hemisphere) (°C)	(16.5?) 17.5 to 22	18.5	18.3	18.3	17.1	17.2	17.5	16.5
Mean daily temperature in winter (January in Northern Hemisphere, July in Southern Hemisphere) (°C)	1 to 8	9.2	9.0	7.9	8.3	6.5	6.5	5.2
Annual sunshine hours	1900 to 2800	2169	2172	1704	2043	2397	1999	1892
"Summer" sunshine hours (April to September in Northern Hemisphere, October to March in Southern Hemisphere)	1200 to 1800	1227	1238	1079	1227	1377	1175	1092

New Zealand Soils

Much of the North Island is covered with soils derived from acidic volcanic ash while many soils in the South Island are derived from low pH greywacke, argillites, schist, granites, diorites, gneiss, amphibolites, gabbros, basalts, andesites, dacites or loess (Natureandco.com 2009). While New Zealand does have soils derived from limestone and marble, these cover a relative small part of the country compared with the truffle growing countries of Europe (Institute of Geological & Nuclear Sciences, 2008; Riddolls, 1987).

Because of the lack of high pH soils in New Zealand research was carried out to determine if truffles could be grown in New Zealand on naturally low pH soils to which lime had been added. As a rough guide between 1.5 and 2 tonnes of lime per hectare per 10 cm soil depth was found to raise the soil pH by 0.1, i.e., to raise the top 30 cm of a soil from a pH of 5.9 to 7.9 (the optimum for *Tuber melanosporum*) required between 90 and 120 tonnes of lime per hectare. This lime had to be worked into the soil so that it was spread evenly throughout the profile. The concentrations of available trace elements generally fell when such large quantities of lime were applied and in many cases one or more had to be applied to control trace element deficiencies, particularly in *Quercus robur*.

Seedling production and host plants

Initially we used *Q. robur* (English oak) and *Corylus avellana* (hazelnut) as the host plants because the seed was readily obtainable, both species grow well throughout New Zealand and were found to readily form mycorrhizas with *T. melanosporum* using the methods developed by Ian Hall. Because *Quercus ilex* (holm oak) is perceived to be a better host plant in Europe and Australia, some New Zealand nurseries are now using it too. However, truffles have not yet been harvested from experimental numbers of *Q. ilex* established in 1990. Another concern is the possible long term impact of *Phytophthora kernoviae* on *Q. ilex* and *Q. robur*, which is widespread in Northland and the central North Island (Ramsfield *et al*., 2007; Brasier, 2008; Forest Research, 2008; Gill, 2008; Tompkins & Beever, 2007).

Despite efforts to exclude *Tuber brumale* by inspecting every truffle macroscopically and microscopically before including it in a batch of inoculum and discarding all truffles that were even remotely questionable *T. brumale* has been identified in some New Zealand truffières (Guerin-Laguette *et al*., 2010). In contrast, despite imported truffle at times containing much more *Tuber indicum sensu lato* than *T. brumale* Guerin-Laguette *et al.,* (2010) did not detect any *T. indicum* mycorrhizas in New Zealand's truffières. It is assumed that the *T. brumale* contamination originated from small pieces of *T. brumale* trapped inside cracks on the surfaces of *T. melanosporum* truffles. However, it is also possible that *T. brumale* may have been accidentally introduced by early European settlers on the roots of imported plants like *Tuber maculatum* which is now widespread in New Zealand on birch (*Betula*), common lime (*Tilia europaea*), Douglas fir (*Pseudotsuga menziesii*), poplars (*Populus*), radiata pine (*Pinus radiata*) and willows (*Salix*).

In 1987, within two years of beginning research, a few hundred *T. melanosporum* infected plants were produced and these were used to establish two small truffières in North Otago (45°S) on rendzina soils with pH 7.8 – 8.1. The considerable publicity surrounding these first experiments generated a demand for *T. melanosporum* infected plants so a small facility was established to produce them. A free consultancy service was provided to these early growers until the first commercial harvest had been made.

Planting densities and management

Initially, Ian Hall relied heavily on early work by French and Italian researchers for information on planting densities and management practices (Hall & Brown, 1989; Hall *et al.*, 1994, 2007). All of the truffières were planted with two *C. avellana* about 2.8 m apart, alternating with a single *Q. robur* along the rows, and with the rows about 4.5 m apart to give a planting density of about 800 trees per hectare.

Overall *Q. robur* was more prone to contamination by *Scleroderma* and *Hebeloma* than *C.*

avellana but only when the trees were planted into areas where these contaminants were already present. Problems from contaminating fungi generally occurred where growers had failed to adjust the pH of their soils completely before planting. A covering of grasses was maintained in truffières established on soils subject to wind or water erosion. However, this depressed soil temperatures, which we suspect, may have inhibited fruiting. The only pests and pathogens encountered were powdery mildew and wood wasps, but generally these did not require treatment.

Q. robur and *C. avellana* in the first truffières were pruned to give one to three leaders, following advice from France. However, this was found to be a mistake because *C. avellana* spread too wide and shaded too much soil, while *Q. robur* tended to split during high winds at the point where leaders separated. Also, in Europe *Q. robur* grows about 20 cm per year, but in New Zealand growth was up to 230 cm in one season. Coupled with the high planting density this required considerable pruning in some parts of the country after only 6 years and thinning out plants about 10 years after planting.

As soil pH was raised there was an invariable fall in plant available trace element concentrations, in particular boron, copper, iron, manganese and zinc. Sometimes this resulted in nutrient deficiencies, particularly in *Q. robur*. This was reversed by the application of relatively small quantities of compounds containing these elements.

Yields

Of the 11 Périgord black truffières covering up to 0.5 ha that had been established up to 1990, 8 produced with yields ranging from about 20 kg/ha to in excess of 200 kg/ha 10 years after planting. Management of these truffières was somewhat varied but some features were common: soil pH was maintained above 7.4, irrigation was applied when conditions were dry, when necessary trace elements and other nutrients were applied in Spring, and the soil was worked once to a few times in Spring and early Summer. The remaining three unproductive truffières were all in North Otago (45°S) where the summer temperatures are marginal, the rainfall low, and where management was suboptimal. More recently established truffières in warmer parts of New Zealand have also failed to produce. This can be attributed to establishing truffières adjacent to trees that carry competing fungi such as *Tuber maculatum*, applying insufficient or excess irrigation water, allowing undecomposed organic matter to accumulate in the soil causing soil acidification, failing to prune causing excess shading leading to low soil temperatures, and poor advice from people who appear to think that growing truffles is akin to growing potatoes or other horticultural crops. Even so, there are growers who have followed best practice to the letter but have still to produce a crop.

Past problems and future potential

During the past 25 years of research on truffles in New Zealand many technical problems were overcome but by far the worst problem came after 1992 when the New Zealand Crown Research Institutes were established (CCMAU, 2009). From this time onwards funding for research became competitive, covered not only research costs but also salaries and was administered by a single funding body – the Foundation for Research Science and Technology (2009). Consequently, a loss of funding could lead to scientific oblivion.

Past research on agricultural and silvicultural crops, on which the prosperity of countries like New Zealand have developed and now depends, took decades to complete. Truffles are the epitome of slow food and hence considerable patience was also needed in establishing a truffle industry in New Zealand. In retrospect we cannot see how the initial research could have been done any quicker. It took less than 2 years of part time work to develop a method for producing truffle infected plants, the first truffles were found in 1993 only 5 years after planting, and then only 4 more years of field experimentation were required to produce the first commercial harvest of *T. melanosporum* – the first in the Southern Hemisphere. To have research funding then stopped the following year can only be described as inept and suggests that the funding

body was advised by the ill informed, myopic and/or risk averse. Or perhaps there was a belief, like in European countries and New Zealand now, that the allocation of research funds was best made not on the establishment of new industries that might generate wealth but on the basis of bibliometrics such as publication record, accumulated impact factors, citation record, etc. (Evidence Ltd, 2008; Garfield, 2005, 2008; Hall, 2008; Reference. com, 2009; Rogers, 2002; Smith, 2008; Tertiary Education Commission, 2008). Whatever the reason piecemeal, short-term or fixed term funding of science is now commonplace and is putting unnecessary stress on researchers that has led to ill health. This is of no benefit to applied science, is immoral and arguably illegal in some countries (New Zealand legislation, 2002).

Despite funding problems, New Zealand now has the beginnings of industries based on the cultivation of *T. melanosporum*, *T. borchii* and other edible mycorrhizal mushrooms including the saffron milk cap (*Lactarius deliciosus*) and shoro (*Rhizopogon rubescens*) (Hall, 2008; Hall *et al*., 2007; Wang *et al*., 2002). In the late 1990s it was joined by Australia and research in Chile, South Africa and Argentina shows promise. Indeed Périgord black truffle production in Australia is now more than one tonne per year (Haslam pers. comm.) and within the next 10 years could exceed French production (12 t in 1994/95; Hall *et al.,* 2007), at least in bad years. It is regrettable that New Zealand lost the six year lead it once had over other Southern Hemisphere countries following the withdrawal of funding in 1998 and the events of 2004 (New Zealand Herald, 2004). It is to be hoped that all those now involved in commercialising truffles and other edible mycorrhizal mushrooms in New Zealand, and the rest of the Southern Hemisphere, will be motivated not too much by personal gain but by the exciting challenges of establishing new industries catering for local and off-season Northern Hemisphere markets.

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VIABILIDAD DE LAS PLANTACIONES TRUFERAS EN CORTAFUEGOS

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Abstract: Feasibility of truffle plantations in firebreaks
Firebreaks are strips of open vegetation inside forested areas, constructed to fight against wildfire. They are frequently affected by controversy concerning high maintenance costs, environmental impact and land use conflicts. On the other hand, Perigord black truffle is mainly produced in open forests that have a structure similar to that sought for firebreaks, and the biocidal effects of the truffle could help to maintain this structure. In addition, truffle ascocarps command high prices on the market. The successful establishment of truffle plantations in firebreaks would represent a multifunctional use which could increase the economic and social sustainability of wildfire prevention. Nevertheless, this establishment is conditioned by several factors: 1) black truffle requires specific soil and climatic conditions, and 2) firebreaks are usually constructed on forest soils, whereas trufficulture is a technique developed for use on agricultural land.
This study aims, first, to identify the main obstacles in establishing young inoculated seedlings on forest soils and, then, to select techniques to overcome these obstacles. Laboratory bioassays were used as pre-feasibility studies. Initial results showed that the ectomycorrhizal inoculum potential was much higher in forest soils than in agricultural soils, with the effect that previously mycorrhized plants were contaminated with native soil fungi and more than half of the truffle mycorrhizas were replaced in less than two years. To reduce the soil inoculum potential, several physical and chemical treatments were assayed. Both overliming and high temperature reduced the inoculum potential, but some doses also affected root growth. Therefore, proper site preparation of the planting hole could be a useful tool for establishing truffle plantations in firebreaks. If no site preparation is performed, there is a serious risk that native soil ectomycorrhizas will replace the truffle ones in the short term.
Key words: *Tuber melanosporum*, firebreak, truffle cultivation, bioassay, land use.

Introducción

Actualmente, el fuego es una de las mayores amenazas para el bosque mediterráneo. En las últimas décadas, el abandono de los aprovechamientos tradicionales (leña, madera, pastoreo) en la Europa mediterránea ha hecho aumentar la espesura del bosque. La incidencia de los incendios forestales ha aumentado y el ataque al fuego desde tierra se ha vuelto más difícil (Quézel y Médail, 2003).

En la defensa contra los incendios forestales, la creación de áreas cortafuegos es una de las medidas selvícolas más frecuentes. Las áreas cortafuegos son franjas de terreno en las que se elimina la vegetación. Normalmente, se dividen en una banda de decapado, una banda de desbroce total y otra de desbroce selectivo y poda. Para que los cortafuegos sean efectivos, son necesarias labores de mantenimiento periódicas, que suponen un gran gasto económico.
En los cortafuegos se busca una vegetación con estructura abierta, semejante a la requerida para la producción de trufa negra (*Tuber melanosporum* Vittad.). Las plantaciones truferas en cortafuegos proporcionarían una serie de ventajas: (1) el efecto fitocida del hongo permitiría controlar la vegetación espontánea y reducir los trabajos de mantenimiento del cortafuegos, (2) la producción trufera permitiría revalorizar el monte: en el mediterráneo, la trufa negra

es uno de los pocos aprovechamientos forestales que mantienen precios elevados y (3) la revalorización del monte permitiría aumentar la vinculación de la población rural con sus bosques. Todo ello supondría una mejora en la sostenibilidad de los cortafuegos.
Sin embargo, la viabilidad de estas plantaciones está condicionada por varios factores. A priori, se consideró que los principales limitantes son: (1) la profundidad del suelo y los fenómenos erosivos no deben comprometer la supervivencia y crecimiento de las plantas, (2) los hongos ectomicorrícicos nativos del suelo no deben desplazar a la trufa de las raíces de las plantas.
Si las ectomicorrizas (ECM) nativas del suelo desplazan a la trufa antes de la formación del quemado (5-7 años), la plantación sería un completo fracaso. Los cortafuegos se ubican sobre suelo forestal, cuyo potencial de inóculo ectomicorrícico es mayor que el de los agrícolas. Las raíces de las plántulas corren el riesgo de contaminarse a corto plazo con hongos nativos del suelo. El potencial de inóculo se define como la capacidad de los hongos micorrícicos de un suelo para colonizar las raíces de las plantas.
Para establecer la influencia de los hongos nativos sobre la viabilidad de las plantaciones truferas en áreas cortafuegos, se han realizado una serie de bioensayos de laboratorio. Los objetivos son: (1) valorar si los hongos ectomicorrícicos nativos de los suelos de cortafuegos desplazan a las ECM de *T. melanosporum* a corto plazo (bioensayo de competencia) y (2) evaluar si la capacidad infectiva de estos suelos puede reducirse tratando el suelo (bioensayos del potencial de inóculo).

Bioensayo de la competencia
Se tomaron suelos de tres cortafuegos con más de 20 años de edad y sobre ellos se plantaron brinzales inoculados con *T. melanosporum* procedentes de un vivero comercial. Los cortafuegos están situados en una región trufera (El Toro, Comunidad Valenciana, este de España) y cumplen las condiciones generales para el cultivo de la trufa, pero no han sido productores de trufa. Se tomó el suelo de los 20 cm superficiales.
Se estudió el efecto de las siguientes variables sobre el estado de micorrización de las plantas inoculadas:

(1) Bosque denso (encinar) vs cortafuegos (sin *Quercus* ni *Pinus* a menos de 6 m de distancia)
(2) Encalado: 1 kg m^{-2} de cal viva
(3) Tratamiento térmico: 30 minutos en estufa, con una temperatura máxima de 65ºC; 1,5 minutos en microondas, con una temperatura máxima de 65ºC
(4) Plantas: encinas (*Quercus ilex* L. ssp. *ballota* Samp.) inoculadas de una savia vs dos savias (Tabla 1)

Tabla 1 Características previas de las plantas inoculadas usadas en el ensayo de competencia (n=12)

Edad de las plantas	Una savia	Dos savias
Altura (cm)	6,9 a	9,8 b
Diámetro cuello raíz (mm)	2,3 a	3,7 b
Peso de raíz (g)	1,1 a	3,0 b
Nº ECM *T. melanosporum* [a]	869 a	1202 a
Porcentaje de ápices infectados por *T. melanosporum* (%)	45,7 a	30,9 b
Porcentaje de ECM por *S brunnea* (%)	2,3 a	0,7 a

[a] Método de Reyna *et al.,* (2001).

En vivero, las plantas inoculadas habían sido cultivadas en contenedores de 650 ml de volumen y 18 cm de profundidad. Para el ensayo, fueron trasplantadas a macetas de 5 l de

volumen y 30 cm de profundidad, que se rellenaron con el suelo del campo (marzo de 2007). Los riegos se dieron de manera que la humedad se mantuvo durante todo el cultivo entre el 15-35% peso/peso (capacidad de campo: 45-50%). Se evitó el cultivo en invernadero para limitar la influencia de los hongos típicos de estas condiciones. Se presentan los resultados del primer año de ensayo, muestreadas durante el invierno de 2007/08.
Después de un periodo vegetativo, el porcentaje de ápices colonizados por los hongos nativos del suelo fue menor al 4% en todas las plantas, a pesar de que las raíces habían colonizado el suelo forestal. *T. melanosporum* representaba de media el 99% de las ECM encontradas, sin diferencias significativas entre los diferentes tratamientos. Esto mismo ya se había observado en ensayos anteriores, en los que los hongos nativos del suelo aumentaban de forma significativa durante el segundo periodo vegetativo (Fig. 1)

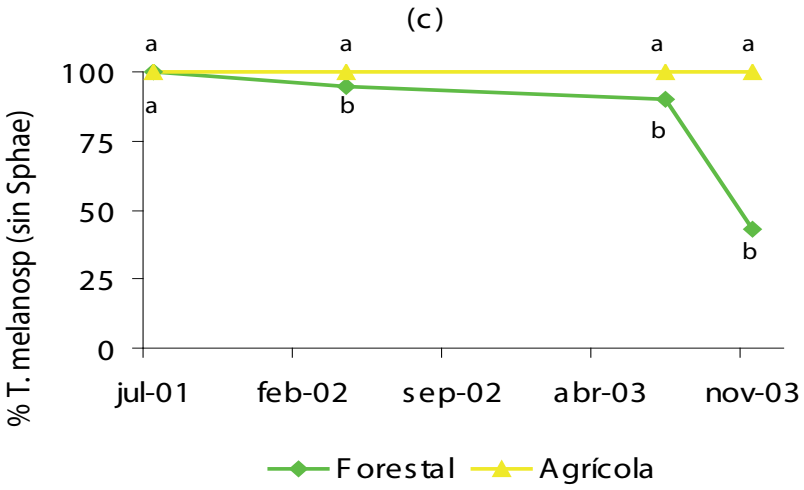

Fig. 1 Evolución del porcentaje de ECM de *T. melanosporum* respecto del total de ECM en suelo de bosque y de cultivo de cereal. Las plantas usadas en el ensayo habían sido inoculadas previamente en vivero (Reyna et al., 2006).

Un 50% de las plantas cultivadas en suelo del bosque presentaban ya ECM de hongos nativos del suelo, mientras que en el suelo del cortafuegos esto sólo se observó en un 25% de las plantas (n=24). Un 50% de las plantas de dos savias presentaban ECM de hongos nativos, por un 25% de las plantas de una savia. Las dosis de cal y calor ensayadas no produjeron diferencias significativas durante el primer año.
Antes del ensayo, la única ECM presente en las raíces, aparte de *T. melanosporum*, era el contaminante de vivero *Sphaerosporella brunnea* Svrcek et Kubicka. Aparecía en un 42% de las plantas y representaban un 5% de las ECM. Un año después, las ECM de *S. brunnea* aparecían en un 42% de las plantas y representaban un 4% de las ECM.
El crecimiento de las plantas no se vio alterado por ninguno de los suelos.

Bioensayos del potencial de inóculo
Se seleccionaron varios tratamientos cuyo potencial como desinfectantes fuera apoyado por la bibliografía científica y que resultaran relativamente fáciles de aplicar en campo:
Ensayo 1: cocción prolongada del suelo (90 minutos), en medio aéreo y sin aplicación directa de llama
Ensayo 2: cal viva a diferentes dosis, entre 0,5 kg m^{-2} y 2 kg m^{-2}
Ensayo 3: microondas (1,5 y 3 minutos, alcanzando el suelo una temperatura máxima de 65ºC y 85ºC respectivamente; potencia de salida: 700 W, frecuencia: 2,45 GHz), hipoclorito sódico (2 y 8 l m^{-2} con una concentración de 35 g l^{-1}), ácido acético (2 l m^{-2} a una concentración del 7,5%).
Los tratamientos se aplicaron en campo y puntualmente: se preparó un hoyo de plantación a la manera tradicional de la repoblación forestal (40 x 40 x 20 cm) y se le aplicó el tratamiento.

En el caso de la cal, el hipoclorito sódico y el ácido acético, el tratamiento del suelo se realizó durante el invierno, 2-3 meses antes de montar el bioensayo, de manera que cuando se plantó el pH del suelo había vuelto a ser prácticamente igual al previo (8,0-8,3).
Se propone una aplicación puntual de los tratamientos porque la finalidad principal de los mismos es reducir la competencia ectomicorrícica durante la fase de establecimiento de la plántula (los dos primeros años). La aplicación puntual reduce el impacto sobre el medio natural, respetando la mayor parte de la superficie de suelo.
Los suelos utilizados en el ensayo están situados en una región trufera (El Toro, Comunidad Valenciana, este de España) y cumplen las condiciones generales para el cultivo de la trufa, pero no han producido trufa en las últimas décadas. La vegetación está dominada por la encina y tiene gran espesura (900-2000 pies ha^{-1}). Se eligieron bosques densos porque en ellos el potencial de inóculo es más elevado que en los agrícolas (Reyna *et al*., 2006).
En enero se germinaron semillas de encina en condiciones de esterilidad. Las plántulas se mantuvieron en substrato inerte (perlita) hasta que comenzaron a formarse raíces finas micorrizables (abril). En ese momento se recogió del campo el suelo tratado y se montaron los bioensayos en contenedores forestales de 650 ml de volumen. De esta forma, se intentó reducir el tiempo trascurrido desde que se recogió el suelo hasta que en él vuelven a haber raíces finas.
Las plantas fueron regadas a capacidad de campo cada 2-3 días, manteniendo una humedad superior al 20% peso/peso. Se evitó el cultivo en invernadero para limitar la influencia de los hongos típicos de estas condiciones.
Los muestreos se realizaron 7-15 meses después del trasplante. Para valorar si cada tratamiento reduce la capacidad infectiva de los hongos nativos del suelo (potencial de inóculo ectomicorrícico), se ha medido el porcentaje de raíces finas micorrizadas. También se ha contabilizado la cantidad de especies ectomicorrícicas que aparecen por planta (riqueza de morfotipos).
Con la cocción del suelo (ensayo 1) se intentó simular el calor al que se ve sometido el suelo de una carbonera. Se realizó sobre una chapa de hierro, la cual alcanzó 620ºC a los 15 minutos de comenzar la cocción. El suelo se colocó sobre ella en una capa de 5-7 cm de grosor. El tiempo de cocción fueron 90 minutos, alcanzándose 380ºC a 3 cm de la chapa.
El suelo quedó profundamente alterado: se consumió la materia orgánica, los agregados del suelo se deshicieron y los que quedaron se volvieron más duros, la capacidad de retención de agua se redujo y debido al color oscuro aumentó la rapidez con la que el suelo se calentaba. Sin embargo, en el bioensayo el crecimiento de las plantas y su supervivencia no se vieron afectados. La disposición de las raíces sí se vio afectada: en la zona superior, más seca y con mayor amplitud térmica, las raíces finas eran más escasas.
En las plantas cultivadas sobre el suelo tratado, se eliminó por completo la capacidad infectiva de los hongos ectomicorrícicos. No se encontró ninguna ECM, mientras que en las plantas testigo se encontró una media de 4,5 morfotipos diferentes por planta.
El sobre-encalado (ensayo 2) redujo la diversidad de morfotipos por planta (Fig. 2), al mismo tiempo que la cantidad de raíces finas de la planta. Sin embargo, a las dosis ensayadas el crecimiento total de la planta no se vio afectado.

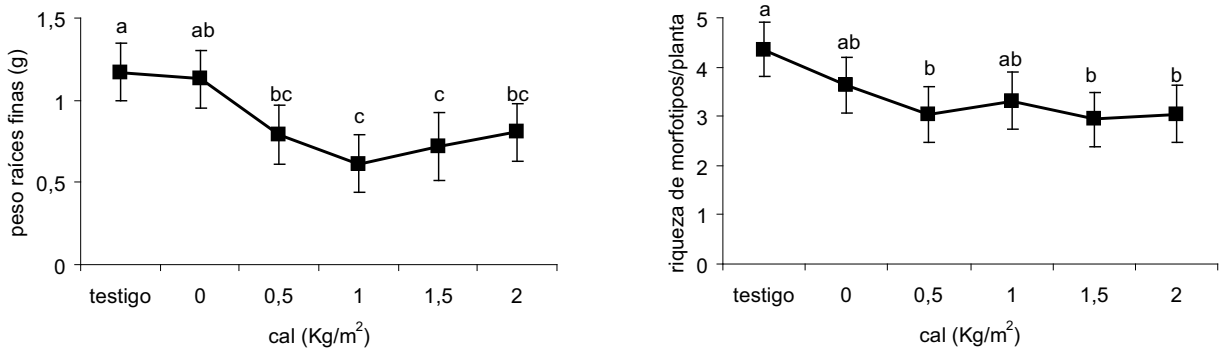

Fig. 2 Efecto de la cal sobre el peso de raíces finas y la cantidad de morfotipos de ECM por planta. Letras diferentes indican diferencias significativas para α=0,05

A las dosis testadas, las microondas (ensayo 3) redujeron el potencial de inóculo del suelo significativamente, pero no eliminaron la capacidad infectiva del suelo. El porcentaje de raíces micorrizadas pasó del 46% en el testigo al 38% y el 26% en los tratamientos de 1,5 y 3 minutos respectivamente. El número de morfotipos diferentes por planta se redujo de 4,8 en el testigo a 3,2 y 1,8 respectivamente. Mientras que el tratamiento de 1,5 minutos no alteró el crecimiento de las plantas, el de 3 minutos redujo significativamente el crecimiento total de la planta.
El hipoclorito sódico a las dosis usadas no ha alterado significativamente ni el porcentaje de raíces micorrizadas ni la diversidad de especies ectomicorrícicas. Tampoco ha afectado al crecimiento de la planta.
En cambio el ácido acético ha reducido significativamente el porcentaje de raíces micorrizadas del 46% al 32%.

Conclusiones.
Diseño de una plantación experimental en áreas cortafuegos.
Las plantas inoculadas con *T. melanosporum* mantienen las ECM durante el primer año de plantación tanto sobre suelo forestal como sobre el cortafuegos. Sin embargo, la presencia de ECM nativas del suelo ya es más frecuente en el bosque denso, sugiriendo que en el cortafuegos las ECM de *T. melanosporum* tendrán más posibilidades de persistir.
El calor y el sobre-encalado reducen la capacidad infectiva de los hongos ectomicorrícicos del suelo forestal. Sin embargo, algunas dosis han alterado el crecimiento de la planta durante los bioensayos, a pesar de que en estos el riego es abundante. En campo, el régimen hídrico es más limitante. Esto, unido a la alteración de las propiedades del suelo, podría aumentar la mortalidad de las plantas o reducir su crecimiento durante los primeros años.
La siguiente fase del proyecto es evaluar en campo el efecto de los tratamientos del suelo. Para ello se ha realizado una plantación experimental, que permitirá estudiar este efecto en un plazo de tiempo más prolongado (Reyna *et al.*, 2008). El periodo más decisivo son los primeros 5-7 años, hasta que aparece el quemado. Si un porcentaje elevado de plantas forman quemado, la plantación habrá alcanzado el primero de sus objetivos: disminuir la cobertura herbácea y arbustiva del cortafuegos.
Al mismo tiempo, las plantaciones en campo permitirán analizar la supervivencia y crecimiento de las plantas en las condiciones realmente existentes en los cortafuegos.

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THE PROJECT "DESARROLLO INTEGRAL DE LA TRUFICULTURA DE TERUEL (SPAIN)"

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Abstract
We present this research Project named "Desarrollo Integral de la Truficultura de Teruel" included in the "Plan de Actuación Específico para Teruel" to impulse the development of some strategical activities for Teruel province as trufficulture with more than 3000 ha of cropping surface.
The institutional support of the Spanish State through "Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria" (INIA) lies in the finantial of scientific research and technical development carried by the main trufficulture R+D Spanish teams with the aim of studying the less known aspects that are delaying the development of this activity.
This project is multidisciplinary based, it counts with a finantial support near to one million euro distributed for 3 years (2008-2010) and insists on the need of a knowledge flow among the members of the trufficulture sector and the participant research teams in order to convert Teruel into a Reference Laboratory of European trufficulture.
Key words: Research Project, Black truffle Teruel, *Tuber melanosporum*.

Introducción

El cultivo y la recolección de la trufa negra (*Tuber melanosporum* Vittad.) en Teruel tiene unas características excepcionales, no solo por la extraordinaria aptitud de sus tierras al cultivo de la trufa negra sino porque genera grandes valores económicos, ambientales y socio-culturales. El desarrollo de dicha actividad supone un complemento de renta en áreas deprimidas o marginadas, ante los precios elevados que alcanza el producto dentro de un mercado sin excedentes y con una gran demanda.
La posibilidad de establecer plantaciones cultivadas para la producción de trufa, justo en aquellas zonas marginales Turolenses donde cualquier cultivo resulta difícil o imposible, está contribuyendo a la forestación de superficies agrarias, con la introducción de especies forestales autóctonas, frondosas micorrizadas, como la encina, el quejigo o el roble, lo que evita la erosión, contribuye a la formación de paisaje y favorece la formación del suelo. Estas características citadas contribuyen a que su cultivo esté subvencionado por la mayoría de las administraciones, locales, autonómicas o central, así como por los fondos europeos, con la posibilidad de obtener ayudas a la diversificación agraria de las zonas rurales, tanto del objetivo 1 como las del objetivo 5, e incluso la de ser apoyado por la iniciativa Comunitaria LEADER, como cultivo de carácter ambiental e innovador. El gancho final está en su condición de producto agrícola de carácter ecológico y natural, al no necesitar su cultivo ningún apoyo químico o fitosanitario.
Es innegable que el conocimiento de las técnicas de micorrización a gran escala, por parte del sector viverista, ha provocado en la provincia de Teruel una gran expansión de los cultivos dedicados a la producción de trufa negra que alcanzan la cifra estimativa de 3.000 ha, frente a las 500 ha que existían hace siete años. La mayor información técnica existente, las ayudas y subvenciones anteriormente mencionadas, la existencia de Asociaciones de truficultores, las modificaciones producidas en el ámbito político-geográfico de la Unión Europea con un reparto más complicado de las ayudas y con unos horizontes difíciles en torno a la PAC, en lo que se refiere a cultivos extensivos de secano, han sido el desencadenante para este gran desarrollo de la truficultura moderna, a partir de plantaciones, constituyéndose en una alternativa posible y rentable.

Sin embargo este gran desarrollo experimentado no ha ido acompañado de los avances científicos en torno a las grandes incógnitas que la truficultura ha planteado desde sus inicios como son, entre otros muchos, los factores bióticos y abióticos y su influencia en la producción de cosecha.
El apoyo institucional del Estado, dentro del Plan de Actuación Específica para Teruel, se concretó en la financiación de aquellos Proyectos de Investigación, vitales y estratégicos para sectores clave de la economía Turolense, entre los cuales se encontraba la Truficultura, lo que dio origen al nacimiento del *Proyecto: Desarrollo Integral de la Truficultura de Teruel*, financiado por el Ministerio español de Ciencia e Innovación a través del Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), que aquí se presenta de forma resumida.
El Proyecto, integrado por 7 Subproyectos (Tabla 1), se financió para el periodo de tiempo comprendido entre 2007 y 2010, concediéndole una dotación económica de 935.000€.

Tabla 1. Plan general del Proyecto de investigación coordinado: "Desarrollo integral de la truficultura de Teruel".

PROYECTO DE INVESTIGACIÓN COORDINADO 7 SUBPROYECTOS	
1.	Análisis y estudio de los factores bióticos y abióticos que afectan al establecimiento de plantaciones y a la producción y a la calidad de la trufa negra de Teruel.
2.	Inventario de los hongos micorrícicos competidores y contaminantes de las plantaciones de trufa negra, en las comarcas productoras turolenses. Evolución del estatus micorrícico de las truferas en función de actuaciones externas.
3.	Desarrollo de métodos de lucha para el control de parásitos, patógenos y contaminantes, tanto de la trufa negra como de sus huéspedes.
4.	Métodos de mantenimiento y recuperación de truferas naturales. Su utilización como áreas cortafuegos. Las carboneras.
5.	Catalogación de plantaciones truferas. Estudio de suelos. Elaboración de un mapa provincial de potencialidad para la truficultura.
6.	Incremento de la vida comercial en fresco de *Tuber melanosporum*: utilización de métodos combinados. Aplicación de nuevas tecnologías.
7.	Establecimiento, desarrollo y coordinación de que cobije todas las investigaciones y experimentaciones del Proyecto.

Memoria de actividades previstas
Cada uno de los 7 Subproyectos coordinados se realiza en diferentes Centros de Investigación y Universidades y en muchos casos los investigadores implicados, lo están en más de uno de ellos a la vez.
Estos son sus objetivos:

Subproyecto 1 - Inventario de los hongos micorrícicos competidores y contaminantes de las plantaciones de trufa negra, en las comarcas productoras turolenses. Evolución del estatus micorrícico de las truferas en función de actuaciones externas.
Este Subproyecto está dirigido por el Dr. Carlos Palazón, del Centro de Investigación y Tecnología Agroalimentaria de Aragón, coordinando un Equipo Investigador de 6 participantes pertenecientes al propio Centro, a la Universidad de Navarra y al Instituto Técnico de Gestión Agrícola (ITGA) de Navarra.

Objetivos:

a) Conocer el estado de micorrización y el cortejo micorrícico de las parcelas truferas turolenses.
b) Estudio de la flora acompañante y de otros esporocarpos presentes.
c) Desarrollo de bioensayos con las tierras de las truferas.

Subproyecto 2 - Análisis y estudio de los factores bióticos y abióticos que afectan al establecimiento de plantaciones y a la producción y a la calidad de la trufa negra de Teruel.
Este Subproyecto está dirigido por el Dr. Carlos Colinas, del Centro Tecnológico Forestal de Cataluña (CTFC), coordinando un Equipo Investigador de 7 participantes.

Objetivos:

a) Impacto del laboreo (Fig. 1).
b) Influencia del riego y sus dosis (Fig. 2).
c) Influencia de la M.O. y textura.
d) Influencia del contenido miceliar del suelo en la previsión de cosecha.

Fig. 1 Laboreo en un campo de trufa cultivada

Fig. 2 Riego en una trufera cultivada

En todos estos factores estudiados se realizará un seguimiento de la producción de las plantas huésped y de la presencia de hongos ectomicorrícicos.

Subproyecto 3 - Desarrollo de métodos de lucha para el control de parásitos, patógenos y contaminantes, tanto de la trufa negra como de sus huéspedes.

Este Subproyecto está dirigido por el Dr. Juan Barriuso, de la Escuela Politécnica Superior de la Universidad de Zaragoza, coordinando un Equipo Investigador de 6 participantes.

Los objetivos generales que se pretende alcanzar en este Subproyecto son los siguientes:

1. Establecimiento de un inventario con los principales patógenos, parásitos y contaminantes de la pareja simbionte *T. melanosporum* - *Q.ilex* (Fig. 3).

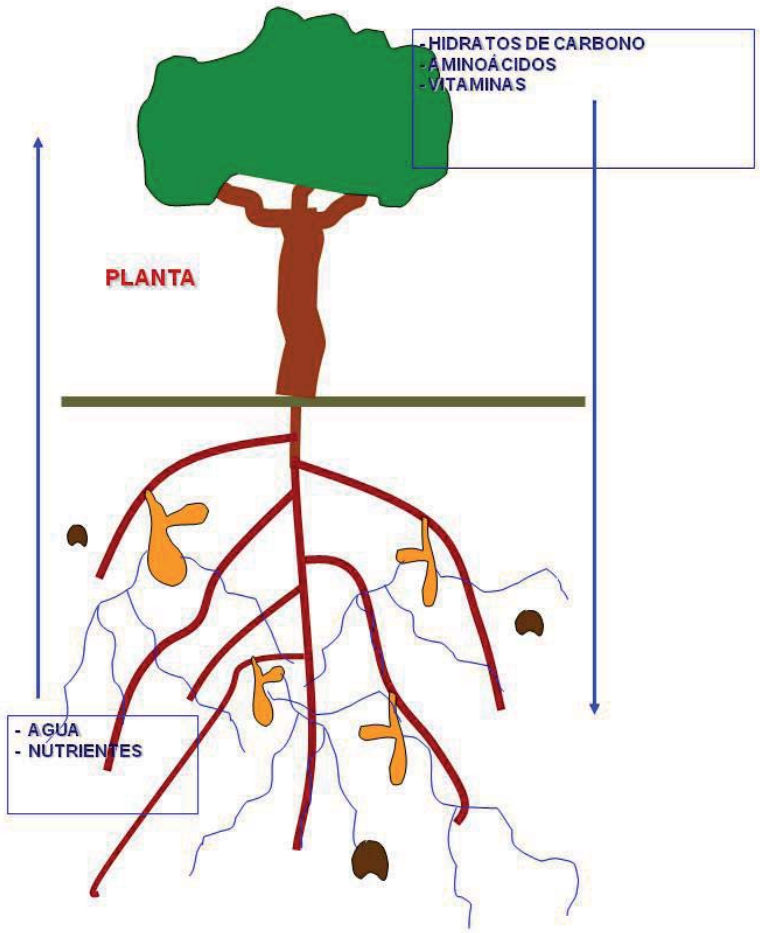

Figura 3. Esquema de un árbol trufero con micorrizas, micelio y carpóforos.

1.1 Identificar los principales hongos patógenos presentes en el sistema radical de las plantas simbiontes.

1.2. Identificar los principales parásitos del hongo simbionte, particularmente de los esporocarpos de *T. melanosporum*.

1.3. Identificar los principales contaminantes de las plantas simbiontes, en su fase de vivero.

1.4. Identificar los principales competidores de *T. melanosporum* presentes en el sistema radical de las truferas cultivadas. Este objetivo se desarrolla extensamente en el subproyecto nº5.

2. Conocer la epidemiología y los ciclos biológicos de los organismos implicados.

2.1 Caracterización de los factores iniciadores de la enfermedad. Influencia de la temperatura y humedad. Estudio de la interacción huésped-patógeno.

Caracterización del ciclo biológico de los insectos que parasitan los ascocarpos de *T. melanosporum.*

3. Desarrollo de posibles métodos de control.

Desarrollo de métodos de control, mediante lucha química.

Desarrollo de métodos de control, mediante lucha biológica.
Desarrollo de métodos de control, mediante técnicas de confusión sexual.
4. Producción en masa de micelio de trufa
Ensayar diferentes medios de cultivo, según la bibliografía previa, y optimizar aquel en donde mejor crezca el micelio de *T. melanosporum* mediante la presencia de extractos de raíces de posibles plantas hospedantes (*Quercus ilex, Q. coccifera*, etc.).
4.1. Estudiar cómo afecta la presencia de hongos competidores al crecimiento in vitro de *T. melanosporum*, mediante co-cultivos en placa Petri de dichos hongos (Fig. 4).

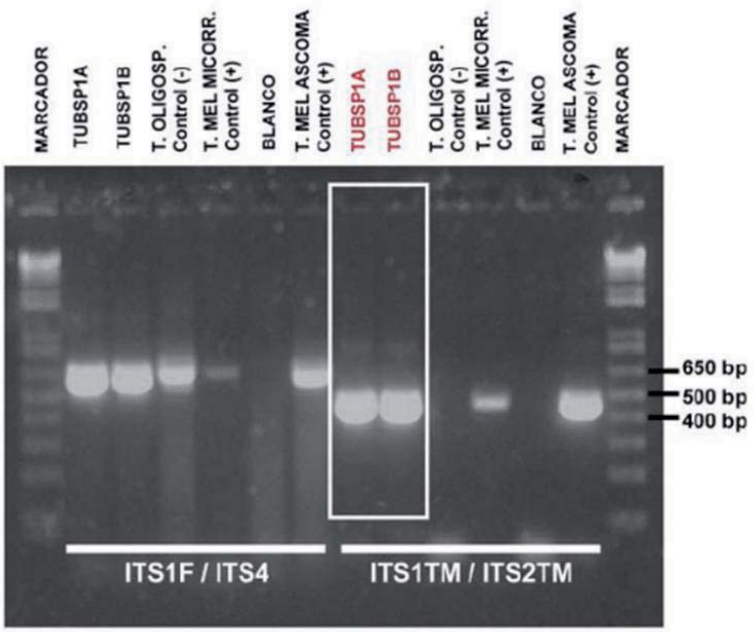

Figura 4. PCR para la identificación molecular de trufas.

Ensayo de diferentes cepas de bacterias del suelo, aisladas de suelo de las zonas de estudio, que sean capaces de estimular el crecimiento de *T. melanosporum* frente a sus competidores.
4.2. Cultivo de micelio de *T. melanosporum* en biorreactor, para su producción en masa.

Subproyecto 4- Truficultura y Gestión Forestal: recuperación de truferas y aplicación de la truficultura en áreas cortafuegos.
Este Subproyecto está dirigido por el Dr. Santiago Reyna, de la Escuela Técnica Superior de Ingenieros de Montes de la Universidad Politécnica de Valencia, coordinando un Equipo Investigador de 3 participantes.

Objetivos:
a) Evaluación y diagnóstico de los montes truferos de titularidad pública en Teruel. Bases para su gestión. Caracterización de truferas: historial productivo, análisis de suelo, vegetación, micorrización. Análisis de factores limitantes de la producción. Establecimiento de criterios prácticos de gestión de truferas en base a su estado actual y los resultados de proyectos de investigación previos. Seguimiento de la producción trufera en los montes inventariados.
Redacción de proyectos pilotos de selvicultura trufera en dos montes, con carácter demostrativo y posibilidad de ejecución por parte de la Administración Forestal.
b) Plantaciones truferas en áreas cortafuegos.
La evaluación definitiva de estas plantaciones sólo puede realizarse a largo plazo, pero se plantean una serie de medidas para seguir su evolución durante las primeras fases:
Para parametrizar en campo los efectos fitocidas de la trufa sobre la vegetación espontánea

y evaluar su utilidad en la defensa contra incendios forestales, se cuantificará la superficie afectada por los efectos alelopáticos de la trufa y la reducción de vegetación producida, a través de métodos no destructivos (método del punto intersectado) y destructivos (siega de la vegetación).
Para evaluar la viabilidad de las plantaciones truferas (posibilidad de que produzcan trufa en el futuro) y la efectividad de los distintos métodos de preparación, se analizará el estado de micorrización de las plantas introducidas.
Dado que los resultados de este ensayo serán evaluables a largo plazo, se pretende establecer unas parcelas de carácter permanente. El proyecto se dimensiona, pues, con el objetivo de que las plantas no queden agotadas por los muestreos del presente proyecto y que el tamaño de la población que quede sea suficiente como para que los resultados obtenidos a largo plazo sean representativos y significativos (reproducibles en otras condiciones diferentes).
c) Caracterización de los efectos de las carboneras sobre el suelo sobre la producción trufera.
Realización de dos carboneras. Antes y después de las mismas, se realizarán análisis de suelos (físicos, químicos y biológicos, además de análisis de sustancias volátiles destiladas) y bioensayos para determinar el efecto sobre las micorrizas autóctonas. Así mismo, se realizarán análisis de suelos y bioensayos sobre carboneras antiguas (truferas y no truferas) para conocer sus condiciones actuales.
Diseño de un horno quemador metálico de pequeño volumen y fácilmente transportable, con objeto de realizar los tratamientos de quema de suelo necesarios para los experimentos.

Subproyecto 5 - Catalogación de plantaciones truferas. Estudio de suelos. Elaboración de un mapa provincial de potencialidad para la truficultura.
Este Subproyecto está dirigido por el Dr. Fernando Martínez y se realiza en Departamento de Investigación Forestal de Valonsadero (Soria). Los objetivos planteados son los siguientes:
a) Inventario y catalogación. Estratificación en función de la Clasificación Biogeoclimática y de la producción.
b) Caracterización ecológica y dasométrica de plantaciones >10 años. Estudio de los parámetros edáficos del suelo y % de micorrización.
El análisis del estatus micorrícico de las plantas será siempre cualitativo, pero con el paso de los años nos permitirá conocer la evolución en sentido positivo o negativo hacia el desarrollo y proliferación de las micorrizas de *T. melanosporum*, frente a otras competidoras, que va a conducir al inicio de la producción trufera o a su desaparición, cuantificando la proporción de árboles con presencia de *T. melanosporum* en las diversas parcelas estudiadas.
Para ello, en todas las parcelas objeto del estudio, se recogerán muestras estacionales (primavera, verano, otoño e invierno) de micorrizas de un total de 2 encinas por parcela, lo que implica el muestreo de 144 árboles productores o no, seleccionadas al azar, tanto entre las productoras como entre las que no producen. Para el posterior estudio e identificación en el laboratorio de las distintas especies encontradas se mantendrá la metodología seguida anteriormente a lo largo de varios años de investigación.
La metodología de recolección de muestras es laboriosa y complicada. Para conocer el estado de la micorrización es necesario la toma periódica de muestras estacionales, con especial atención a la primavera y el otoño, por ser estas épocas en las que el micelio se desarrolla más rápidamente y con mayor actividad. Se trata básicamente de tomar muestras de las raíces en la zona superficial (10-20 cm de profundidad) en los límites del quemado y, en el caso de árboles jóvenes, en proximidad del cuello ó en la proyección de la copa sobre el suelo. El muestreo debe ser representativo del desarrollo vegetativo de las plantas (Fig. 5).
c) Elaboración de un mapa provincial de potencialidad para la truficultura.

Fig. 5 Recolleción de muestras

Subproyecto 6 - Incremento de la vida comercial en fresco de *Tuber melanosporum*: utilización de métodos combinados. Aplicación de nuevas tecnologías.
Este Subproyecto está dirigido por el Dr. Domingo Blanco, de la Facultad de Veterinaria de la Universidad de Zaragoza, coordinando un Equipo Investigador de 6 participantes pertenecientes dicha Institución.

Objetivos:

a) Caracterización y tipificación microbiológica de trufas frescas.
b) Prolongar la vida útil de trufas frescas mediante el empleo de métodos de conservación adecuados que preserven las características organolépticas del producto a lo largo del tiempo.
c) Determinación de parámetros de calidad.

Incremento de la vida comercial de la trufa negra: utilización de métodos combinados. Aplicación de nuevas tecnologías para su conservación. Dada la elevada similitud genética, fisiológica, morfológica y ecológica existente entre la trufa blanca y la trufa negra, consideramos a priori, que las experiencias y conclusiones derivadas de las investigaciones son aplicativas y extensibles a ambas especies. Ello nos va a permitir disponer de muestras durante casi 8 meses al año y poder experimentar inicialmente con un producto mucho más barato como es la trufa de verano. Una vez seleccionado el procedimiento más apropiado para la conservación, el mismo será aplicado y verificado en la trufa negra. Los objetivos se pretender alcanzar en base a:

Subproyecto 7 - Establecimiento, desarrollo y coordinación de la RED EXPERIMENTAL que cobije todas las investigaciones y experimentaciones del Proyecto.
Este Subproyecto está dirigido por el Dr. Rogelio Castaño, de los Servicios Agropecuarios de la Diputación Provincial de Teruel, coordinando un Equipo de 2 participantes pertenecientes dicha Institución.

Objetivos:
Proporcionar al resto de los Subproyectos las parcelas y el soporte logístico para la realización de los trabajos y experimentos (Fig. 6).

Fig. 6 Elecciòn de las plantaciones

a) Parcelas Historial: parcelas “feed back” de las que se debe extraer el historial cultural y las producciones.
b) Parcelas Colaboradoras: Parcelas antiguas en las que se dispone del historial completo y la total colaboración del propietario incluso para arrancar árboles completos o la apertura de pozos de seguimiento fuera de temporada.
c) Parcelas Forestales: parcelas en zonas forestales de monte público (Autonomía o Ayuntamientos) en áreas cortafuegos.

Características del proyecto
El Proyecto *Desarrollo Integral de la Truficultura de Teruel* se caracteriza por una *gran envergadura económica*, cercana al millón de euros, motivada fundamentalmente por la necesidad de refuerzo de los medios humanos disponibles para poder acometer los trabajos del Proyecto.
Presenta igualmente un coste muy alto en el capítulo de personal y en el de dietas y desplazamientos, en virtud del alejamiento geográfico entre la zona objeto del Proyecto y los distintos Grupos participantes.
Su *marcado carácter pluridisciplinar* plantea la exigencia de una eficaz coordinación entre todos los Grupos integrantes así como la necesidad de fluidez de conocimientos e intercambio recíproco de información entre los componentes del Sector trufero y los Grupos de investigación participantes.
El Proyecto intenta profundizar sobre la situación actual y las posibilidades de expansión de la truficultura turolense, despejando incógnitas sobre la precocidad y los plazos de entrada en producción. Abarca igualmente el estudio de los enemigos naturales (parásitos, patógenos y contaminantes) para un eficaz control de los mismos y participa en nuevos trabajos sobre la gestión de los montes en aras al mantenimiento de una selvicultura trufera que permita mantener las producciones silvestres y su incorporación al medio como áreas cortafuegos. También aborda trabajos inéditos en este producto agroforestal referentes a la postcosecha con el fin de aumentar la perdurabilidad de los carpóforos de la trufa negra, de gran importancia para la reestructuración del sector comercial.
Resumiendo este apartado diremos que el Proyecto expuesto se caracteriza por desarrollar una investigación aplicada cuya finalidad es generar nuevo conocimiento que permita transferir resultados al sector de la producción de trufa negra. Este objetivo tiene una alta relevancia científica dado que implicará hacer operativo, evaluar y determinar los pesos específicos, y el modo en que interaccionan entre si, de los factores más relevantes en el manejo de las plantaciones y en la postcosecha de sus producciones, con un impacto socio-económico muy importante para la economía turolense, del que se beneficiarán las 3.500 ha de cultivo de todas las Comunidades y provincias españolas dedicadas a la truficultura.

L'ANALYSE TERRITORIALE DE LA PRODUCTION TRUFFIÈRE: UN PRÉALABLE À L'EXPÉRIMENTATION BIOTECHNIQUE

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Abstract: **The territorial analysis of the truffle production: a prerequisite to the biotechnical experimentation**
The part of the program of PSDR (for and on the regional development) research "INGEDICO" (Instruments of management and collective devices of conservation and recovery of naturals and renewable ressources) deals with a concerted management of limestone hills with environmental and landscape devices of ecosystems and associated practices. The purpose of this communication is to expose the first results of the research carried out in the natural Park of limestone hills the region of Quercy (Lot, France).
The analysis of environmental issues of those territories favorable for truffle production (issues of fire risks and biodiversity, issues of erosion risks and water) is essential before establishing a mapping of experimental areas, selected with the multi-criterions method. This delimitation has been obtained with a multi-criteria analysis with the Electre III method built with different types of data: the pedo-climatic truffle potential, the right location of environmental areas, the analysis of mobilized and mobilizable management instruments for truffles and the analysis of human dynamics and practices connected to the concerned networks of actors. Fifty interviews of truffles growers and managers of expert data (Agricultural, forestry, and environmental) reinforce those first results allowing an assessment of practices of truffles growers and take advantage of existing expertise and tools linked to each environmental issue, while implying local actors to the research approach. Concretely, this territorial approach of truffle practices makes it possible:
- to study the organization of actors and whether or not they contribute the preservation of these natural resources with their practices.
- to propose mobilizable instruments for a concerted management of limestone hills and ecosystems of truffles according to present environmental issues.
- to implement experimental bio-technological protocols to promote environmental practices.
Key words: Sustainable development, Truffle Cultivation, forestry truffle, Lot (France).

Résumé
Le volet «truffe» du programme de recherche PSDR (Pour et sur le développement régional) INGEDICO (Instruments de gestion et dispositifs collectifs de conservation et de valorisation des ressources naturelles renouvelables) interroge la mise en place et la pérennisation d'une gestion concertée des coteaux calcaires, associée à la valorisation environnementale et paysagère des pratiques et des écosystèmes truffiers lotois et périgourdins. Cette intervention se propose d'exposer, pour plus de concision, les résultats obtenus sur le PNR Causses du Quercy (Lot, France) à savoir la détermination de zones d'actions prioritaires susceptibles de promouvoir la truffe et certains modèles de production comme leviers de développement territorial raisonné et durable.
L'analyse des enjeux environnementaux de ce territoire favorables à la production truffière (incendie et biodiversité, qualité de l'eau et érosion) est un préalable indispensable à l'établissement de la cartographie des zones d'expérimentation truffière. Cette délimitation géographique a pu être obtenue grâce à une analyse multicritère Electre III construite sur l'imbrication de différents types de données: le potentiel pédoclimatique truffier, la localisation exacte des zones à enjeu environnementales, l'analyse des instruments mobilisés et

mobilisables pour la truffe, des dynamiques humaines et des pratiques reliées aux réseaux d'acteurs concernés. Une cinquantaine d'entretiens réalisés auprès de trufficulteurs des deux départements viennent conforter ces premiers résultats, mettre à profit les savoir-faire et les outils existants tout en impliquant les acteurs locaux à cette démarche de recherche. Cette approche territoriale des pratiques truffières permet concrètement:
- d'étudier l'organisation des acteurs et la manière dont ils contribuent à la préservation de cette ressource naturelle ou non,
- de proposer des outils mobilisables pour une gestion concertée des coteaux calcaires dont les écosystèmes truffiers,
- et d'impulser l'établissement de protocoles expérimentaux biotechniques visant à favoriser les pratiques truffières à fort potentiel environnemental (plantations selon une conduite raisonnée, entretiens des truffières naturelles et réhabilitation de boisements associée à un pacage extensif).

Mots clés: Développement durable, Trufficul-ture, Sylviculture truffière, Lot (France).

Introduction

La première phase du projet «truffe» du programme de recherche INGEDICO (Instruments de gestion et dispositifs collectifs de conservation et de valorisation des ressources naturelles renouvelables) souhaite interroger, par le biais des sciences humaines et sociales, la valorisation socio-économique, environnementale et paysagère des pratiques et des écosystèmes truffiers dans le Lot et la Dordogne. Nous nous attachons dans la présente communication à développer l'approche méthodologique suivie, les premiers résultats obtenus ainsi que les premières propositions d'actions potentielles sur le Parc Naturel Régional des Causses du Quercy (Lot, France), à savoir la détermination de zones d'actions prioritaires susceptibles de promouvoir la truffe et certains modèles de production comme leviers de développement territorial raisonné et durable.

Si nous nous appuyons sur la réflexion de A. Fischer (1999) traitant de la correspondance entre l'espace et le territoire, il en ressort des logiques d'appropriation et d'appartenance, d'organisation et de gouvernance, de projets et de stratégies mais également de conflits d'intérêt et de concurrence. A l'origine de cette territorialisation de l'espace et des actions de développement qui le font évoluer, les acteurs (et leur organisation résiliaire) nous paraissent être l'élément-clé de la compréhension du territoire. C'est donc par cette entrée que nous souhaitons aborder la question du développement territorial de cette zone d'étude dont l'une des voies principales est la valorisation d'une ressource spécifique: la truffe du Périgord (*Tuber melanosporum* Vittad.). Ce territoire est, de fait, caractérisé par une très forte mobilisation de la filière trufficole. Trois syndicats, associations ou groupements, selon le statut choisi pour la représentation locale des trufficulteurs et trois Centres d'expérimentation sur la truffe* dessinent le premier réseau de compétences spécifiques sur cette zone. En termes de stratégie de concertation, de valorisation de la ressource et de réseaux d'acteurs, il est indispensable d'observer comment ce produit et son milieu sont pris en compte dans les différentes actions de valorisation patrimoniale, touristique, ou environnementale à échelles multiples (échelon régional, départemental et intercommunal). L'analyse territoriale qui en découle, forcément multicritères, croise les enjeux environnementaux et humains, à dire d'acteurs, coexistant sur la zone. Elle devrait permettre concrètement:
- d'étudier l'organisation des acteurs et la manière dont ils contribuent à la préservation de cette ressource naturelle et plus largement de son biotope,
- de proposer des outils mobilisables pour une gestion concertée des coteaux calcaires dont les écosystèmes truffiers,
- et d'implémenter des protocoles biotechniques dans des zones pertinentes pour promouvoir des pratiques truffières durables.

*Trois, si l'on tient compte du CETA de Gignac et surtout de la Station trufficole du Montat dont le site est extérieur au PNR mais dont les expérimentations et les compétences guident les pratiques observées dans le Lot et au-delà. De plus certains de leurs terrains expérimentaux se situent au sein même du PNR.

Coexistence d'enjeux environnementaux et de pratiques truffières au sein du Parc naturel des Causses du Quercy (Lot, France)

Un Parc naturel régional (P.N.R) possède un patrimoine naturel et culturel remarquable reconnu comme tel par l'Etat. Pour autant, les chartes constitutives de tels territoires, établies entre les collectivités locales et territoriales concernées pour dix ans, loin de vouloir muséifier ce territoire, tentent de concilier préservation de l'environnement, développement économique et social. Classé en 1999, le parc naturel en question compte 97 communes, 26 000 habitants et s'étend sur plus de 175 717 hectares. Terre de contrastes, les Causses du Quercy sont l'un des derniers grands ensembles de pelouses sèches de France alors que son taux de boisement approche les 50% et s'accroit inexorablement. Outre le couvert végétal, il convient dans un premier temps de faire un état des lieux des enjeux environnementaux avérés sur le PNR de façon à les mettre en corrélation avec les pratiques trufficoles existantes ou potentielles.

Ainsi, en termes de biodiversité ordinaire ou remarquable (Cf. Fig. 1), les inventaires et zonages officiels ou réglementaires sont constitués par les Zones naturelles d'intérêt écologique, faunistique et floristique (ZNIEFF), les arrêtés de protection Biotope, les réserves naturelles volontaires, la directive Habitats et le réseau Natura 2000 ainsi que les espaces naturels sensibles (ENS). 74 ZNIEFF sont ainsi identifiées en 1988, soit 32 907 hectares.

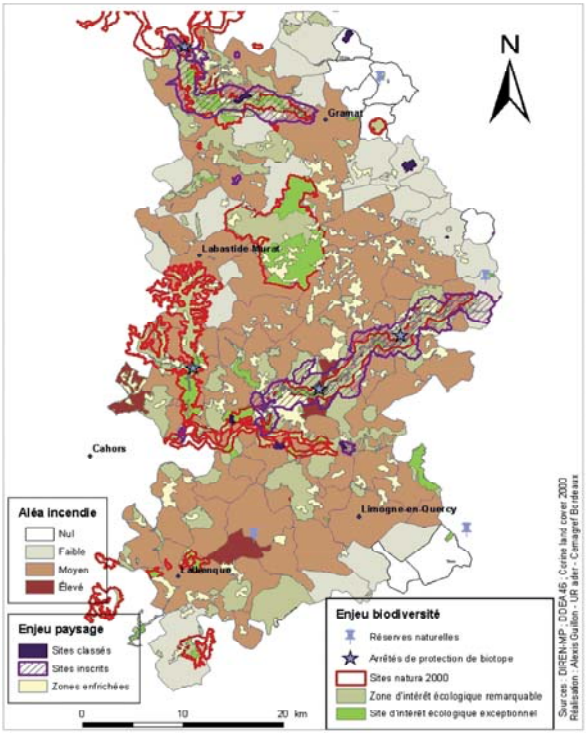

Fig. 1: Croisement des enjeux Biodiversité, Paysage et Aléa Incendie sur le PNR

Depuis, le Parc a réactualisé en 1999 ces zonages en sites d'intérêt écologique exceptionnel (SIEE) et zone d'intérêt écologique majeur (ZIEM). De plus, l'inventaire des habitats a permis de recenser 11% du territoire, concernant 47 communes, susceptibles d'être intégrés au réseau Natura 2000 (Source: PNRCQ, 2008). Malgré la multiplicité de ces inventaires, le principal enjeu pour la biodiversité de ce territoire réside cependant dans la préservation des milieux ouverts ou dans la conduite de pratiques ré-ouvrant les milieux. Le même constat a pu être établi en cartographiant l'aléa incendie sur l'ensemble du périmètre. Le rythme d'expansion de la forêt lotoise s'accroit régulièrement depuis près d'un siècle passant de 1% par an au début du siècle dernier à plus de 1,3% par an à la fin des années 1980. Plus de trois quart des boisements actuels du PNR pourraient devenir, sans intervention, non seulement impénétrables

mais également plus sensibles au risque incendie. Lutter contre cet aléa concourt également à préserver une mosaïque paysagère de qualité améliorant tant la visibilité que l'accessibilité des différents milieux. C'est finalement tout l'enjeu des réflexions construites autour d'une meilleure coexistence des activités agricoles, forestières et de loisir. Les activités truffières favorisant la réouverture d'anciens peuplements présenteraient donc ici un réel intérêt en termes de réhabilitation de milieu, si appliquées à des zones en cours de fermeture où résident encore des communautés végétales de milieux ouverts d'intérêt patrimonial ou bien à des boisements anciens non entretenus. Les techniques de sylviculture truffière pourraient donc, à juste titre, être considérées sur le PNR comme des pratiques parmi tant d'autres préservant les habitats caussenards menacés par l'uniformisation des paysages, l'embroussaillement et l'abandon des exploitations agricoles ou forestières.
Si le relief karstique caractéristique du modèle paysager dominant des Causses du Quercy est en partie responsable de la faiblesse du risque érosif sur l'ensemble du Parc, exceptée sur les contreforts des vallées, il est en contrepartie très perméable aux multiples polluants qui rejoignent l'aquifère quasi instantanément (Cf. Fig. 2).

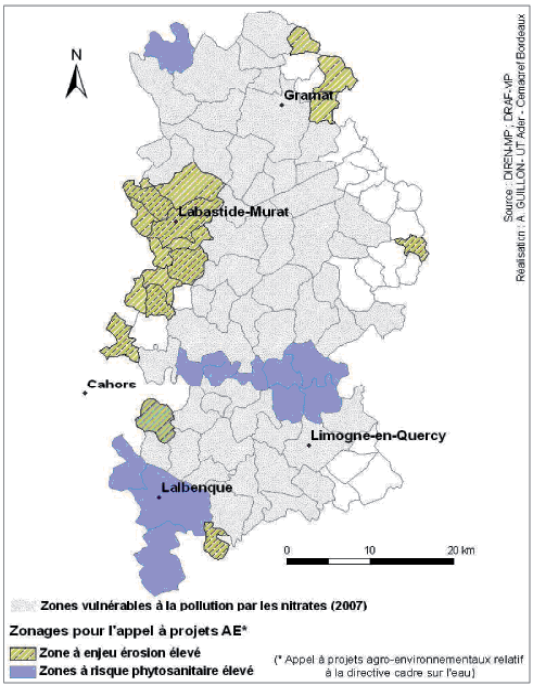

Fig. 2: Zones vulnérables à l'érosion et au risque phytosanitaire sur le PNR

Bien que ces deux problématiques environnementales ne doivent pas être négligées dans notre analyse territoriale, elles peuvent difficilement être corrélées, dans le cas des contreforts des vallées, aux plantations truffières en raison de l'exigüité des surfaces disponibles. Ce modèle cultural, associé à un itinéraire technique raisonné, saurait par contre répondre tant aux attentes des acteurs qu'au risque phytosanitaire de la région de Lalbenque, au sud-ouest du PNR.
Dans le premier cas, l'enjeu «érosion» est loin d'être une priorité pour le Parc et de plus la cartographie réalisée par l'INRA Orléans prenant en compte la géologie, la pédologie, la battance, les pentes et l'occupation du sol confirme l'inadéquation entre les pratiques trufficoles et les zones pentues à risque érosif fort en raison justement de l'incompatibilité du relief avec une telle exploitation. Dans le deuxième cas, les plantations truffières intensives sont difficilement valorisables pour leur intérêt vis-à-vis de la qualité de l'eau. Toutefois, les pratiques de plantations raisonnées d'arbres mycorhizés en milieux ouverts seraient sans doute plus valorisables à l'ouest de notre zone d'étude, le Quercy Blanc, en raison d'une sensibilité accrue aux risques érosifs et de pollution aquifère. La plantation d'arbres pourrait

y limiter l'érosion, filtrer les eaux d'écoulement tout en faisant office de corridors écologiques; ne pas éluder cependant le fait que de nombreux trufficulteurs s'interrogent sur l'impact des intrants en milieu truffier. Le cycle biologique du champignon en serait-il affecté? Les truffes produites stockeraient-elles les substances biocides?
Ces données environnementales peuvent être interrogées en regard des pratiques truffières en raison du fort potentiel truffier de la quasi-totalité du PNR (Cf. Fig. 3).

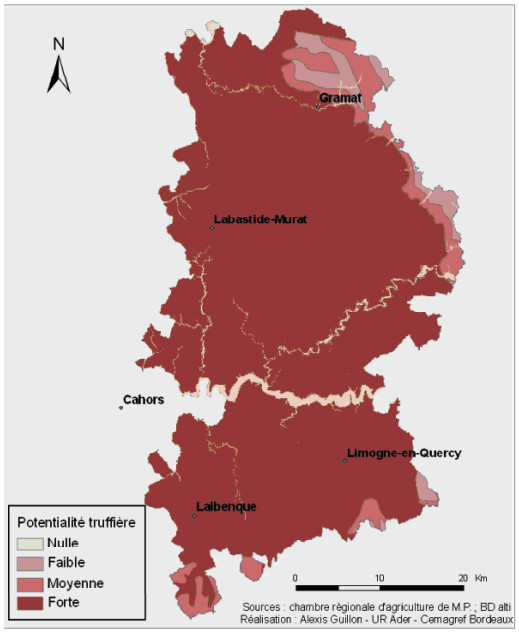

Fig. 3: Potentialité truffière sur le territoire du PNR

Le substrat est calcaire en tout point du territoire, excepté dans les vallées du Lot et de la Dordogne qui possèdent des alluvions siliceuses. Ainsi qu'ils soient calcaires ou calciques, les sols possèdent des pH optimum pour la truffe, entre 7,7 et 8,3 (Olivier *et al.*, 1996). Leur principal écueil est un taux d'argiles élevé variant entre 30 et 38%. Cet inconvénient peut toutefois être compensé par des conditions locales favorisant le drainage comme la présence d'éléments grossiers, la fissuration de la roche-mère, une topographie convexe ou une pente élevée. N'existant pas de carte pédologique à l'échelle du parc, il n'a malheureusement pas été possible de prendre en compte ces paramètres dans une cartographie des potentialités truffières; seule la carte des grands ensembles morpho-pédologiques de la région Midi-Pyrénées au 1/500 000ème permet d'apprécier et de tenir compte de la lourdeur relative des sols dans l'établissement des potentialités édaphiques sur le PNR, l'altitude et le climat n'étant pas limitant sur la zone. Cette typologie conforte la classification par communes déjà réalisée par les techniciens de la Station trufficole du Montat et établie en croisant les données géologiques et celles acquises par une connaissance accrue du terrain. Il reste néanmoins à affiner la typologie afin de gagner en précision et d'investir ainsi les meilleures stations à l'heure des changements climatiques. La typologie des différentes pratiques truffières réalisée confirme cependant bien l'intérêt des pratiques truffières vis-à-vis des enjeux environnementaux étudiés.

Prise en compte des dynamiques locales, des réseaux d'acteurs et des instruments mobilisables pour la gestion truffière

Complémentaire à l'analyse spatiale précédente, le niveau d'analyse suivant est fondamental à l'approche territoriale et à la détermination de potentialités de travail sur le territoire défini comme un «*construit d'acteurs pourvus de ressources spécifiques liées à un héritage collectif, à une mémoire partagée*» (Bessière, 1998, p. 53). Ainsi, l'abandon des exploitations, la fragilisation du tissu rural, le morcèlement du foncier, privé aujourd'hui à 97%, les savoir-

faire ancestraux toujours ancrés dans les pratiques paysannes et les innovations qui se concrétisent et renversent certains a priori sont autant de faits incontournables qui sont le témoignage de la réalité effective des réseaux d'actions qui s'étendent sur un espace limité forcément socialisé. Nous retiendrons de fait le concept qui fait du territoire une manifestation de «l'*appropriation à la fois économique, idéologique et politique (sociale donc) de l'espace par les groupes* [enjoignant] *donc les notions d'espace de vie, d'espace social et d'espace vécu en y ajoutant celles de l'insertion de chaque individu dans un groupe, d'appartenance et d'identité collective*» (Di Méo, 2000).
Au cœur du territoire donc, les acteurs. C'est autour d'eux et notamment autour de leur témoignage (Cf. Encadré 1) que s'articule notre analyse des pratiques paysannes mobilisées en matière de trufficulture. Un développement territorial reposant sur «*des dynamiques collectives d'organisation des acteurs*» (Angeon *et al.,* 2007) impose de fait la prise en compte d'une nouvelle variable, «la dynamique», instaurée par des partenariats de cesse renouvelés.

Questionnement autour des acteurs impliqués dans la filière «truffe»: connaissance des différents types d'acteurs mobilisés (trufficulteurs, techniciens, scientifiques, pépiniéristes…) et mobilisables (forestiers, chasseurs, propriétaires fonciers…), organisation et mise en réseau de ces acteurs, convergences et divergences d'approche et d'objectifs…
- Questionnement autour des stratégies de concertation mises en place susceptibles d'enclencher des dynamiques de développement pérennes: choix des produits valorisables, concurrence entre leviers de développement et réseaux d'acteurs, implication des acteurs et valorisation des produits spécifiques hors territoire....
- Questionnement autour de l'appartenance territoriale du produit: alors qu'aujourd'hui 76% de la production nationale provient du sud-est de la France, importance des processus de spécification du produit, la qualité passe-t-elle par la reconnaissance systématique de l'origine locale?

Encadré 1: Délimitation du champ des interrogations

Dans un premier temps, en s'appuyant sur les dires d'acteurs autour des pratiques «paysannes» mobilisées en matière de production truffière, à savoir les trufficulteurs eux-mêmes ainsi que les représentants des syndicats, des chambres d'agriculture et des stations d'expérimentation, nous avons souhaité mettre en exergue les premiers résultats de la gestion des couverts arborés truffiers sur le triptyque «arbre hôte/espaces agricole et forestier/acteurs de la filière truffe et autres acteurs» en termes de politiques et de règlements, d'innovation technique, de modalités de gestion de l'arbre, d'information, de coopération, de protection de l'environnement et de valorisation paysagère. Cinquante entretiens ont ainsi été réalisés sur la base des listes des trufficulteurs syndiqués sur l'ensemble des zones d'études (Lot, Dordogne) dont plus de 31 heures d'entretiens sur le seul PNR.
Outre l'étude des dynamiques truffières, une vingtaine d'entretiens auprès d'acteurs institutionnels (experts agricoles, forestiers et environnementaux) ont permis de collecter des données expertes socio-économiques et environnementales concernant la spatialisation fine des enjeux environnementaux et des instruments de gestion mobilisables (Guillon, 2008). Les dynamiques foncières, forestières, pastorales et de DFCI[1] permettent de localiser la présence d'acteurs et d'instruments de gestion potentiellement mobilisables en faveur d'un développement de pratiques truffières favorables à l'environnement et au paysage. Ayant mis en évidence l'intérêt des pratiques ré-ouvrant les milieux boisés, il est important de tenir compte des plans de massif[2] existant, des superficies concernées par des plans simples de gestion[3] ainsi que des parcelles ayant déjà bénéficié d'une opération de réhabilitation ou de rénovation de taillis par le CRPF. Pour les mêmes raisons, la question du sylvo-pastoralisme est à étudier tant dans une optique d'entretien simple des couverts que couplé à la culture de la truffe, d'autant qu'un grand nombre de trufficulteurs sont en attente d'une réponse concrète quant à l'impact réel du pacage sur la virulence des truffiers. De telles pratiques pourraient de

plus être complémentaires au PPFCI[4] départemental notamment dans le cas des boisements couvrants les contreforts des vallées du Lot et du Célé.
Cet état des lieux permet de sélectionner les éléments qualitatifs et quantitatifs définissant le territoire à un instant, nécessaires à l'utilisation spatiale de l'analyse multicritères Electre III, méthode qui permet de classer, deux à deux, les 97 communes en fonction des critères retenus et visant à définir les zones les plus favorables sur le plan socio-économique, environnemental et paysager à l'expérimentation truffière (Cf. Encadré 2). Différents types d'instruments mobilisés ou mobilisables y sont explorés (contractuels, paysagers, fonciers etc.) par rapport à l'enjeu du développement de la production truffière ainsi que les dynamiques d'acteurs associés**.**

Méthode ELECTRE I: méthode élaborée par Roy, 1968. Le but vise à obtenir un ensemble d'actions aussi réduit que possible, parmi lesquelles figure la meilleure solution de compromis.
Méthode ELECTRE II: méthode élaborée par Roy et Bertier (1971, 1973). Le but de cette méthode est de classer les actions potentielles, depuis les meilleures jusqu'aux moins bonnes, en acceptant les ex-æquo.
Méthode ELECTRE III et IV: développées par Roy en 1978 et 1982. Le but est de classer les actions potentielles des meilleures aux moins bonnes, mais en prenant en compte la crédibilité des hypothèses et en intégrant des critères qualitatifs. La procédure de classement correspond à une distillation descendante et à une autre ascendante.
Adaptation au contexte de l'étude: dans le cadre de notre étude, il s'agit d'effectuer une comparaison deux à deux des communes du territoire à l'aide de notes attribuées pour les différents critères afin de définir les zones à enjeux favorables à l'expérimentation truffières. La commune est considérée comme une unité territoriale potentielle d'action en lien avec la présence d'enjeux environnementaux et socio-économiques favorables à l'expérimentation truffière.
La méthode Electre III permet une comparaison deux à deux des communes du territoire à l'aide de notes attribuées pour les différents critères.
Cinq pondérations différentes des critères ont été utilisées afin de classer les communes en fonction de l'importance de telle ou telle catégorie de critères (poids des enjeux environnementaux, de la connaissance du foncier, de la présence d'acteurs mobilisables, d'acteurs trufficoles et forestiers, de la présence d'actions de réouverture déjà initiées et de maitrise foncière publique).
- Critères de type 1: le potentiel truffier et dynamiques associées (Acteurs et instruments).
- Critères de type 2: Enjeu de la biodiversité, Aléa incendie et dynamiques agro-environnementales, pastorales, forestières ou cynégétiques associées (Acteurs et instruments).

Encadré 2: Presentation de la methode Electra (d'apres Macary 2003)

Il convient ici de préciser la définition d'outils de gestion retenue dans le projet INGEDICO: «*tout dispositif formalisé permettant l'action organisée*» (David, 1998); l'outil devenant seulement instrument à travers l'usage qui en est fait par les acteurs. Nous faisons l'hypothèse qu'améliorer l'adéquation entre les outils de gestion et les situations d'action dans lesquels ils sont mobilisés peut aider à améliorer l'efficacité des dispositifs collectifs. Ceci oblige à travailler sur des instruments (Lascoumes, Le Gales, 2004) (mobilisés, mobilisables ou à concevoir) adaptés aux situations particulières de conservation et de valorisation des ressources naturelles. A. Hatchuel (2000) distingue des instruments orientés «connaissances», contribuant à la co-construction de connaissances nouvelles, de ceux orientés «relations» c'est-à-dire contribuant à la coordination d'acteurs et à leur mise en présence. Des instruments mixtes peuvent cependant exister, agissant à la fois sur les savoirs et sur les coordinations d'acteurs.

Résultats et discussion
Un certain nombre de communes ressortent systématiquement quelque soit la pondération des critères retenus. Ceci confirme la robustesse de la méthode et compense le caractère

artificiel de la pondération. Ceci permet d'identifier trois zones principales d'expérimentations biotechniques possibles pour la truffe (Cf. Fig. 4).

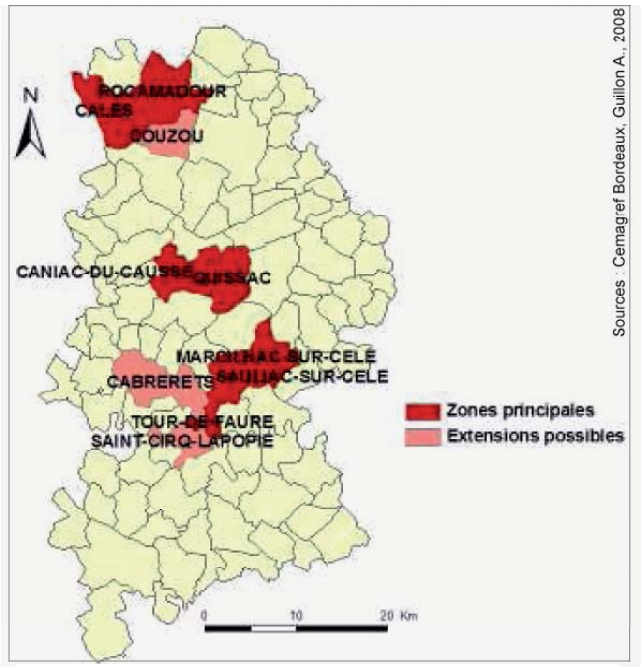

Fig. 4: Territoires d'étude sélectionnés par l'AMC Electre III

Grâce à l'exploration des instruments de gestion mobilisables, différentes propositions ont pu être faites en concertation avec les acteurs concernés afin de mieux prendre en compte la production truffière.

A l'issue de cette analyse du territoire croisée avec les pratiques truffières actuelles et les demandes d'expérimentation, une première grille réflexive (Cf. Encadré 3) d'interventions biotechniques est proposée. Elle tient compte de l'expérience des trufficulteurs sur le terrain et complète les travaux de la «Station truffe» du Montat. Cette grille présente une pratique encore empirique en Europe méditerranéenne mais qui tend à se développer en Languedoc-Roussillon, la «*sylviculture truffière*» (Diette, 2003; Lauriac, 2004). Elle s'inspire plus précisément des modalités techniques que les «sylviculteurs truffiers» appliquent dans le sud-est de la France, en les adaptant cependant aux conditions pédoclimatiques lotoises; il convient en effet de considérer les premiers retours sur expériences effectuées ces dernières années dans ce département afin de ne pas réitérer les essais souvent infructueux de rénovation qui consistaient en des élagages et émondages[5] sévères associés à un travail du sol (Sourzat, 2006).

Au-delà des interventions techniques, il serait intéressant d'aller plus loin dans le suivi des dispositifs expérimentaux afin d'évaluer les impacts des pratiques. Au niveau truffier, un suivi de la production et de l'évolution de la présence de mycorhizes et de spores de truffes dans le sol pourrait être lié à l'analyse des concurrences fongiques. Au niveau floristique, un suivi de l'évolution de la flore du site devrait permettre également de capitaliser des connaissances sur l'écosystème truffier**.** Une attention particulière sera portée à la présence de plantes compagnes: ont-elles une valeur mellifère et un potentiel valorisable dans une micro-filière de bois artisanaux (genévriers, buis, fruitiers, aubépine…)? Quel rôle jouent-elles dans la gestion fine de l'eau pour la truffe? Quels sont les méthodes alternatives de gestion de l'eau possibles (paillage, arrosage raisonné, etc.)? De plus, un suivi de la petite faune sauvage (lapin, perdrix) permettrait d'évaluer l'impact des lapins sur la «virulence» de *T. melanosporum* notamment par l'effet qu'aurait leur système digestif sur la germination des spores. Enfin, l'évaluation des dépenses et des recettes occasionnées au cours du temps devrait permettre de mieux

prendre en compte les atouts et les contraintes de ce type d'expérimentation afin d'adapter au mieux les instruments utilisés pour financer la restauration et l'entretien des milieux.

- Maintenir un taux de couvert arboré entre 10 et 30%: cet élément est fondamental dans les interventions à tester.
- Effectuer des essais d'éclaircie et de recépage: ces deux opérations peuvent être réalisées avec un arrachage des souches surnuméraires
- Conserver voire planter des arbustes et des plantes compagnes (genévriers, lavande, prunelliers, aubépine, églantier, alisier torminal, alisier blanc, cerisier de Sainte Lucie et pommiers, poiriers, buis…).
- Regarnir par des plants mycorhizés: ces plants seraient intéressants pour régénérer le peuplement. D'autre part leur mycorhization par *T. melanosporum* est assurée et ils peuvent être utiles pour inoculer les autres arbres.
- Travailler le sol de façon localisée et superficielle: ce travail est en effet à réaliser autour des arbres conservés sur une profondeur de 8 à 10 cm, contrairement au travail profond et en plein qui a pu être réalisé pour certaines rénovations. Ce travail permet de redynamiser les radicelles sectionnées, d'aérer le sol tout en conservant les communautés végétales herbacées.
- Réensemencer les truffières par inoculation avec des spores de *T. melanosporum*: cela pourrait en effet s'avérer intéressant pour favoriser la mycorhization ou la reproduction de la truffe.
- Tester les différents types de paillage et d'arrosage: ces interventions ne sont pas spécifiques à la sylviculture truffière mais sont intéressantes pour pallier aux trop fortes sècheresses estivales. Elles doivent être prises en compte dans les itinéraires expérimentaux.
- Expérimenter la présence d'animaux domestiques (ovins principalement): le passage des animaux sur les truffières permet d'entretenir les milieux truffiers après restauration.

Encadré 3: Principaux éléments de la grille d'expérimentation proposée

Pour pouvoir réaliser ce suivi complet des parcelles expérimentales, on s'intéressera donc prioritairement aux sites en maîtrise foncière publique. On essaiera de plus, si possible, de sélectionner les sites au sein des zones d'études définies grâce à l'AMC Electre III; d'autant que les essais seraient l'occasion d'y relancer des dynamiques locales. De plus, le Conservatoire Régional de l'Environnement, la fédération départementale des chasseurs, l'Office National des Forêts et certains propriétaires ont été identifiés au cours des entretiens comme partenaires potentiels.
Sachant que la mise en place d'expérimentations requiert un processus relativement lourd, il est également envisageable de mettre en place des suivis plus opportunistes comme par exemple la recherche de la potentialité truffière sur des sites où le milieu a été ouvert dans un tout autre objectif. Cela permettrait d'ailleurs d'étudier la compatibilité entre les activités trufficoles et les autres activités travaillant pour la réouverture des espaces fermés. Ces suivis consisteraient au relevé des indicateurs les plus simples de la présence du champignon c'est-à-dire la présence de brûlés et la production de carpophores. Ils pourraient être réalisés au niveau des parcelles:
- ayant fait ou allant faire l'objet de réhabilitation d'habitat pour le petit gibier par la fédération et les sociétés de chasse,
- coupées ou éclaircies, notamment dans le cadre des contrats de réhabilitation de chênaie pubescente mis en œuvre par le CRPF,
- en site Natura 2000 où ont été contractualisées des Mesures Agro-Environnementales Territorialisées (MAETER) de gestion pastorale des espaces boisés,
- qui feront l'objet de réouverture à un stade pré-bois à l'occasion des aides du programme «espaces embroussaillés» piloté par le Conseil Général du Lot,

- en lisière de coupures de combustible réalisées dans les années 1990.

Ces zones présentent l'avantage d'être déjà suivies puisque la souscription aux différents contrats exige un enregistrement des travaux, des pratiques et même la réalisation d'un plan de gestion. Il serait nécessaire, à ces occasions, d'évaluer comment ces actions de coupe, d'élagage, de débroussaillage et de pâturage correspondent aux exigences techniques de sylviculture truffière et dans quelle mesure il serait envisageable de les adapter en vue du déclenchement d'une production truffière.

Outre le fait que la restauration et l'entretien de milieux semi-fermés ont un coût économique, ils font l'objet de règlementations strictes (défrichement, protection des milieux, etc.). Cela implique de réfléchir à l'utilisation expérimentale d'instruments de gestion financiers (contractuels ou non) afin de faciliter leur expérimentation. L'étude des instruments existants mobilisés et mobilisables pour l'expérimentation a donc permis de formuler des préconisations qui mériteraient d'être débattues et enrichies avec les acteurs du territoire. Il s'agirait de mieux prendre en compte l'environnement dans les aides à la rénovation (de ne pas les supprimer) en réfléchissant par exemple aux précédents culturaux et en adaptant les itinéraires au milieu. Il serait également possible d'inscrire des actions d'expérimentations en sylviculture truffière dans la charte du Parc, le SRGS[6], une charte forestière ou le programme «espaces embroussaillé» du conseil général et d'inclure des parcelles expérimentales dans le réseau expérimental des CRPF. Des actions truffières innovantes pourraient être expérimentées au titre de Natura 2000 et/ou pour le risque incendie, tout comme pourraient l'être des contrats innovants au profit d'espèces ou d'habitats incluant la truffe comme espèce indicatrice de qualité de milieu. Enfin, les contrats agro-environnementaux pastoraux existants et ceux de réhabilitation de taillis de chêne pubescent seraient susceptibles d'être adaptés à la problématique truffière tout en s'inscrivant dans la lutte contre l'embroussaillement, contre le risque incendie et pour la préservation de la biodiversité.

D'autres instruments d'accompagnement sont cependant possible comme l'intégration de la problématique «truffe» au sein des programmes LEADER+ ou LIFE environnement, la mise en place, pour les bénéficiaires, de formations adaptées aux précédents culturaux et aux milieux investis ainsi que la valorisation économique des espèces concurrentes telle que *Tuber brumale*.

Cette méthode d'analyse territoriale a permis de mettre en évidence les zones à enjeux environnementaux ainsi que les pratiques et dynamiques truffières existantes sur le territoire du parc naturel régional des Causses du Quercy. Elle a aussi permis de déterminer quelles pratiques pourraient avoir un intérêt par rapport à ces enjeux et quels instruments paysagers et financiers pourraient être mobilisés pour l'expérimentation truffière sur les territoires identifiés comme favorables du fait des dynamiques d'Acteurs et des instruments de politiques publiques existants. Ces propositions devront être débattues et adaptées par les acteurs du territoire afin de déboucher sur une expérimentation de pratiques truffières durables.

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[1] **DFCI:** Outil de Défense des Forêts Contre l'Incendie

[2] **Un Plan de Développement de Massif de forêts privées (PDM)** est une approche territoriale et concertée du développement durable de la gestion multifonctionnelle des forêts privées: gestion économique, gestion environnementale et gestion sociale, emploi. Le périmètre du PDM est choisi avec les acteurs locaux. Sa surface permet les rencontres entre acteurs sur le terrain: de 500 à 5.000 ha (ordre de grandeur).

[3] **Un Plan Simple de Gestion** est un outil de prévision et de suivi de la gestion forestière. Présenté par le propriétaire, le Plan Simple de Gestion (PSG) est un document qui fixe les règles de conduite de sa propriété boisée. Il comprend trois parties: une analyse des peuplements dans leur contexte économique, environnemental et social; la définition des objectifs de gestion; un programme de coupes et travaux. Le plan de gestion précise la stratégie cynégétique du propriétaire pour les grands animaux soumis au plan de chasse.

[4] **PPFCI:** Plan de Protection des Forêts contre les Incendies

[5] **L'émondage** est une forme de taille consistant à supprimer les branches latérales ou la cime d'un arbre pour favoriser les rejets.

[6] **SRGS:** Schéma régional de gestion sylvicole

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TRUFICULTURA Y GESTIÓN FORESTAL EN TERUEL

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Abstract: Trufficulture and forest management in Teruel

In the framework of the Socioeconomic Development Plan for the province of Teruel (Spain), a coordinated trufficulture research project is currently being carried out. Perigord black truffle has been a significant agroforestry resource in this region since 1950, when people began collecting naturally growing forest truffles. The main goals of this research project are related to the management of truffle plantations, which have spread very quickly since 1990. However, there are also other goals related to the management of forests that spontaneously produce black truffles and whose productivity is currently experiencing a pronounced decline. In this subproject, the concrete aims are: 1) to adapt management techniques to the ecological conditions in Teruel and disseminate said techniques among forest managers and owners, 2) to evaluate the feasibility of establishing truffle plantations in firebreaks, and 3) to improve the understanding of the ecological relationship between former charcoal kilns and the spontaneous formation of truffières.

With respect to the first aim, several truffle silviculture pilot projects have been proposed for two forests with naturally occurring truffles. The ecological conditions in the truffières and the factors limiting their productivity have been analyzed and improvement actions have been proposed. Regarding the second aim, three firebreaks have been experimentally planted for demonstrative purposes. They have also been designed to evaluate the efficacy of several types of site preparation. Finally, with regard to the third aim, the effect of ancient charcoal kilns on current soil properties and on their potential for truffle cultivation are to be studied. In addition, two experimental charcoal kilns will be set up to evaluate their short-term effect on soil and ectomycorrhizal fungi.

The research project for the “Integral Development of Trufficulture in Teruel” is not intended to establish definitive conclusions on many of these research questions during its duration (2007-2010), since the life cycle of the truffle is much more prolonged. Nevertheless, this project has already been successful in encouraging collaboration between researchers, public administrators and truffle growers and in opening communication channels for scientific dissemination and exchanges of empirical experiences.

Key words: *Tuber melanosporum*, Teruel, truffle silviculture, firebreak, charcoal kiln.

La trufa negra en Teruel

La provincia de Teruel, en el este de España, tiene unas condiciones ambientales mediterráneas de montaña que limitan notablemente la producción agrícola. La superficie forestal ocupa un 64% del territorio y las explotaciones agrícolas tienen pequeño tamaño y baja productividad, siendo muy escasos los regadíos. La población ha emigrado masivamente a las ciudades durante el siglo XX y actualmente la densidad de población es de 9 habitantes km^{-2}, con más de un 25% de ciudadanos mayores de 65 años. Todo ello lastra el desarrollo económico, basado principalmente en el sector primario (Guillén y Lozano, 2005).

Para contrarrestar la situación, el Gobierno de España puso en marcha un Plan Específico para el Desarrollo Socioeconómico de Teruel. Entre las medidas adoptadas se incluye el fomento de la productividad en el sector agroalimentario a través de la investigación científica y el desarrollo tecnológico.

En este marco, se aprobó el proyecto de investigación Desarrollo Integral de la Truficultura de

Teruel (Palazón *et al*, 2008). La Asociación de Truficultores de Teruel estima que la producción trufera provincial varía entre 1,5 y 15 Tm año^{-1} y que supone un 30-50% del total de España. El objetivo general del proyecto es resolver los problemas técnicos más urgentes del sector. Para ello, dedica los mayores esfuerzos en establecer las bases científicas de la truficultura para las condiciones ambientales de Teruel, estudiando aspectos como la edafología, la micorrización y los tratamientos culturales. En Teruel, las plantaciones truferas comenzaron a extenderse a finales de los años 1980. Actualmente se estima que ocupan más de 3000 ha, todas ellas de propiedad privada.

La trufa negra (*Tuber melanosporum* Vittad.) también aparece de forma natural en los bosques de Teruel. Se recolecta desde finales de los años 1950. En España, se estima que la producción silvestre supone aproximadamente un 70% del total, aunque se encuentra en declive desde los años 1970 (Reyna, 2007). En Teruel, el 60% de la superficie forestal es de titularidad privada y no se dispone de datos sobre su producción trufera. El 40% restante es de titularidad pública y de ésta, un 10% han tenido aprovechamiento trufero en los últimos 15 años (39600 ha, Fig. 1).

La trufa de los montes públicos se adjudica por subasta y se aprovecha mediante arrendamientos por un periodo de 3-5 años. El precio total (en euros constantes) por el que se arrienda el aprovechamiento trufero de los montes públicos de Teruel ha disminuido un 35% en los últimos 15 años, mientras que el precio medio de la trufa pagado al recolector español ha aumentado un 27% en el mismo periodo (Reyna, 2007). Actualmente, la trufa aporta a estos montes un ingreso medio de 2,78 euros ha^{-1} año^{-1} (euros de 2007).

Dentro del proyecto de investigación, se plantearon tres objetivos concretos relacionados con la producción silvestre: (1) establecer criterios de mantenimiento y mejora de las truferas silvestres, adaptados a las condiciones ambientales de Teruel, (2) desarrollar una tecnología para producir trufa en las áreas cortafuegos, y (3) determinar los factores ecológicos que favorecieron en su momento la formación espontánea de truferas sobre las carboneras abandonadas.

Estas líneas de trabajo intentan comprender mejor la ecología de las truferas silvestres y desarrollar técnicas de gestión que permitan conservar y mejorar la producción trufera de las áreas forestales. Otro objetivo igual de importante es divulgar estas técnicas entre los propietarios y gestores forestales.

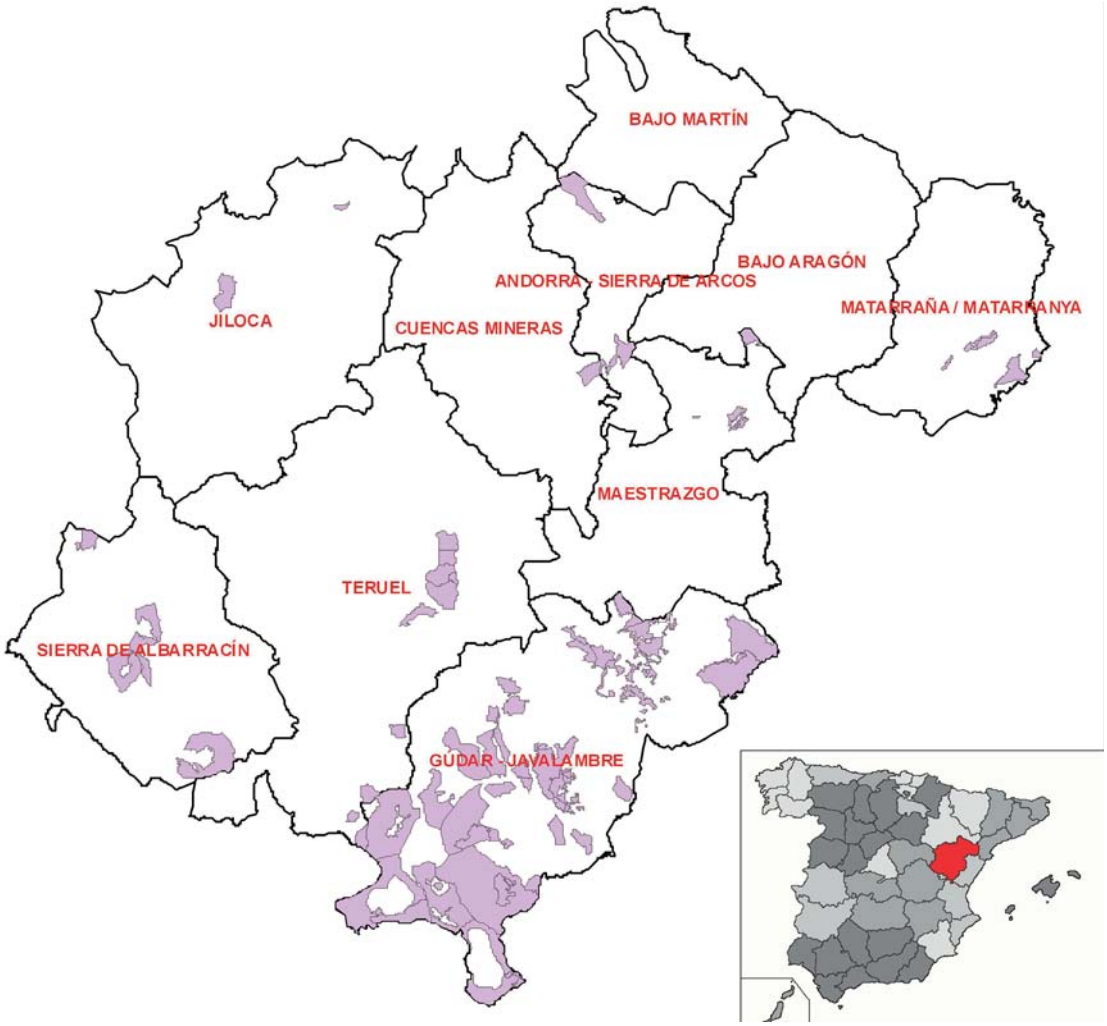

Fig. 1 Provincia de Teruel y montes de propiedad pública con aprovechamiento trufero en los últimos 15 años.

Mejora de truferas silvestres

Se escogieron dos montes truferos de propiedad pública en la comarca de Gúdar-Javalambre. Ambos han visto reducida su producción en las últimas décadas. Con la colaboración de los arrendatarios se localizaron sus truferas: 55 de *T. melanosporum* y 15 de *Tuber aestivum* Vittad.. Se hizo un inventario de sus características ecológicas y productivas más importantes. Basándose en esta información y la ofrecida por los recolectores, se determinaron los factores que limitaban la producción silvestre. Se redactaron sendos proyectos de mejora, que incluían principalmente tratamientos de la vegetación y del suelo.

El monte Carrascal se encuentra a 1000-1575 m de altitud. La temperatura media anual es de 11-12º C y la precipitación media anual 500-550 mm. El relieve es montañoso, pues los suelos se han formado sobre roca caliza jurásica dura. Los suelos son calcáreos, poco profundos (en general, con menos de 40 cm) y muy pedregosos (cubriendo más del 70% del suelo). La vegetación es abierta (fracción de cabida cubierta variando entre el 10-75%) y está dominada por la encina (*Quercus ilex* L. ssp. *ballota* Samp.) y matorrales bajos.

Las truferas se forman en encina. Históricamente, las encinas eran cortadas para leña, lo que provoca que actualmente cada mata tenga un elevado número de pies que proyecta una intensa sombra sobre el quemado. Las truferas de *T. melanosporum* tienen una producción en regresión, mientras que las truferas de *T. aestivum* han mantenido su producción en los últimos años. Un 50% de las truferas se encuentran en pendientes mayores del 10% y en orientación sur. Estas laderas tienen pequeños muretes de piedra en un estado ruinoso, que servían antiguamente para acumular suelo y cultivar cereal.

Las actuaciones que se propusieron fueron puntuales, limitadas a las truferas en producción y a aquéllas que han dejado de producir recientemente:

(1) resalveo y poda de los *Quercus* productores de trufa y los que rodean el quemado
(2) restauración de muretes de piedra seca, para conservar el suelo
(3) picado del suelo en el borde externo del quemado, para acumular suelo en truferas con escaso volumen de tierra fina (diámetro de las partículas menor de 2 mm).

El monte Rebollar-Loma Royuela se encuentra a 900-1100 m de altitud. Presenta 12-13ºC de temperatura media anual y entorno a 450 mm de precipitación. El suelo está formado por sedimentos terciarios sin consolidar, capas alternas de arenas calcáreas y arcillas. La cantidad de materia orgánica es muy escasa (0,5-1,1%) y el suelo es muy erosionable, formándose regueros en las pendientes. El relieve es de lomas suaves (pendientes menores del 10%) y fondo de valle, aunque atravesado por algunos barrancos encajonados, con fenómenos erosivos activos. La vegetación es abierta en una parte del monte, mientras que en la otra es densa porque se realizó una repoblación de pinar. En los relieves suaves, las truferas se forman sobre coscoja y encina. En los taludes de los barrancos cerrados, domina la coscoja (*Quercus coccifera* L.) como especie simbionte.

En este caso, las actuaciones que se propusieron fueron:

(1) en la zona de pinar denso, abrir claros alrededor de las truferas como propone la selvicultura trufera (Reyna *et al.*, 2004)
(2) en zonas con pendiente o en el lecho del barranco, muretes de piedra seca para conservar el suelo y desvío de los regueros por fuera del quemado
(3) en las zonas de vegetación abierta, picado y subsolado del borde externo del quemado para evitar el encostramiento de la capa superficial del suelo y enmiendas orgánicas puntuales con restos vegetales compostados.

En los montes estudiados, la aparición de nuevas truferas ha sido muy escasa en las últimas décadas. Las que han aparecido han tenido una producción escasa y han dejado de producir en pocos años, debido al intenso aprovechamiento de las trufas. La regeneración de la producción trufera a medio-largo plazo (más de 20 años) depende de que se formen nuevas truferas, que tomen el lugar de las que van desapareciendo. Con objeto de fomentar esta regeneración se localizaron áreas adecuadas para la plantación trufera (zonas sin vegetación ectomicorrícica, cultivos abandonados, etc.) y se propuso realizar pequeñas plantaciones inoculadas.

Truficultura en áreas cortafuegos

Los cortafuegos son estructuras lineales de defensa contra los incendios forestales. Son muy frecuentes en los montes mediterráneos, pero para ser efectivos requieren un mantenimiento continuado. La espesura vegetal en los cortafuegos debe mantenerse reducida, ya que su finalidad es crear una discontinuidad en la vegetación. La producción de trufa negra requiere una espesura reducida (Reyna *et al.,* 2004), lo que permite considerar a priori que el uso como cortafuegos y para truficultura son compatibles (Reyna y Garcia-Barreda, 2005).

Con objeto de aumentar el valor económico del monte y contribuir al mismo tiempo a la defensa contra incendios, se propuso implantar truferas en zonas con interés para la defensa contra incendios. Para desarrollar la técnica, se realizaron tres plantaciones experimentales en diferentes ambientes:

(1) La banda de desbroce selectivo y poda de un área cortafuegos. Zona culminal, altitud: 1400 m, pendiente: 0-10%, suelo poco profundo (20-40 cm) sobre roca caliza jurásica dura, vegetación clara de *Juniperus*, *Pinus* y *Quercus* (Fig. 2).
(2) Cultivos abandonados junto a una pista forestal. Fondo de valle, altitud: 1300 m, suelo llano y profundo (más de 60 cm) de origen coluvial, vegetación clara de *Juniperus* con elevada densidad de aliagas rebrotadoras (*Genista scorpius* DC)
(3) Loma de vegetación clara junto a una pista forestal. Altitud: 900 m, pendiente: 0-30%. Suelo formado sobre capas poco consolidadas de arena calcárea y arcilla. Profundidad efectiva de suelo: 20-40 cm, limitado por encostramientos calcáreos. Vegetación clara de coscoja y *Juniperus*.

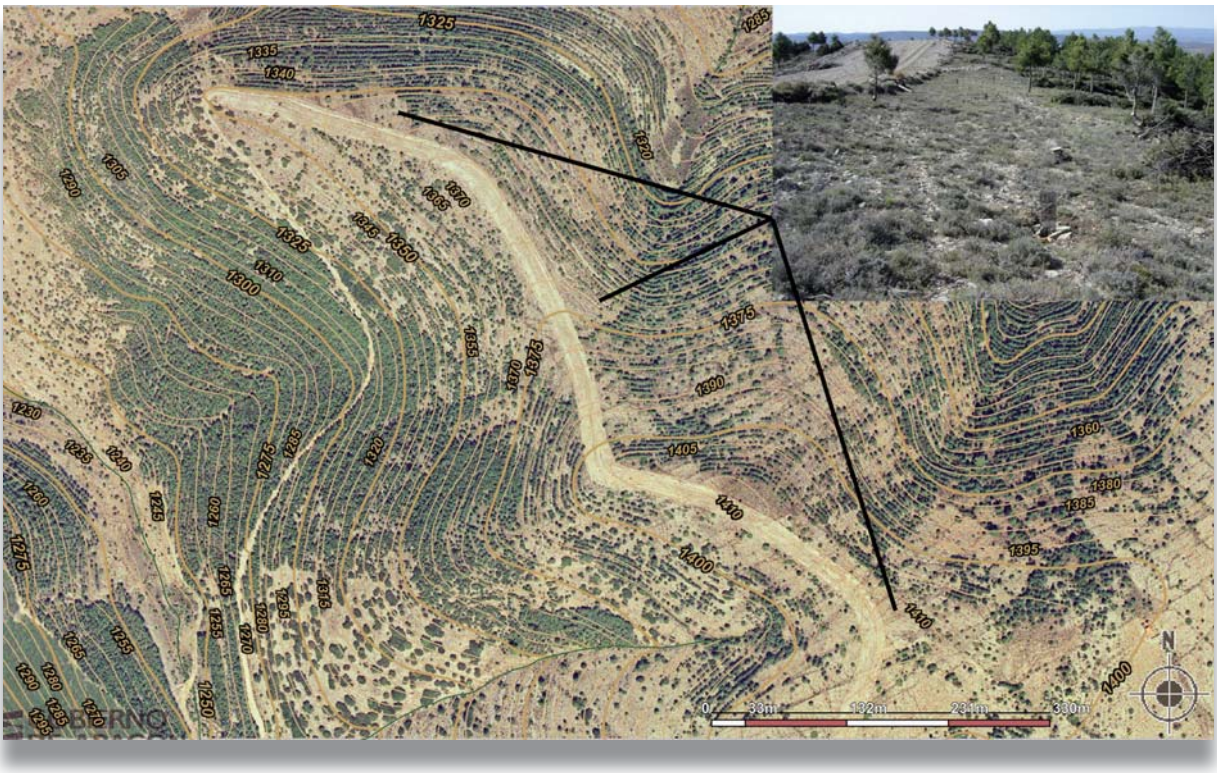

Fig. 2 Plantación inoculada en el ambiente 1 (imagen aérea extraída de www.sitar.aragon.es)

La plantación se realizó con un marco al tresbolillo, con distancias medias entre las plantas de 5-7 m. En los ambientes (1) y (2) se desbrozó la vegetación previa (respetando los Juniperus) y el suelo se preparó por subsolado. En el ambiente (3) no se hizo dado que el suelo es muy erosionable y la dinámica de colonización vegetal es lenta. En los tres casos el punto de plantación se preparó mediante ahoyado manual (40 x 40 x 30 cm).

La planta utilizada fue encina inoculada de una savia, cultivada en contenedor forestal de 650 ml. En el momento de la plantación, las plantas presentaban como media 12,5 cm de altura, 4,6 mm de diámetro en el cuello de la raíz y 3390 ectomicorrizas de *T. melanosporum*, colonizando un 45% de las raíces finas (supervisada mediante el método de Reyna *et al*., 2001). Un 3,5% del total de las ectomicorrizas eran *Sphaerosporella brunnea* Srveck et Kubicka, la única contaminante. Las plantas se protegieron en campo mediante protectores de malla.

Se consideró que los dos principales amenazas para el éxito de la plantación eran que las raíces de las plantas se contaminaran con hongos nativos del suelo durante los primeros años (Reyna y Garcia-Barreda, 2008) y que las plantas no tuvieran un crecimiento significativo durante los primeros años. Por ello, se probaron cuatro tratamientos del suelo en los hoyos de plantación: tratamiento térmico (quema sobre el hoyo de 25 kg de leña durante 4 horas), hipoclorito sódico (1 l por hoyo, con una concentración de 35 g l^{-1}), sobre-encalado (4 kg m^{-2} de cal viva) y enmienda orgánica (9 l m^{-2} de residuos vegetales compostados). Estos tratamientos se llevaron a cabo un mes antes de la plantación. Antes de la misma, se comprobó que el suelo había recuperado su pH previo.

La plantación se ejecutó en abril de 2008. En el ambiente (3) las plantas recibieron un riego de apoyo durante el primer verano (8 litros por planta) y se hubo de reconstruir algunos alcorques después de las lluvias de primavera y otoño. El plan de seguimiento incluye muestreos de micorrización, crecimiento de la plantación y evolución de la vegetación espontánea. La supervivencia durante el primer verano fue del 98% y un 98% de las plantas supervivientes tuvo elongación del tallo el primer año.

Ecología de las carboneras

En España, muchos recolectores de trufa señalan que son frecuentes las carboneras

abandonadas que han albergado o albergan truferas. En estas carbonera se producía carbón de leña mediante parvas (hornos de tierra). Estaban situadas en el monte, junto a las masas de las que se obtenía la leña (FAO, 1983).
El proceso de carboneo consiste en apilar la leña, cubrirla con ramas, hojas y suelo y encenderla de forma que se produjera una combustión sin llama. El proceso dura 3-10 días, dependiendo del tamaño de la carbonera, y en el interior de la misma se pueden alcanzar temperaturas de 400-500ºC.
Los principales factores que pueden explicar la relación entre carboneras y trufa son, según Reyna (2007):

(a) la carbonera requiere un claro en el bosque (Fig. 3), lo que permite la insolación del suelo que la trufa necesita; años después de ser abandonada, el suelo aún tiene una escasa cobertura de herbáceas y arbustivas (Mikan y Abrams, 1995)
(b) el calor esteriliza el suelo, eliminando los hongos ectomicorrícicos
(c) el calor altera las características del suelo: pH, color, estructura e incluso textura; años después del abandono, los suelos mantienen unas propiedades físicas diferentes a las de su entorno
(d) los destilados de la madera tienen efecto biocida
(e) los jabalíes usan las carboneras para revolcarse y desprenderse de los parásitos; como el jabalí es un vector de dispersión de las esporas de la trufa, podría haber introducido inóculo de trufa en estas zonas.

Para estudiar los diferentes procesos que están actuando y entender su efecto sobre *T. melanosporum*, se van a llevar a cabo dos experimentos:
En primer lugar, se estudiarán antiguas carboneras abandonadas, con el objetivo de conocer si el suelo y su comunidad ectomicorrícica siguen alteradas tras más de 50 años. Para ello se estudiará el potencial de inóculo, la actividad microbiana, la cobertura vegetal y la circulación del agua, las propiedades térmicas del suelo, propiedades mecánicas.
En segundo lugar, se realizarán dos carboneras experimentales sobre suelos que nunca habían sido carboneados y se analizará el efecto del calor y de los destilados sobre las ectomicorrizas nativas.

Fig. 3 Foto antiga carbonera

Perspectivas del proyecto

El proyecto Desarrollo Integral de la Truficultura de Teruel tiene una duración limitada a tres años (2007-2010). El cultivo de la trufa negra, sin embargo, se realiza en plazos de tiempo mucho mayores, por lo que es difícil que en tres años se obtenga respuesta a muchas de las cuestiones planteadas en el proyecto de investigación.

En España, el sector de la trufa ha acumulado un importante bagaje de conocimientos empíricos durante 50 años de recolección de trufa silvestre. Además, todas las plantaciones son de propiedad privada. El presente proyecto tiene también el objetivo de establecer lazos de colaboración con estos truficultores y con los gestores de los montes públicos. La comunicación es imprescindible para que la investigación trufícola avance y para que los truficultores se beneficien de los resultados de la investigación científica.

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INOCULATION OF HAZELNUT GROVES WITH *TUBER BRUMALE* & *TUBER MELANOSPORUM* VITTAD.

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Abstract
The hazelnut tree has a great social an economic value in the NE of Spain, nowadays sunk in economic crisis. 15000 Hectares of these groves lay on potential truffle producing areas. *Tuber melanosporum* and *Tuber brumale* fruit in some of them naturally. We studied the ecology and soil parameters on hazel truffieres. No significative differences were found on the fine and coarse oxidable organic materia between *T. melanosporum* and *T. brumale* soils. Our aim is to develop inoculation techniques in mature groves and later to coordinate the truffle and hazelnut cultivation, trying to get an added value to the traditional crop. In this work, large inoculations using the *Mycoforest Technology* have been carried out in mature hazels with *Tuber brumale* on 3230 hazels, and *Tuber melanosporum* on 1300 hazels, in a total area of 11,3 hectares. They were carried out two inoculations with sporal inoculum in all the trial fields, spring-fall within the same year or spring-spring with one year delay. Percentage of trees that got mycorrhizae of *Tuber melanosporum* one year after the first inoculation are between 28,6% and 45,2% of the inoculated hazels. The hazels that present mycorrhizae of *Tuber brumale* are between 24,1% and 56,2% after the first inoculation. In both cases, two years later infection degree raised.
Key words: *Tuber, Corylus avellana,* truffles, field inoculation, *Mycoforest Technology*.

Introduction

Hazelnut production in Spain is nowadays sunk in an economic crisis, because of the prizes and imports especially from Turkey. In Catalonia (North East of Spain) there are 18.537 hazelnut groves (*Coryllus avellana*) (Cens Agrari, 1999), most of them on truffle potential areas. *Tuber melanosporum* and *Tuber brumale* fruits on them naturally.
In 1995 we start the present work with the aim to develop methods to inoculate those nature hazel groves with truffle, in order to get an added value to the traditional hazel crop.
We found that the production and presence of mycorrhizae of *Tuber* and other fungi in these groves are scarce, probably due to the use of pesticides and fertilizers.
The hazel tree has a high number of shallow fine roots, so it has been easy to inoculate and check the mycorrhizae later.
Field inoculation from spores or soil from truffle producing areas is quite old, with some good results 1-2 years later, although is complicated to know if the fruiting comes from the soil plough or from the inoculation. From mycorrhized seedling outplanted on field, the truffle infection can develop and infect new neighbouring plants (Chevalier & Grente, 1978).
Reinoculation directly in the field of *Tuber uncinatum* already mycorrhized plants one year after outplanting, improves Bourgundy truffle production. The inoculation with *Tuber uncinatum* of 14 years old trees that just produce *Tuber brumale*, leads 5 years later to a production of the *Tuber uncinatum* in the reinoculated areas (Chevalier *et al*., 2002; Frochot *et al.*, 2001).
Reyna, with sporal inoculation on mature Holm oaks, got truffle mycorrhizae on 10 of the 17 samples (Reyna *et al*., 2002). Lo Bue studied the inoculation with root fragments on nature trees (Lo Bue *et al*., 1990). On inoculated *Quercus pubescens* with *Tuber melanosporum* and *Tuber aestivum* at the outplanting time, two years later the whole root system had high levels

of mycorrhizae from the inoculated truffles, with few other fungi (Tanfulli *et al.,* 1997).
We started studying the truffle ecology on hazel groves where truffles fruits naturally. *Tuber melanosporum,* fruits on those hazels at higher elevations, where watering is not possible. Those trees are smaller and never reach a full canopy with almost no weeds. Soil has a higher pH and with less organic matter (Fig. 1).

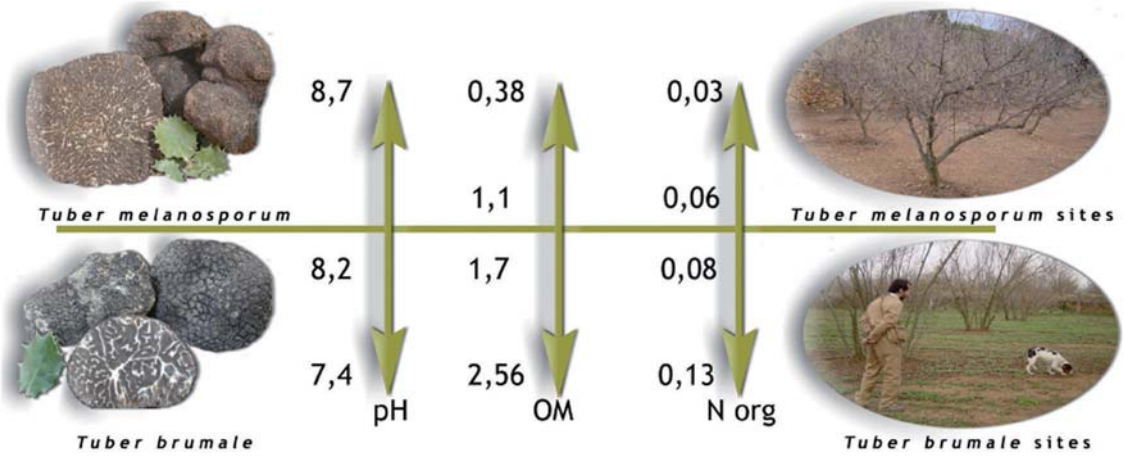

Fig. 1 Soil parameters on natural hazel groves where truffles grow naturally

Some studies carried in France (Ricard, 2003) show differences between oxidable organic materia on *T. melanosporum* and *T. brumale* truffieres, although they come from just four soil samples. Those differences were focused on a higher values of >50µm fraction on *T. brumale* than in *T. melanosporum*.
Our aim was to develop an agronomical way of inoculation, in order to be cheap, easy and fast. Inoculations with tractor gave the results showed in Tab.1 (Morcillo *et al.,* 2007).

Tab. 1 First inoculated fields and first truffle productions 1, 2 and 3 years after the inoculations.

TRIAL FIELDS					
Trial field	Number of trees	Age	Elevation	Orientation	Mycorrhization 2 years later
A	110	35	950	SW	55%
B	40	17	908	S	69 %
C	235	24	995	W	50 %

BLACK TRUFFLE PRODUCTION			
Trial field	First year	Second year	Third year
A	400g (3 hazels)	580g (3 hazels)	150g (2 hazels)
B	30 g (1 hazel)	450g (5 hazels)	310g (6 hazels)
C	0 g*	0 g*	0g*

*Mycorrhization level four years later: 73%

Material and methods
We did soil analysis for fine (<50µm) and coarse (50-200µm) organic materia on *Tuber melanosporum* and *Tuber brumale* soils under hazels in order to compare with the previous results obtained at the french studies (Ricard, 2003).

In this study, following previous results, we have inoculated 1300 adult hazels with *Tuber melanosporum* and 3230 with *Tuber brumale.* We choose five trial sites for *T. melanosporum* and 11 trial sites for *T. brumale* from the Prades Range, located 120 km southwest from Barcelona, Spain (Fig. 2).

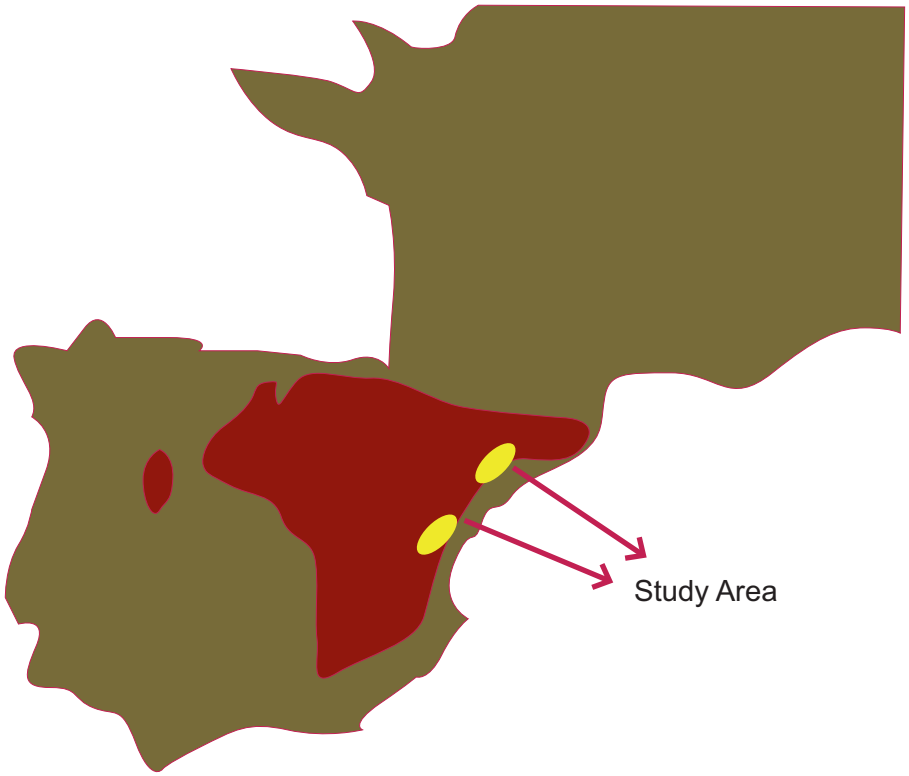

Fig. 2 Study area in NE Spain

Before any inoculation, a root sample was taken from 10% of all the trees to check the initial level of mycorrhizae of each truffle, as a control. Roots were taken in October-November and stored with FAA until their observation at microscope.
Inocula was made from nature truffles, harvested in the Prades Range, at the end of truffle season (February-march). Inocula is mixed with hydrogels, root promoting factors and spore germination promoting factors (following the *Mycoforest Technology* method).
Inoculations were done by tractor, usually at 1-1,5 m from the tree trunk. Two systems were used: a simple one with a 50L bottle over a plough, with a tap at the bottom and two hoses tied at each side of the ploughing machine. As tractor runs forward, inoculum drops by gravity and get buried into the soil. The second machine was a large bottle of 600L with an air pump that allows us to adjust pressure inside the tank, adjusting at the same time the inoculation dose. With this system the time of inoculation is reduced to 1h/Ha/500 trees.
Six months after each inoculation, roots are sampled at a level of 10% of inoculated trees. Root samples are taken at 1-1.5m from the trunk of the tree at both sides of the tree, at the same place where they were inoculated. Roots are stored in FAA and checked at microscope for a qualitative analysis: we just checked presence/absence of the inoculated truffle, according with previous studies (Agerer, 1987-1998; Etayo & De Miguel, 1998; Saez & De Miguel, 1995; Verlhac, 1990).

Results and discussion

Soil analysis results for fine (<50µm) and coarse (50-200µm) organic materia on *Tuber melanosporum* and *Tuber brumale* under hazels showed no significative difference nor on the <50µm neither on 50-200µm between both truffles (Tab. 2).

Tab. 2 Oxidable organic materia for fine (<50µm) and coarse (50-200µm) on *Tuber melanosporum soils* (left) and on *Tuber brumale* soils (right) under hazels. (values on %)

Sample		*Tuber melanosporum*		Sample		*Tuber brumale*	
	< 50µm	50-200µm	ratio		< 50µm	50-200µm	ratio
B	**4,6**	5,59	1,22	L	**3,19**	3,6	1,1
C	**1,58**	1,33	0,84	M	**1,84**	1,27	0,7
D	**2,48**	3,13	1,26	N	**4,04**	3,89	1
E	**1,9**	2,16	1,14	O	**3,36**	3,51	1
F	**6,21**	5,44	0,88	P	**6,72**	8,23	1,2
G	**2,51**	2,6	1,04	Q	**7,63**	7,02	0,9
H	**2,93**	3,47	1,18	R	**1,75**	1,65	0,9
I	**1,42**	1,97	1,39	S	**3,35**	3,46	1
J	**0,83**	0,94	1,13	T	**3,7**	6,02	1,6
K	**4,97**	4,88	0,98	U			
Average	**2,94**	3,15	1,11	Average	**3,95**	4,29	1,1
S.D.	**1,75**	1,67	0,17	S.D.	**2**	2,35	0,3
There are no significative differences between *T. melanosporum* and *T. brumale* neither for <50µm, nor for 50-200µm, nor for the ratio (P<0,05).							

These results need further study as they can be useful for the later management on hazelnut groves infected with truffles. We present the mycorrhizae level six months after the first inoculation (done on september-october 2006). Second inoculation was done on spring 2007 and hazels were sampled again on november 2008 (1,5 years after the second inoculation).
Between 7.1% and 17.6% of the hazel trees have naturally (without any artificial inoculation) mycorrhizas of *Tuber melanosporum* and between 0 and 17.6% have *T. brumale* naturally in the studied area. We have proved that disinfection pre-treatment decrease the level of some mycorrhizal fungi before the inoculations, but they seem to be not necessary as the disinfected trial fields get the same levels of truffle infections as non disinfected fields. Similar results were found by our team in previous tests (Morcillo *et al*., 2003; Frochot *et al*., 1990).
All trial fields had an increase in the level of truffle mycorrhizae after the first and second inoculation, despite there was no rain at all during 4 months after inoculations (tab. 3 & 4).

Tab. 3 Trial Fields inoculated with *Tuber brumale*. There are significative differences between mycorrhization levels before and after the first inoculation (P<0,05), but no differences between first and second inoculation (P<0,05).

Trial Fields	Number Hazels	Age	Elevation (m.o.s.l)	pH	DP[1]	MLBFI[2]	MLAFI[3]	MLASI[4]
B	240	26	810	7,64	YES	17,24	53,8	35,7
C	400	23	580	7,66	YES	12	45,6	42
D	150	29	1005	7,76	NO	11,1	48,3	78
E	150	29	995	7.49	NO	0	42,3	ND*
F	400	27	90	7,15	NO	0	32,7	35,7
G	225	17	700	7.51	NO	10	50	35,3
H	450	18	890	7.43	NO	10	42	40
I	175	15	595	7,18	NO	17,6	24,1	38,9
J	275	24	750	7.78	NO	12,9	40	28,6
K	400	20	620	7.78	NO	13,8	56,2	50

[1] DP: Disinfection pretreatment - [2] MLBFI: % Mycorrhizae level before first inoculation - [3] MLAFI: % Mycorrhizae level after first inoculation - [4] MLASI: % Mycorrhizae level after second inoculation - * ND: No data

Tab. 4 Trial fields inoculated with *Tuber melanosporum*. There are significative differences between mycorrhization levels before and after the first inoculation (P<0,05), but no differences between first and second inoculation (P<0,05).

Trial Fields	Number hazels	Age	Elevation (m.o.s.l.)	pH	DP[1]	MLBFI[2]	MLAFI[3]	MLASI[4]
L	50	26	810	7,64	YES	17,24	42,8	35,7
M	150	18	890	7,43	NO	10	ND*	33
N	550	6	700	7,87	NO	7,1	45,2	44,8
O	75	15	595	7,18	NO	17,6	28,6	38,9
P	455	24	750	7,78	NO	ND*	40	69,4

[1] DP: Disinfection pretreatment - [2] MLBFI: % Mycorrhizae level before first inoculation - [3] MLAFI: % Mycorrhizae level after first inoculation - [4] MLASI: % Mycorrhizae level after second inoculation - [*] ND: No data

Percentage of trees that got mycorrhizae of *Tuber melanosporum* one year after the first inoculation are between 28,6% and 45,2% of the inoculated hazels, and between 33% and 69,4% after the second inoculation. The hazels that present mycorrhizae of *Tuber brumale* are between 24,1% and 56,2% after the first inoculation, and between 28,6% and 78% after the second inoculation.

Traditionally hazel groves have been planted at densities 5x5 m, 6x4 m or 6x6 m, the same we use for black truffle culture. Some of these hazel groves produce truffles naturally despite the higher levels of fertilizer (N:P:K 13:13:15) at 125 Kg/Ha, nitrofosca and several phytosanitary treatments against hazelnut plagues. Hazelnut harvest is made with heavy machines during September-October, fallen leaves are swallowed and powdered back to soil. Usually leaves and branches are burnt and the ashes are spread. Ashes can raise pH and all decreases fresh organic matter levels, that ould improve truffle fruiting. More studies are being carried out with the aim to coordinate hazel and truffle production.

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IMPACT OF THE CULTURAL PRATICES THE TREE FIRST YEARS OF THE PLANTATION ON THE TRUFFLE-PRODUCING POTENTIAL

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Abstract
Setting up truffle plantations needs to take account of different factors to be sure of satisfactory results when they enter into the production phase. The parameters generally taken into consideration are the environment, previous cultivation, the orientation, the topography and the nature of the soil. Using plantations aided by grants from local authorities in Midi-Pyrenees, this study aims to determine the effect on the truffle-producing potential of data recorded before planting and of cultivation methods.
We chose to study a certain number of plantations in the department of the Lot on the basis of environmental and farming factors. The results concern the development of the trees, the formation of burnt areas, the mycorrhizal status and the general state of cultivation. The mycorrhizal status was checked on a sample of trees.
The initial results of this study show that working the soil under young trees planted on former uncultivated land gives the best presages of future production. In the best situations burnt zones are seen from the third year onwards under ever green and downy oaks. The mycorrhizal status (with excellent indices) of these trees is dominated by *Tuber melanosporum*.
Key words: *Tuber melanosporum* Vittad., soil, burnt areas, mycorrhizal status.

En trufficulture, il existe plusieurs façons de réaliser une plantation et de l'entretenir les premières années avant son entrée en production. Certains privilégient une bonne préparation du terrain au préalable et un entretien soigné, au moins jusqu'à l'apparition des brûlés comme le préconisaient De Bosredon et le Docteur Pradel. D'autres préfèrent maintenir le milieu naturel de la pelouse calcicole, intervenant le moins possible, en particulier dans les environnements boisés marqués par une forte pression de contaminations mycorhiziennes (*Tuber brumale*). Dans la conception de la plantation truffière, le précédent cultural est un autre facteur qui semble influer fortement sur son évolution.
Nous avons cherché à évaluer l'impact de ces différents paramètres sur le potentiel truffier.

Objectif de cette étude

Il s'agit de déterminer quels types de préparation et d'entretien les premières années de la plantation truffière semblent les plus adaptés pour assurer une production future. Nous essayerons également de mettre en évidence les précédents culturaux qui semblent être les plus favorables.

Méthodologie

Pour mener cette étude, une enquête de terrain s'est avérée nécessaire. Elle a porté sur 27 plantations en âge de présenter des brûlés, réalisées en 2002, 2003 ou 2004 en partenariat avec la Station trufficole du MONTAT et avec l'aide de l'Europe (FEOGA).
Ces jeunes truffières se situent dans les départements du LOT et de l'AVEYRON et ont chacune une surface comprise entre 20 et 50 ares. Elles ont été implantées sur des calcaires du Jurassique (Kimméridgien, Bathonien, …) de l'Oligocène (calcaires lacustres) ou des éboulis du Quaternaire prédisposés à la production de truffes. L'existence de truffières spontanées à proximité immédiate atteste de la valeur du milieu trufficole. On rencontre sur ces plantations deux types de sols reconnus comme truffiers:

- des sols peu profonds (moins de 40 cm de terre), calcaires ou au moins calciques, caillouteux, à structure plutôt grumeleuse appelés rendosols ou rendisols,

- des sols plus profonds (plus de 50 cm de terre), caillouteux, appelés colluviosols. On retrouve ce type de sol le plus souvent dans les combes sèches ou au pied des versants.

Chaque truffière est constituée de 50 plants mycorhizés par *Tuber melanosporum* et 10 plants pièges non mycorhizés permettant d'identifier plus tard par prélèvement de racines la concurrence fongique présente naturellement sur le terrain et d'en évaluer son agressivité.
Les essences plantées sont le chêne pubescent, le chêne vert et le noisetier.
Toutes ces jeunes plantations ont été réalisées avec des plants issus d'une même pépinière. Leur provenance ne pourra donc expliquer les différences de résultats.

Méthode

Pour chaque jeune plantation, les éléments suivants ont été relevés:

- précédent cultural
- préparation avant plantation
- type d'entretien après plantation
- nombre de brûlés formés.

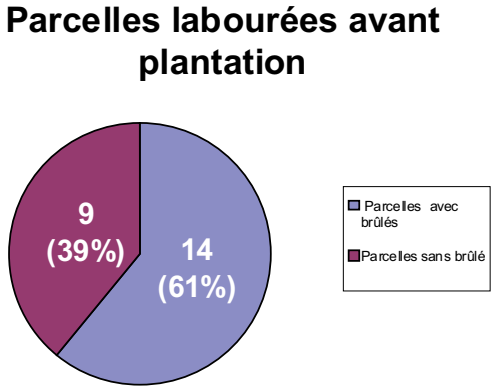
La formation des brûlés annonce souvent une entrée en production imminente de l'arbre. Le nombre de brûlés formés est donc certainement la variable quantifiable exprimant le mieux le potentiel d'une truffière. Nous avons donc utilisé cette donnée pour évaluer la situation de chaque plantation visitée pour l'enquête.

Résultats de l'enquête

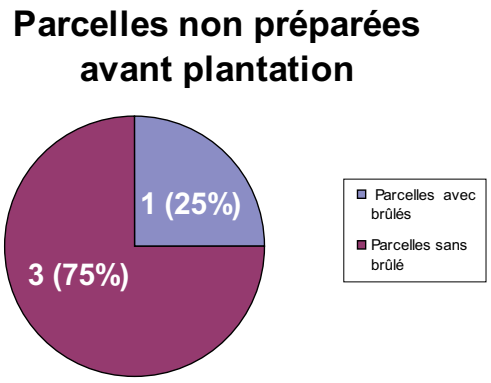
1) <u>Influence de la préparation du terrain avant plantation</u>

Au cours de la visite des 27 jeunes truffières, 2 types de préparation avant plantation ont pu être distingués:

- 23 parcelles ont été totalement labourées ou travaillées en profondeur
- Les 4 autres parcelles n'ont subi aucune préparation préalable.

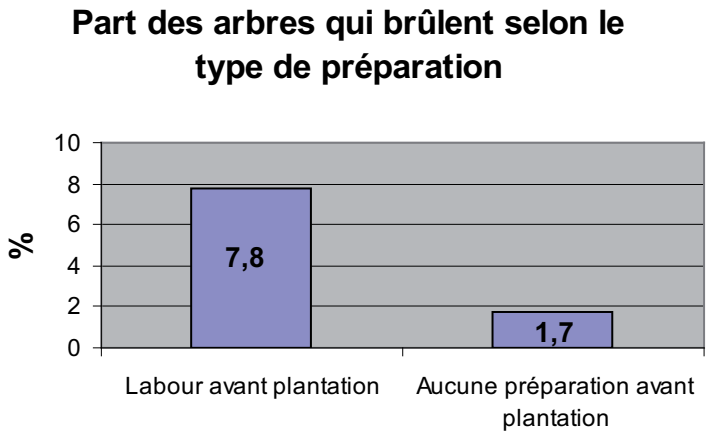


Graph. 1



Graph. 2

Plus de 60% des parcelles labourées avant plantation présentent un ou plusieurs brûlés (graph. 1). Par contre, parmi les jeunes truffières n'ayant bénéficié d'aucune préparation préalable (travail du sol), seulement une d'entre-elles en possède (graph. 2).



Grap. 3

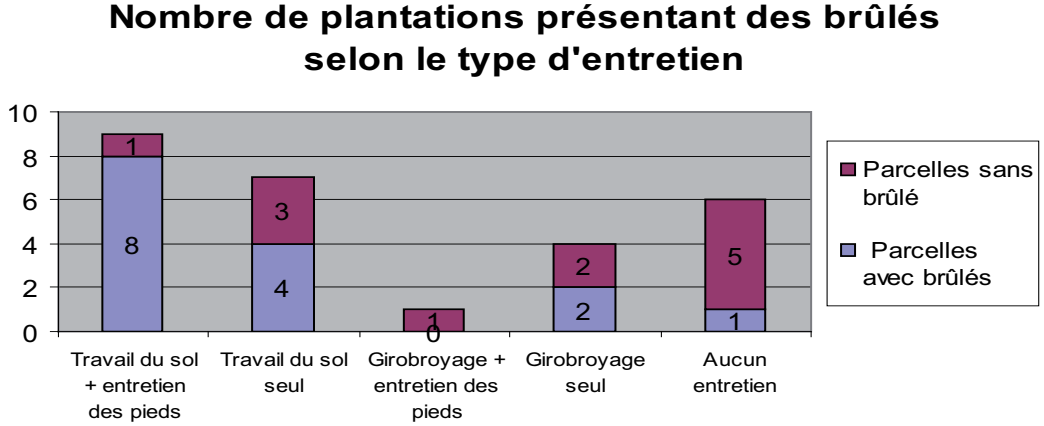
Quantitativement, on observe en moyenne presque 5 fois plus de brûlés dans les jeunes plantations ayant bénéficié d'un travail du sol que dans celles où la pelouse a été préservée au moment de leur création (graph. 3).
Un travail du sol avant plantation semble donc promettre un meilleur avenir aux jeunes truffières ou du moins permet aux brûlés de s'installer plus précocement, laissant présager ainsi une entrée en production plus rapide.

2) <u>Influence de l'entretien des premières années après plantation</u>

Au cours de l'enquête, 5 différents types d'entretien appliqués dans les jeunes truffières ont été recensés. Nous n'en retiendrons que 4, le cinquième étant trop peu pratiqué pour être représentatif et permettre d'émettre des conclusions significatives à son égard.

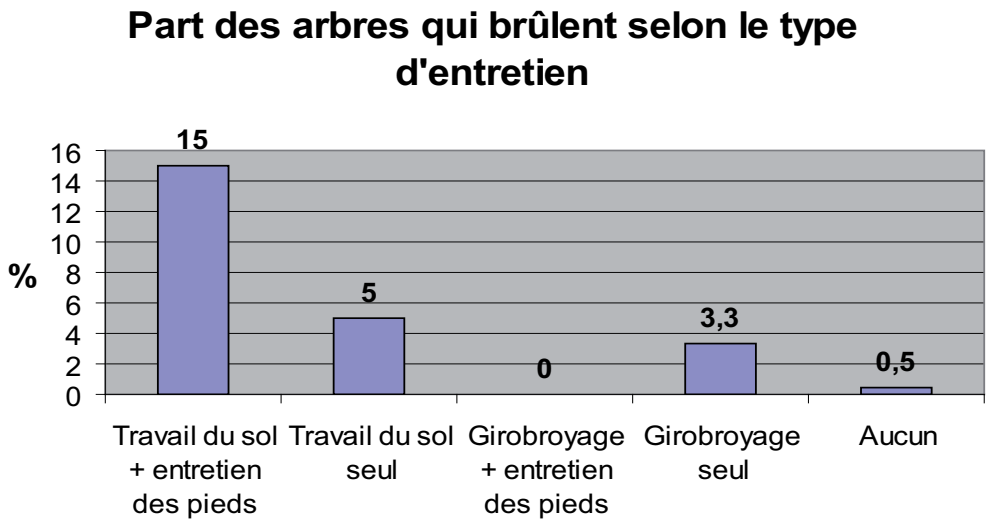
- 9 parcelles bénéficient d'un travail du sol en surface entre les rangs, à l'aide d'un outil à dents tracté tel qu'un cultivateur ou vibroculteur. A cet entretien mécanique s'ajoute pour ces 9 parcelles un sarclage ou désherbage manuel autour de chaque arbre de la plantation pour maintenir cette zone propre.
- Dans 7 parcelles, seuls les inter-rangs sont travaillés.
- Quatre trufficulteurs se contentent de girobroyer ou tondre la végétation entre les rangées, laissant s'installer la pelouse (écosystème de truffière naturelle) à proximité immédiate des jeunes plants.
- Enfin, 6 plantations n'ont bénéficié d'aucune forme d'entretien.

Le nombre et la fréquence des interventions varient selon les truffières. Chaque trufficulteur passe à l'action dès lors que c'est nécessaire afin d'éviter un embroussaillement ou enherbement trop envahissant. Certaines parcelles ne demandent qu'une intervention par an car leur sol est peu fertile et l'herbe pousse peu, d'autres en nécessitent 3 ou 4.



Grap. 4

Un travail du sol entre les rangs associé à un sarclage ou désherbage manuel du pied des arbres apparaît comme le type d'entretien le mieux adapté pour permettre au potentiel truffier de la plantation de se développer pleinement. En effet, 8 jeunes truffières conduites de cette manière sur 9 présentent des brûlés. Seulement 50 à 60% des parcelles entretenues autrement ont également ces signes avant-coureurs prometteurs (graph. 4).
La main de l'homme paraît donc bénéfique, sinon indispensable pour que le «système truffier» s'installe correctement et rapidement avant de s'exprimer. L'absence d'entretien confirme cette hypothèse puisqu'on ne retrouve des brûlés que dans une seule des 6 plantations livrées à elles même. Cette exception s'expliquerait par la pauvreté du sol qui permet à la végétation herbacée de ne pousser que de manière très clairsemée. Ainsi, même en l'absence d'entretien, l'installation de brûlés ne semble pas être contrariée lorsque le couvert végétal de la parcelle est peu dense.



Grap. 5

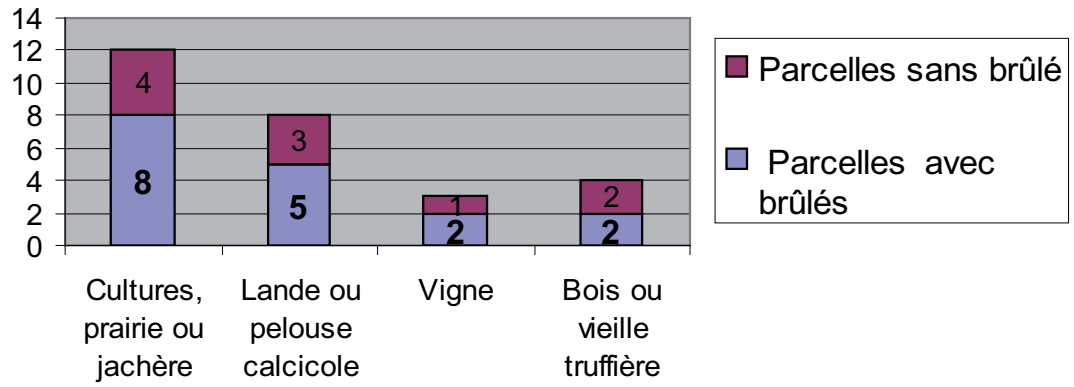
En nombre de brûlés, le constat est encore plus flagrant. Plus d'un arbre sur 7 brûle lorsque sont à la fois travaillés les inter-rangs et le pied des jeunes plants. L'avenir des plantations entretenues différemment semble nettement moins prometteur: on observe des signes de bon présage sous 0,5 à 5% des arbres seulement suivant le type de conduite culturale (graph. 5).

3) <u>Influence du précédent cultural</u>

Quatre catégories de précédents culturaux sont représentées dans cette enquête:

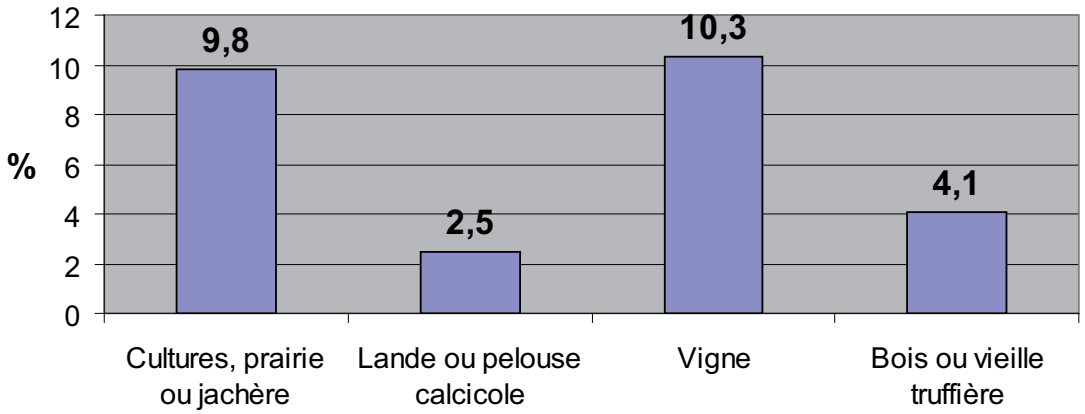
- cultures de causse (non intensives), prairie artificielle ou jachère (12 parcelles concernées)
- lande à genévriers ou pelouse calcicole (8 parcelles concernées)
- vigne (3 parcelles concernées)
- bois ou ancienne truffière abandonnée (4 parcelles concernées).

Nombre de plantations présentant des brûlés selon le précédent cultural



Grap. 6

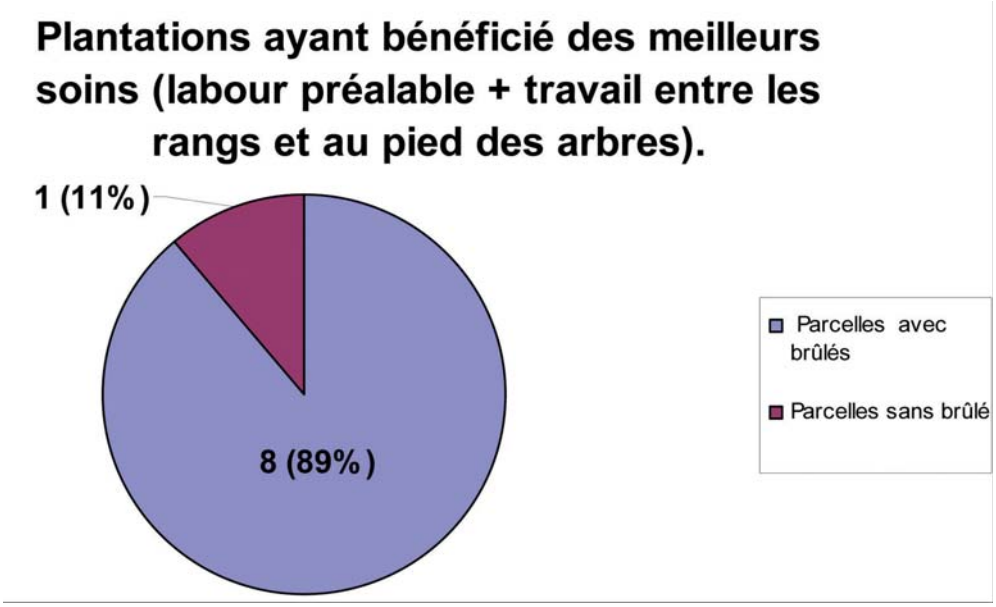
Part des arbres qui brûlent selon le précédent cultural



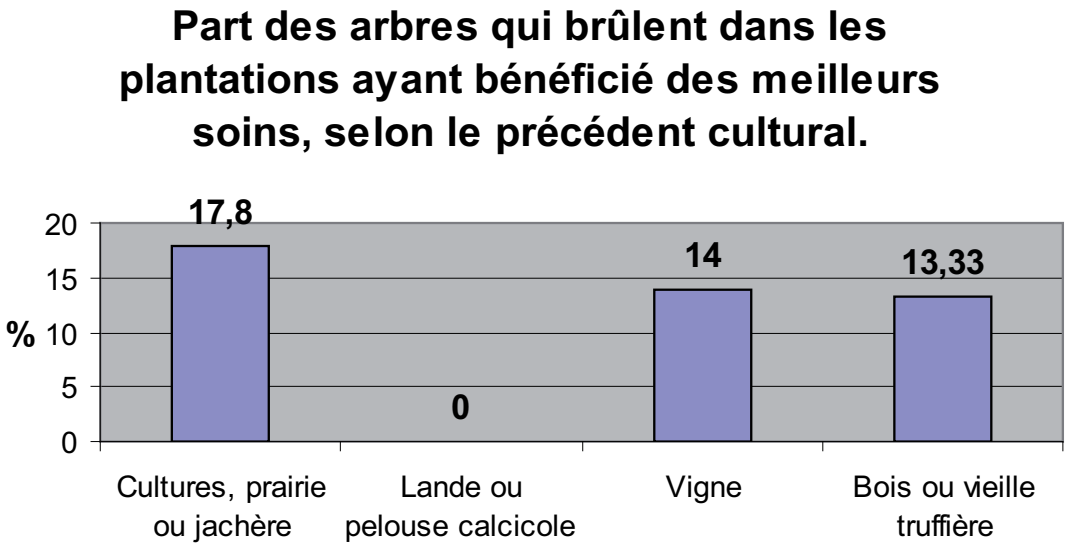
Grap. 7

Quel que soit le précédent cultural, la moitié ou les deux tiers des jeunes truffières présentent des brûlés (graph. 6). Par contre, quantitativement, on observe en moyenne beaucoup plus de brûlés dans les plantations réalisées après des cultures, prairies, jachères ou une vigne (graph. 7). Il apparaît donc que les brûlés s'installent plus facilement et plus rapidement lorsque la parcelle était déjà travaillée avant d'être plantée en chênes truffiers. Si le terrain a connu une longue période de repos sans aucune intervention de l'homme, comme c'est le cas surtout dans les landes ou pelouses calcicoles, et n'est pas remis en culture, le «système truffier» semble rencontrer des difficultés à s'installer et à se révéler par le biais des brûlés. Ce phénomène se confirme en milieu naturel quand on voit le temps qu'il faut à une truffière pour se former spontanément.

Parmi les 9 jeunes plantations ayant bénéficié de pratiques culturales soutenues les premières années, le plus convaincant mis en avant précédemment dans l'enquête (labour avant plantation + travail du sol entre les rangs et au pied de chaque arbre les premières années), seule la jeune truffière réalisée après une lande ne compte aucun brûlé (graph. 8 et 9). Toutefois, trop peu de parcelles parmi l'échantillon étudié présentent à la fois ce type de précédent et cet entretien. Aucune conclusion les concernant ne peut donc être avancée.



Grap. 8



Grap. 9

Quantitativement, on remarque que des 8 parcelles possédant des signes avant coureurs, ce sont celles réalisées après une culture, prairie ou jachère les mieux pourvues (graph. 9).
On s'aperçoit également grâce à cette étude que le bois n'est pas forcément un mauvais précédent, à condition que la parcelle fasse l'objet d'une bonne préparation et que la truffière soit entretenue soigneusement les premières années.

Conclusion

Au travers de cette enquête, il apparaît clairement que le passé cultural et le bon entretien des trois premières années conditionnent directement la réussite d'une jeune plantation comme il est souligné dans le Guide pratique et le Principe de précaution en trufficulture.
Un labour de la parcelle avant plantation, complété les premières années par un travail du sol entre les rangs et un sarclage du pied de chaque arbre, semble être le début d'itinéraire technique avant production le plus approprié. Cette conception devrait être privilégiée pour assurer une production future à la truffière.
Les arbres qui brûlent font tous partis, sans exception, des plus développés de la plantation. Il est donc nécessaire, voire primordial d'assurer la bonne reprise et l'installation des jeunes

plants pour leur donner plus de chances de production et leur permettre de débuter celle-ci précocement.
En appliquant cette méthode et en arrosant en cas de sécheresse, les conditions optimales de développement pour l'arbre sont réunies. Le labour avant plantation va décompacter le sol et permettre aux plants de s'enraciner plus facilement. Le travail du sol entre les rangs les premières années va ensuite limiter l'enherbement et ainsi empêcher l'installation d'un tapis végétal trop dense pouvant contrarier la formation des brûlés. Enfin, le sarclage ou désherbage manuel au pied des arbres va éliminer toute forme de concurrence herbacée et nutritionnelle. Si l'herbe s'installait petit à petit, on peut craindre la formation d'une couche racinaire très épaisse dans les 10 premiers centimètres, diminuant d'autant la ressource hydrique pour le jeune arbre truffier et limitant probablement la propagation de son système racinaire.
Certes, en milieu naturel, des truffières se forment spontanément sans l'intervention de l'homme à un certain stade (pelouse, lande à genévriers) de l'évolution vers le climax. Mais le contexte est différent lorsque les truffières naturelles sont pérennes. Les brûlés s'installent là où l'enherbement est clairsemé dans des milieux qui évoluent très lentement, d'autant que les sols sont superficiels. En plantation, l'évolution est plus rapide en raison des soins apportés par le trufficulteur.
Avec une culture, jachère ou prairie artificielle en tant que précédent cultural, les perspectives d'avenir semblent meilleures pour une truffière. La plantation après une vigne, un bois ou une lande est aussi une solution appropriée. En réalisant une jeune plantation sur une parcelle travaillée, les arbres se développeront plus aisément et la production devrait se déclencher plus précocement, à condition qu'un entretien soigné soit appliqué.
Plus que le précédent cultural, une préparation avant plantation et des pratiques culturales soutenues les trois premières années semblent prépondérantes pour l'avenir de la plantation truffière. A ce jour, 2 des 27 plantations visitées sont entrées en production au cours de leur 5ème année. Toutes les deux ont bénéficié d'un début d'itinéraire technique prometteur comme le souligne l'étude.
On ne saurait toutefois préjuger des contaminations mycorhiziennes pouvant intervenir, notamment par le *Tuber brumale* qui apparaît être la truffe la plus compétitrice à l'égard du *Tuber melanosporum* dans le département du Lot. Durant l'enquête, des échantillons racinaires ont été prélevés dans certains brûlés et ont tous révélé la présence exclusive du *Tuber melanosporum*. L'absence de mycorhizes du *Tuber brumale* sous ces arbres est de bonne augure bien que cette truffe puisse s'installer *a posteriori* si la pression de contamination par l'environnement est forte et le travail mécanique linéaire (cultivateur) est régulier. Le contrôle des plants piège dans une étape ultérieure devrait apporter des informations complémentaires sur le pouvoir contamination du milieu.

Remerciements
Les travaux d'étude et d'expérimentation en trufficulture à l'origine de cette communication ont été réalisés en Midi-Pyrénées au cours des 11e et 12e Contrats de Plan Etat-Région avec les concours notamment de l'Inra, du Ctifl, du Ministère de l'Agriculture et de la Pêche, de Viniflhor, de la Région Midi-Pyrénées, de l'Europe, du Conseil général du Lot, sous l'égide de la Fédération régionale des trufficulteurs de Midi-Pyrénées et de la Fédération Française des Trufficulteurs. Tout le personnel de la Station trufficole a été impliqué dans les travaux qui ont permis de bâtir le contexte de l'étude ainsi que dans les discussions qui ont enrichi la réflexion a priori et a posteriori.

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TRUFFLE CULTIVATION IN SPAIN

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Abstract
In Spain truffle cultivation takes on a special value because it fits fully within the concept of sustainable development due to the ecological conditions in which it is produced and the economic and social environment that profits from its production. In Spain truffles have two clearly differentiated provenances: they are either produced naturally in spontaneous truffières or they are produced in the numerous truffle plantations established during the last 25 years with mychorrized plants, many of which have already entered in production.
The fundamental change in Spanish trufficulture is considered to have occurred in the decade of the 1970s, when an alarming descent in natural truffle production was first observed. This coincided with increasing rural depopulation.
At present, it is estimated that there are more than 4000 ha of truffle plantations in Spain. The current annual rate of planting is estimated between 250 and 500 ha a year. The most active regions are located in the east (Teruel, Castelló) and the northeast (Soria, Huesca). The most planted tree species is evergreen holm oak, although hazel, downy oak and Portuguese oak (*Quercus faginea* Lamk.), Especially notable is the 600 ha Arotz plantation in Soria, which produces 2500 kg of truffles a year.
In this paper we describe the typical truffle plantation in Spain: ecology, cultivation techniques, pruning, irrigation systems, nurseries of mycorrhized plants etc,
The extension and public promotion of trufficulture in Spain is widely supported from the technical and scientific fields with the collaboration of the various truffle-collectors associations.
This paper includes also information on production, prices, truffle distribution, markets and trade, plantations, research activities and publications. Finally we do a diagnosis of future perspectives for the truffle sector in Spain.
Key words: Cultivation, *Tuber melanosporum*, ecology, sustainable development.

Introduction

In spite of its current status as one of the three largest truffle-producing countries in the world, Spain has only recently incorporated the truffle into its popular gastronomy. In fact, it was not until the 1950s that the systematic collection of Spanish natural truffles began. Nevertheless, much earlier references to the truffle can be found in Spanish literature; the botanist and physician Andrés Laguna (Segovia, 1510-1559) wrote a genuine diatribe on the properties of the truffle "... *They cause a heaviness of the stomach that is reflected in the humours; they breed stones; they engender palsy, apoplexy, side pains and infinite health problems."*

This association between truffle consumption and a wide spectrum of health disasters (from strokes to kidney stones) may have contributed to the fact that Spain never developed a gastronomic culture with respect to this valuable fungus.
In 1882, Chatin reported the export from Spain to France of 877 k of black truffle (Nicolas, 1971), probably from the Catalonian Pyrenees.
Also around this time (end of the 19th century and beginning of the 20th century), the politician Joaquín Costa (1912) stands out as one of the most important advocates of forestry activities and hydraulic policies. He wrote numerous publications in which he passionately and determinedly proposed steps he thought would contribute to the progress of Spain, and, in so doing, he helped to make the public aware of the importance of forests. In his book El Arbolado y la Patria (The Forest and the Homeland) he wrote the following in reference to the truffle:
"Since 1860, the truffle oak has been propagated at a large scale in several French

departments. Ten years after planting, these trees generate a net profit of 500-2000 reales per hectare in truffles. One municipality (Bédouin) has already planted truffle oaks on around 3000 hectares of Mt. Ventoux, which offers detestable conditions for any vegetation.....With such a simple combination, the inconveniences we traditionally found in cultivating the holm oak, disappear."

Not long after Costa's writings, Bellpuig published Truffles, Mushrooms, Asparagus and Strawberries (Bellpuig, 1900), which correctly reflected all the up-to-date knowledge collected on the subject, mainly from France.
Up to 1990, initiatives and publications in the field of Spanish trufficulture were very limited. We can cite the works by Fité (1962), Nicolas (1971), Recio (1972), Abreu (1975), Reyna (1982, 1992), Aguilar (1982), Oria (1989), Rodriguez Barreal *et al*., (1989) and Martinez y Grijelmo (1991). Subsequent to these dates, considerable advances have been made in the field and there is a growing research dynamism.

In Spain trufficulture takes on a special value because it fits fully within the concept of sustainable development due to the ecological conditions in which it is produced and the economic and social environment that profits from its production.

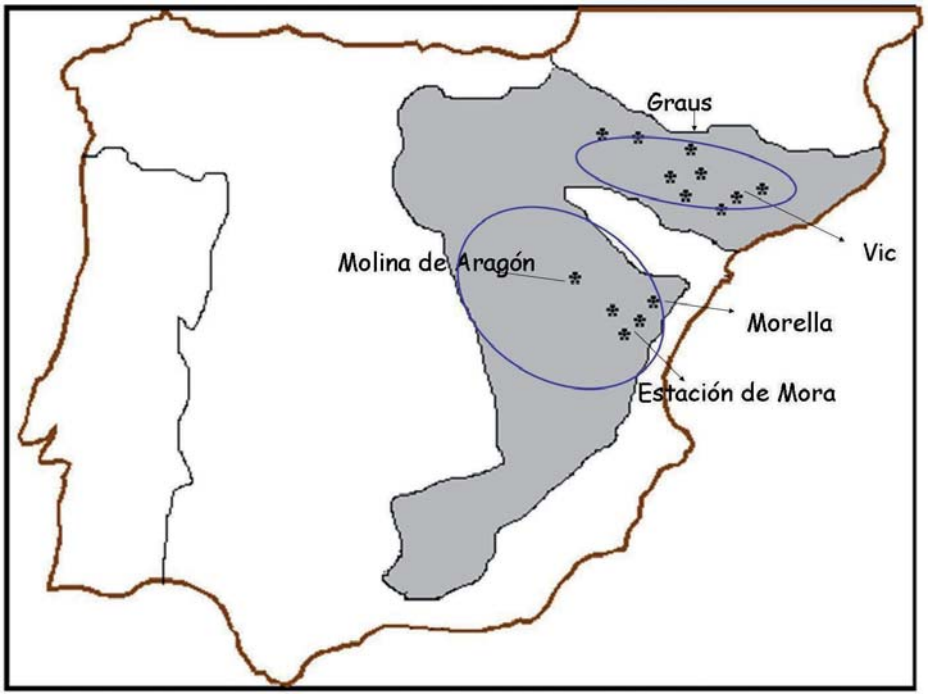
Distribution, Production and Market prices
In Spain truffles have two clearly differentiated provenances: they are either produced naturally in spontaneous truffières or they are produced in the numerous truffle plantations established during the last 25 years with mychorrized plants, many of which have already entered in production. Making a correct evaluation of the total truffle production is frankly difficult because of the lack of market transparency and the obscurantism that generally surrounds the sector.
Tab. 1 shows the mean 16-year truffle production of each Autonomous Community, according to official statistics from the Ministry of Agriculture (Mapa, 1986 - 2002).

Tab. 1 Distribution of production by Regions. Sixteen -year mean. Source: MAPA (1986-2002)

Region	Kg	%
Aragon	3696	14.5
Cataluña	9225	36.1
Castilla-Leon	623	2.4
Castilla-La Mancha	4929	19.3
C:Valenciana	6925	27.1
Andalucia	150	0.6
Rioja	382	1.5
TOTAL	25532	100.0

Truffles can be found in the eastern half of the Iberian Peninsula, where soils are mainly calcareous, as reflected in Fig. 1. with the principal markets and productions areas. In the northern areas (piedemont of the Pyrenean mountains, Catalonia etc) the production has a wild origin, nevertheless in Teruel – Castellon (Iberian Mountain) the plantations have a role more important. We estimate on the Mora market (Teruel) 30-40% of the production comes from plantations and in Vic (Barcelona) less than 5% comes from plantations.

Fig. 1 Truffle distribution areas in Spain. Points indicate the location of the main markets and blue circle indicate the principal production area.



Tab. 2 shows European truffle production in the last 20 years, according to the data provided by the European Tuber Group (GET) and our own data. The maximum production, estimated at 126 Tm, occurred during the decade of 1970-1980 (Reyna, 2007).

Tab. 2 Annual production of black truffle in Tm. Sources: French Trufficulture Federation, European Tuber Group and our own data*

Year	Spain	France	Italy	Total Europe
90/91	30	17	5	52
91/92	10	20	5	35
92/93	23	31	3	57
93/94	9	22	2	33
94/95	4	12	30	46
95/96	20	19	25	64
96/97	25	50	20	95
97/98	80	30	24	134
98/99	7	14	4	25
99/00	35	40	10	85
00/01	6	35	4	45
01/02	20	15	5	40
02/03*	40			
04/05*	22			
05/06*	14			
06/07*	12			
07/08*	15			
AVERAGE	21.8	25.4	11.4	59.3

Fig. 2 shows the evolution of Spanish production of truffle in the last 50 year with a general decreasing trend. In spite of this in the last 15 years (fig. 3) production has a little increasing trend. Specially it can appreciates a stabilization in the last 5 years due to the production of new plantations in Teruel and Castellón areas (eastern Spain).

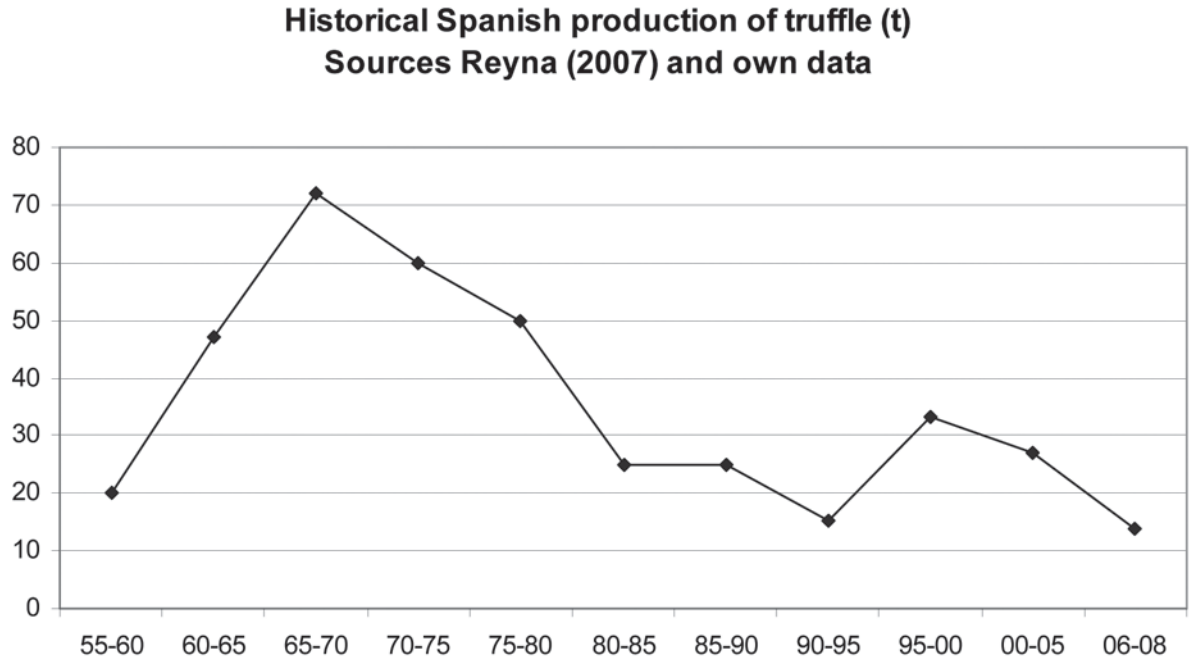


Fig. 2 shows the trend of the Spanish production is decreasing in last 50 years

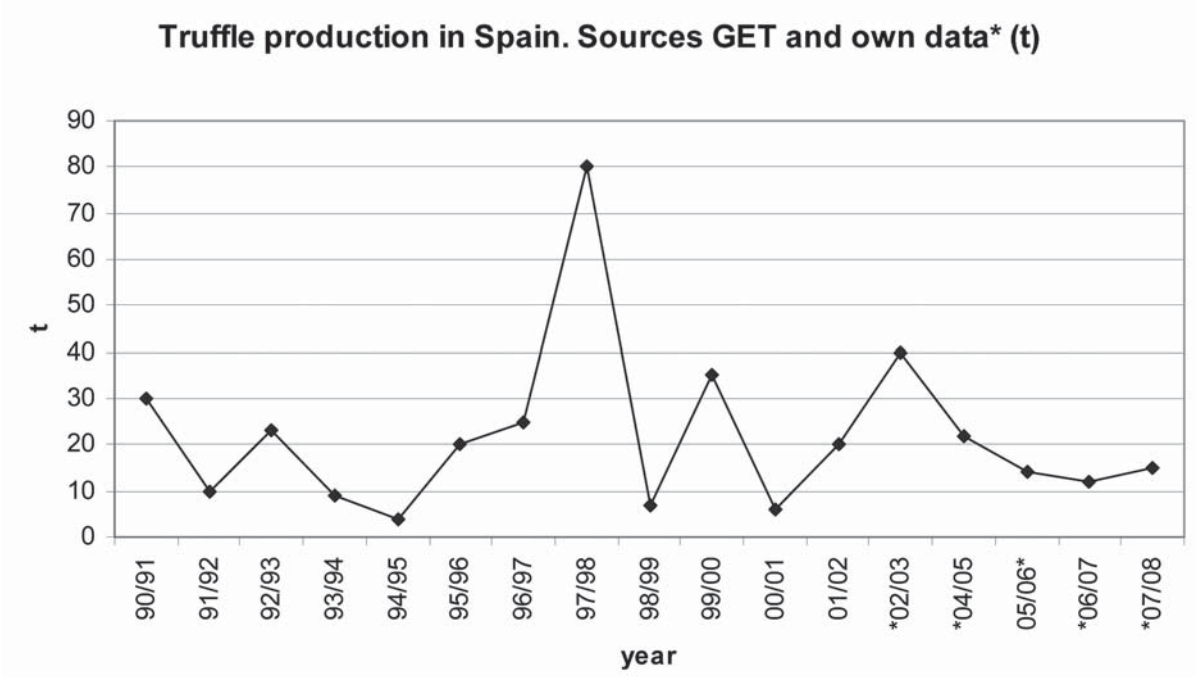


Fig. 3 In the last 5 years due to the production of many new plantations a trend to stabilization is apreciated

Fig. 4 shows the relatively constant increase in truffle prices (in Euros) from 1955 to 2006, with some logical interannual variations. In fact, if we compare the five-year truffle prices from 1985 to the present, the annual price increase in constant Euros of 2005 is 4.4%.

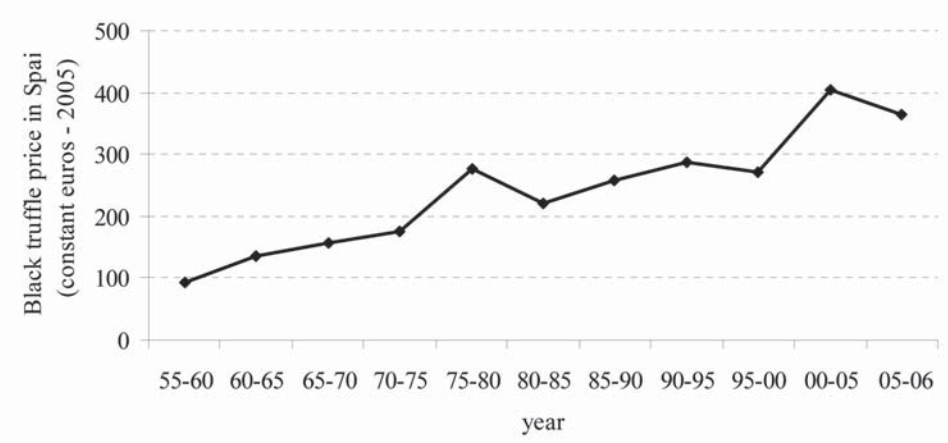


Fig. 4 Evolution of Spanish truffle prices in constant euros

Truffle prices are consistently lower in Spain than in France (Fig.5) around a 38% cheaper (Reyna, 2007).

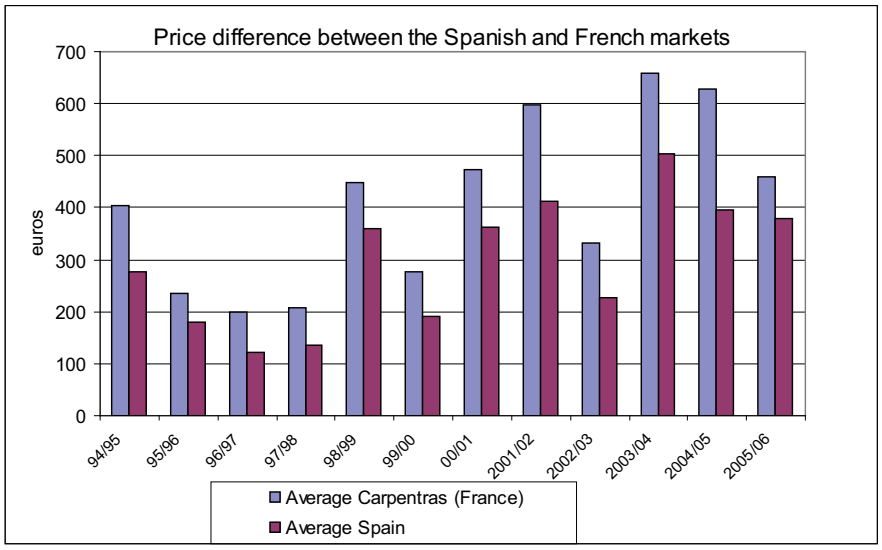


Fig. 5 Price difference between Spanish and French truffle markets in Euros (1955 2005)

Mycorrhized plant production. Control and certification

The production of quality mycorrhized plants is one of the pillars of contemporary truffle cultivation practices. In Spain numerous specialised nurseries (Fig. 6) offer quality plants that are not only well-mycorrhized but also well-adapted to the ecological conditions in the different truffle areas. The Spanish market is supplied almost exclusively from the symbiotic pair *Quercus ilex* L./*Tuber melanosporum* Vittad., but other less-frequent combinations are also present in which the Lusitanian oak (*Quercus faginea* Lam.), the hazelnut (*Corylus avellana* L.), the evergreen oak (*Quercus coccifera* L.) and combine (almost always) with *T. melanosporum.*

Mycorrhized plants did not appear on Spanish truffle markets until the end of the 1980s (Rodriguez Barreal *et al.,* 1989); they were packed in plastic bags, with sterilized soil, and either the seed or the seedling were inoculated by means of a sporal suspension. Production methods related to both the plant and the mycorrhization itself have been constantly improved and adapted to quality requirements; nevertheless, these techniques continue to be protected by industrial patents, constituting one of the main values of the companies dedicated to these pursuits (Palazón y Barriuso, 2007).

At present, there are no official directives regulating either the production in Spanish nurseries

of plants mycorrhized with black truffle or the certification of their quality and purity. At several meetings coordinated by the National Institute of Agrarian and Alimentary Technology (INIA), different methodologies have been proposed for evaluating and controlling *Quercus* plants (especially *Quercus ilex*) mycorrhized with *T. melanosporum* (Fischer y Colinas, 1996, 1997; Palazon *et al*., 2001b, 2007; Reyna, 1997; Reyna *et al*., 2000, 2001). These proposals coincided in the need to establish controls over production factors such as seeds, substrates, water for irrigation, containers, inoculum (Palazon *et al*., 2001a; Reyna *et al.,* 2000), and they differed mainly on the types of sampling, the minimum mycorrhiza thresholds admissible, and the possible contaminants. This situation is not exclusive to Spain; it has not been possible to establish one preferred criterion regarding truffle production in Italy or France either. At the 2002 meeting of the European Tuber Group (GET) in Graus (Spain), members were encouraged to prepare a European-scale truffle certification protocol, the GET protocol, to serve as reference for European truffle controllers.

At any rate, the present situation in Spain is complicated by the existence of Autonomous or Regional Governments, each of which has the authority to establish the standards for plant quality and certification within its territory or delegate this mission to local institutions or entities (universities, foundations, etc.).

One would hope and expect that, with time, the different norms will be unified and the Spanish Central Government will establish some obligatory controls to guarantee the absence of invasive mycorrhizae within our ecological environment, paying special attention to Asian *Tuber* species like *T. indicum* and *T. himalayensis*.

Plantations

The fundamental change in Spanish truffle cultivation is considered to have occurred in the decade of the 1970s, when an alarming descent in natural truffle production was first observed. This coincided with increasing rural depopulation, decreased grazing, abandonment of firewood exploitation and the conversion of non-mechanizable croplands to shrub and forestlands, resulting in the large increase in wildwood density which not only facilitated the initiation and propagation of forest fires but also impeded the soil aeration and insolation that are so important for truffle production. In addition to all this, reforestation activities were carried out using conifers in areas that pertained to evergreen oaks, and wild fauna data indicate that there was also a spectacular increase in the number of wild boars.

A similar situation with similar results had already occurred in France; the 1950 Tm of natural truffles harvested in 1889 has been drastically reduced to a present harvest of 20-50 Tm. In Spain the current mean annual truffle production is around 22 Tm, which is far lower than the theoretical potential of Spanish woodlands.

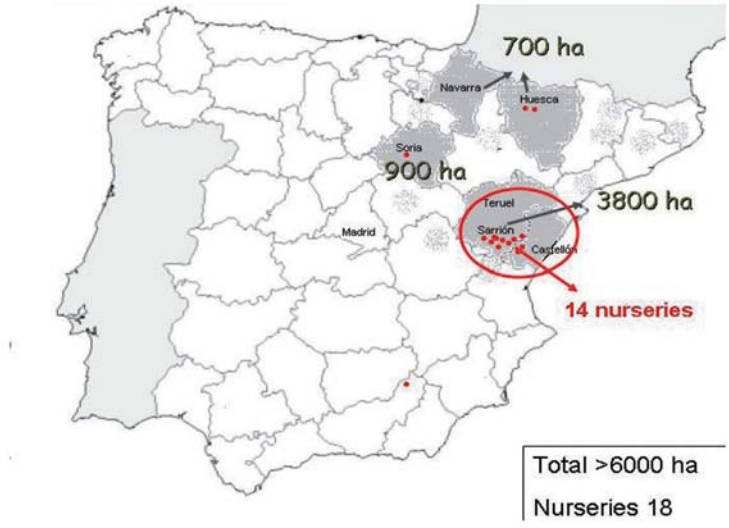


Fig. 6 Truffle plantation and nurseries in Spain

As a consequence of the above and in an attempt to mitigate to a certain extent the worldwide scarcity of the truffle and prevent its possible disappearance, plantations of species that had been mycorrhized with black truffles were initiated. These plantations, which began in France and Italy almost 40 years ago and have shown a certain continuity in Spain for 15 years, represent a milestone within the truffle field as they are the starting point of what we now know as modern truffle cultivation.

The oldest known truffle plantation in Spain dates to 1968 and is Mr Lizandra property located in Castellón (Fig. 7). Although the landowner performed no previous inoculations and simply planted acorns from a truffle-producing evergreen oak stand and in subsequent years buried from 50-100 g of truffles around each plant, his plantation of 139 evergreen oaks has 96 truffle-producing trees (though very late in producing).

Fig. 7 The oldest plantation in Spain dates to 1968

In 1979, the world's largest truffle plantation (Fig. 8). belonging to a single landowner was initiated in the province of Soria. It consists of 600 ha dedicated exclusively to truffle production. It produces around 2000 kg per year 250 ha are irrigated by water cannons from a reservoir with a capacity of 400.000 m3 (5 ha). Irrigation dose is around 25 l/m^2 every 15 days in July and August (if there is no rain). The ground is usually ploughed 3-4 times per year (Reyna & Hernandez, 2007).

Fig. 8 Arotz plantation is the largest one in the world

Privately initiated truffle plantations have progressively multiplied in Spain. According to García Rodríguez (1994), since the decade of the 1990s, these plantations have grown at an annual rate of 40.000-50.000 plants, keeping in mind that this rate is highly influenced by the subsidies and incentives established by the different official administrations with respect to these plantations.
Nowadays around 100.000-150.000 mycorrhized plants are introduced every year. In 2008, it is estimated that there are more than 6.000 ha of cultivated truffle trees in Spain, 80% of which are concentrated in the provinces of Teruel, Castellón and Huesca. Most (95%) of the nurseries that produce truffle plants in Spain are also located in Teruel and Castellón. The annual rate of growth in these areas is very high and is estimated between 250 and 500 ha a year.

The typical truffle plantation in Spain can be described as follows:

- It is located within the Iberian mountain range at altitudes between 800 and 1200 m and has a Mediterranean climate regime with some summer storms (mean annual temperatures from 10-14°C and annual precipitations from 400-800 mm). The climate is irregular, both annually and interannually. Outside the Iberian mountain system, even in potentially excellent zones like the Catalonian provinces, truffle plantations are practically inexistent because the climate and soil permit less uncertain alternatives.
- The plots are 0.2- 3 ha in size and are often hard to mechanise.
- Soils are always calcareous with a pH of 7.5-8.5, and they are generally loamy in texture.
- Previous land use was agricultural and almost always cereal-producing agriculture, although there are some cases of almonds, aromatics or vineyards, and only rarely of recent forest use.
- The planting reference is usually 6x6.
- Plants are small in size and produced in nearby nurseries. The most commonly planted trees are *Quercus ilex* (85%), followed by *Q. faginea*, *Corylus avellana,* and *Q. pubescens*; plantations of *Q. coccifera* are rare.
- During the first years after plantation, some irrigation is usually performed to facilitate establishment; some irrigation is usually performed to facilitate establishment; in later years, irrigation is rare.
- Sometimes the best truffle producing trees are irrigated by means of water tankers. The most used system is micro-sprinkling (Fig. 9).
- The ground is usually ploughed during the first post-planting years, but this practice is very frequently stopped as soon as the truffle burns become intense or truffle production begins (from year 6 to year 12).

Fig. 9 Plantation in Teruel with micro-sprinkling irrigation

- Pruning is almost always carried out to raise the tree crown and favour insolation.

Fig. 10 Air view of a truffle area in Castellón there appreciate plantations and wild trees producers with their brulée marked with red circles

Wild Truffle and forestry

The holm oak, and other *Quercus,* on calcareous soil are presents in the eastern side of Spain. At least a 50% of this forests (5.000.000 ha) has high potential for truffle production.

In recent decades, spontaneous black truffle (*Tuber melanosporum* Vittad.) production in Spain has suffered a generalized decline that has not been counterbalanced by newly planted truffle orchards (Reyna *et al.*, 2004). The most important reasons for this decline are the increasing density of the forests and the over-harvesting of the truffle due to its high market price (Reyna, 2000; Gil *et al.*, 2001). Also other reason like lasts years drought has contributed to decline of truffle production (Saez y De Miguel, 2008).

The truffle is a natural resource of high ecological, economic and social value. The direct economic benefits it provides lead the forest manager to introduce measures aimed at guaranteeing its successful production, and these measures may imply modifying or adapting previous sylviculture concepts.

A pilot project was carried out in El Toro (in the interior of the Valencian region) to guarantee spontaneous truffle production in forests. Some truffle-producing areas had already disappeared in the last years and, according to truffle collectors, some other ones ran the risk of disappearing (Reyna *et al*., 2004). Firstly, it was verified that densification was a limiting factor in truffle-producing forests. Truffle silviculture models have been designed and experimentally applied in Spain (Hernández *et al*., 2001; Reyna *et al*., 2004) and Italy (Gregori *et al*., 2001; Tagliaferro, 2001) to combat this situation. They consist of opening the vegetation and eliminating the non-productive, competitor trees and their associated ECM.

Eight years later, both truffle production and populations of black truffle ectomycorrhizas are recovering, and the holm oak has become the dominant species in the regenerating forest. Nowadays this techniques are used by different Forestry Services of Spain.

Research and development

As we pointed out previously, since 1995 there has been a clear increase in Spain in all activities related to truffle cultivation research and techniques, with numerous practical aspects relating to fieldwork, activity development, technical workshops and research projects. This is reflected

in the increasing participation of Spanish researchers, technicians and truffle cultivators in international congresses, and especially in the Fifth International Truffle Congress, celebrated in Aix-en-Provence in 1999 or in this one of Spoleto 2008.

If we limit our review only to the activities carried out in Spain since the year 2000, we can include various research projects on the truffle, which have led to several doctoral theses, scholarly publications and other work .

At present it is necessary to emphasize the specific *Plan Teruel for Truffle Cultivation*, which coordinates by Dr Carlos Palazón. There take part seven researchers teams from the following Centers and Universities with the indicates topics:

Center	Topics
Centro de Investigación y Tecnología Agroalimentaria de Aragón Dr Carlos Palazón Español Dra Ana de Miguel	Inventory of mycorrhizal fungi competitors and pollutants of the plantations of black truffle, in Teruel. Evolution of mycorrhizal status in trufieres depending on external factors.
Centre Tecnològic Forestal de Catalunya Dr Carlos Colinas	Analysis and study of the factors biotic and abiotic that concern the establishment of plantations, production and the quality of the black truffle. of Teruel.
Universidad de Zaragoza. Escuela Politécnica Superior de Huesca Dr Juan Barriuso Vargas	Development of methods for parasites control, pathogenic and pollutants, of the black truffle and of his symbiotic tree.
CEAM, Centro Estudios Ambientales Mediterráneo. Valencia Dr Santiago REYNA	Truffle Silviculture. Management of Truffles in Wild Forest, Truffle plantation as breakfire.
Departamento de Investigación y Experiencias Forestales de Valonsadero Dr Fernando Martínez Peña	Catalogue of truffle plantations in Teruel. Study of soils and mycorrhizal stock in relation with the production of black truffle
Universidad de Zaragoza .Facultad de Veterinaria Dr Domingo Blanco	Increase of the commercial life of the black truffle: use of combined methods. Application of new technologies for preservation.
Servicios. Agropecuarios de la Diputación Provincial de Teruel D. Rogelio Castaño	Development and maintenance of truffle´s experimental net in Teruel
Other centres and Universities	
Universidad Politécnica de Valencia, Universidad de Navarra, Universidad de Murcia, Instituto Técnico de Gestión Agrícola. Pamplona.	

Activity in the sector is also reflected in the organization of numerous workshops, courses, etc. on the truffle, which have been aimed at practically every level of formation, such as those carried out in Soria, Graus (Huesca), Viver (Castellón), Sarrión (Teruel) and Ares del Maestre (Castellón), etc.
This situation seems to point to the necessity of initiating scientific gatherings at national scale of all those related to research into the truffle.

Social and ecological assessment

In Spain truffle cultivation has an added value in that it falls fully within the concept of sustainable development due to the ecological conditions in which it is produced and the economic and social environment benefitting from its production.

The ecological conditions in which the truffle is developed are found mainly in regions of Spain with relatively poor soil and supramediterranean climatic conditions (according to Rivas Martínez, 1987), implying very limited agricultural potential. In these areas agricultural activity is usually centred in the cultivation of cereals, and production levels rarely exceed 2000 k per hectare (Reyna *et al.,* 1998) for barley, levels which are at the very limit of positive economic returns. Other unirrigated crops such as almond, carob and olive trees are not possible because of the thermal conditions, and vineyards would almost always be outside the areas protected by official guarantees of origin and quality. Moreover, due to poor accessibility, these areas also show little industrial development and, as a result, they have suffered an important depopulation and aging process. At present, truffle cultivation offers a viable development alternative and is helping to stop these processes. Truffle plantations can now be found in areas where it is impossible to grow any other crop, in both the physical and the economic sense.

The truffle contributes to the conservation of natural formations of Holm oak (*Quercus ilex* L.), evergreen oak (*Quercus coccifera* L.), Lusitanian oak (*Quercus faginea* Lamk.) and *Quercus humilis* Miller, as well as to their extension through new plantations, and it is especially esteemed as a product of biological farming with excellent conditions for ecological compatibility and improvement.

Future prospects

Future prospects for spontaneous truffières in Spain are dim unless adequate silvicultural measures are applied in spontaneous truffières of approximately 600.000 ha in size. If this does not take place, these truffières will continue to thicken, thus preventing the permanence of the truffle and, more importantly, the appearance of new truffières. This decay has also been caused by abusive practices that are gradually disappearing, though, unfortunately, they are still being applied in certain areas. There are only two ways to eliminate these unprofessional practices: (1) develop legal regulations that conform better to the reality of the situation and, especially, (2) develop activities of agrarian extension, including courses, workshops, exhibitions, demonstrations, etc., for the people who are directly implicated.

In contrast, from the point of view of plantation truffle cultivation, the Spanish potential is very high, due both to the enormous extension of calcareous soils (>10.000.000 ha) and the climatically appropriate area (>20.000.000 ha). Actual plantation activity continues to increase, with a current rate around 150.000 plants sowed yearly. A very serious problem is the risk of Asiatic truffle expansion; these include *Tuber himalayensis, T. indicum, T. pseudohimalayensis* and *T. pseudoexcavatum*, all of which lack commercial value and can be found in European truffle batches (Garcia Montero, 2000). The confusion or deceit that Spanish, French and Italian nurserymen may be subjected to by those who sell low-value truffle species for inoculation purposes constitutes a grave threat for the sector, not only because of the economic repercussions involved for the agriculturalist, but also because of the serious ecological problems derived from the uncontrolled introduction of very invasive exotic species.

Spanish professional associations with respect to truffle cultivation began with regional associations which have now been constituted in a national federation that participates with its French and Italian counterparts in the GET (European Tuber Group), formed after the Fifth International Congress in Aix-en-Provence. Thus, the various public administrations now have valid interlocutors to help them to both organise the markets and establish co-financing mechanisms for the producing sector with the aim of launching truffle cultivation activities in unirrigated areas. A result of this association is the joint truffle cultivation project being elaborated by France, Italy and Spain with the aim of producing quality truffles, in large amounts, and with long production periods. Within the framework of this GET activity, a Truffle

cultivation Work Group has been formed in Spain and consolidated recently in Valencia to integrate all sectors so as to improve Spanish Truffle cultivation.
Prospects for Spanish truffle cultivation are optimum, given the enormous territorial potential, the increase in research activities, the interest of the Public Administrations and, especially, the increasing dynamism in the private sector.
The advantages of Spanish truffle cultivation can be summarized as follows:

- It is based on soils that are good for the truffle and poor in other mycorrhizae.
- The nursery-produced plant is usually of good quality.
- The climate, although somewhat dry, is quite adequate for truffle cultivation, given its Mediterranean character.
- Agricultural alternatives in the planted areas are practically inexistent.
- The various Public Administration involved provide subsidies to maintain rural populations in their areas.

For these positive prospects to be successful, the negative aspects mentioned will, of course, have to be corrected in a realistic way.
After a short but intense history, truffle cultivation in Spain has now reached practically the same level, in terms of production techniques and research activities, as that found in France and Italy, with centuries of history dedicated to the truffle.

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ECOLOGÍA DE LAS PLANTACIONES DE TRUFA NEGRA EN TERUEL: MÉTODO DE MUESTREO PARA SU CARACTERIZACIÓN

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Abstract
With the aim of going deeply into the ecology of the holmoak (*Quercus ilex* L.) inoculated with *Tuber melanosporum* Vittad. plantations in Teruel (central-east Spain), the method described below was set out. Plantations over 10-years-old are studied, both producing and non-producing black truffle sporocarps and distributed throughout the Teruel province. Near 80 plots are included in this study to cover as much as possible different places.
Field study, conducted in spring, focuses on a representative spot of every plantation as regards the characteristics of soil, vegetation and production of black truffles. This sampling point has nine host trees with variable area depending on the used frameworks for planting. Three soil extraction points are randomly selected in the area, near the line of glass. In each point, two adjoining samples are collected in the South-East of every tree for soil and ectomycorrhizae analysis. A total of 6-cylinder, 4 cm in diameter and 20 cm deep, are extracted in each plantation. In order to estimate the apparent density, outside the burnt area, is made an additional hole. Also, measurements of physiographical and tree parameters are taken.
A full analysis of soil and presence/absence *T. melanosporum* ectomycorrhiza detection is doing in laboratory. Multivariate analysis techniques to detect potential patterns of behaviour of the parameters with the production and/or mycorrhization of black truffle will be done.
Key words: ecology, truffle cultivation, parameter, sampling.

Introducción

Esta acción forma parte de un amplio proyecto denominado **Desarrollo Integral de la Truficultura de Teruel**, incluido en el Plan de Actuación Específico para Teruel. Este proyecto abarca distintos aspectos relacionados con la truficultura, el desarrollo y la conservación. Ha sido financiado por el INIA (Ministerio de Ciencia e Innovación, Gobierno de España) dentro del proyecto PET2007-013-C07.
La provincia de Teruel cuenta con un amplio territorio con una extraordinaria aptitud para el establecimiento de plantaciones encina micorrizada con trufa negra. Este tipo de cultivo, supone en sí mismo un beneficio ambiental en las zonas que lo detentan, ya que contribuye a la forestación de superficies agrarias, con la introducción de especies forestales autóctonas, como la encina, el quejigo o el roble, lo que evita la erosión, contribuye a la formación de paisaje y favorece la formación y estabilidad del suelo. Por otro lado, constituye una muy interesante inversión, con un escaso gasto inicial.
La demanda de trufa negra está actualmente en lo más alto, así que la producción actual, estimada en menos de 100 toneladas anuales a pesar de la incorporación de la producción procedente de las plantaciones, no es suficiente para satisfacer el 10% de la demanda del mercado (Agrobiotruf, 2007).
Además, hay que tener en cuenta la recesión actual en las masas naturales, debida principalmente a la excesiva espesura de los bosques y a la intensa explotación a la que se vieron sometidas (Reyna, 2007), lo que da una mayor trascendencia a la implantación de este tipo de cultivo.
Existen actualmente en Teruel del orden de 3000 hectáreas dedicadas a la truficultura, habiendo aumentado de manera exponencial en los últimos años. Además, hay un número elevado de plantaciones mayores de 10 años que están produciendo o no trufa negra y que pueden ser analizadas.

Sin embargo, estas plantaciones aportan producciones tan variables como su origen, ubicación y los tratamientos a que han sido sometidas, desde 0 a 30-50 kilos por hectárea.
Hoy en día y pese a la falta de controles oficiales, la calidad de la planta micorrizada es bastante aceptable, por lo que cobra una importancia crucial la selección cuidadosa del terreno para hacer la plantación, así como de los tratamientos y cuidados a llevar a cabo desde antes de su implantación y durante toda su vida productiva.
¿Cuáles son los mecanismos y factores que desencadenan la primera cosecha?, ¿qué expectativas de producción se pueden albergar?, son preguntas que aún hoy, más 20 años después de las primeras plantaciones, no tienen una respuesta concreta. Cuestiones que además desvelan que hay muchos factores desconocidos relacionados con la ecología de las plantaciones de *T. melanosporum*. Su conocimiento, al que pretendemos acercarnos con esta acción, podría proporcionar información muy útil para, primero, evitar errores en la selección de los terrenos y segundo, aumentar la producción de carpóforos.

Objetivos

El objetivo concreto de este trabajo es profundizar en el conocimiento ecológico de las plantaciones de encina micorrizada con trufa negra mayores de diez años de la provincia de Teruel. Con ello, y conociendo el estado actual de las mismas en lo que se refiere a micorrización y producción de carpóforos, se pretenden obtener los parámetros ecológicos óptimos para el establecimiento de nuevas parcelas.

Metodología

Trabajo de campo

Se visitan 80 parcelas dedicadas al cultivo de la trufa y repartidas en las comarcas turolenses de Gúdar-Javalambre, Teruel, Calamocha, Cuencas Mineras, Maestrazgo y Matarraña. Estas parcelas han sido cedidas al estudio por propietarios particulares.
De cada parcela se conoce previamente la información relativa a usos anteriores, edad de plantación, origen de la planta y tratamientos a los que ha estado sometida, así como la producción, si la ha habido, de carpóforos de trufa negra. Conocidos estos datos, y teniendo en cuenta las características medias de la plantación, se ubica el punto de muestreo. Esta subparcela consta de 9 árboles y una superficie variable atendiendo a los marcos de plantación, de entre 5 y 7 metros. Dentro de ella, y de manera aleatoria se seleccionan tres árboles junto a los que se toman las muestras de suelo y raíces.
El punto concreto de extracción estará situado entre la línea de copa y el límite del quemado, donde, como subrayan entre otros Barry-Etienne *et al.,* (2008), se detecta el mayor porcentaje de micorrizas de *T. melanosporum* y en dirección sur-sudeste, donde se ha manifiestado una mayor fructificación de carpóforos en distintos trabajos realizados en la Península Ibérica. Con el uso de un cilindro de 20 cm de longitud por 4 cm de anchura, y siguiendo las directrices marcadas por Verlhac *et al.,* (1990) y Taylor (2002) en cuanto a la metodología para la extracción de muestras de suelo para el estudio de las ectomicorrizas, se extraen dos muestras de suelo en cada árbol, con lo que se extrae un total de 6 muestras en cada subparcela. La mitad se dedican a estudio de características edáficas y el resto a la observación de ápices radicales para comprobar la presencia de micorrizas de *T. menalosporum*.
Asimismo, fuera del quemado, se realiza un hoyo de 30 cm de lado y 20 cm de profundidad y del material obtenido, una vez homogeneizado, se toman dos nuevas muestras que se trasladan al laboratorio para el estudio edáfico, que en este caso incluirá densidad aparente.
Por último, se realiza medición de los 9 árboles de la subparcela, en lo que se refiere a sus características biométricas y se observa su estado sanitario. Además, se anotan las características fisiográficas de la parcela.
Es importante observar el entorno en el que se ubica la plantación, por la influencia que éste pudiera tener en el funcionamiento de la misma, de cara a la presencia y evolución de micorrizas de trufa negra. Es interesante, asimismo, testificar la presencia de setas de otros

hongos epígeos, que como sabemos pueden suponer una competencia para el desarrollo de *T. melanosporum* (Donnini *et al.,* 2008).

Análisis de suelo y elaboración de parámetros ecológicos.
En laboratorio, se realiza un análisis de muestras de suelo, con el propósito de detectar parámetros que pudieran ser importantes en los mecanismos de fructificación y desarrollo de la trufa negra. Se estudiarán los parámetros fisiográficos y climáticos y se elaborarán los siguientes parámetros edáficos: densidad aparente, tierra fina, arena, limo, arcilla, coeficiente de capacidad de cementación, coeficiente de impermeabilidad debida al limo y permeabilidad (Gandullo, 1994), humedad equivalente (Sánchez y Blanco, 1985), materia orgánica, acidez actual, acidez de cambio, nitrógeno, relación carbono/nitrógeno, carbonatos, caliza activa, fósforo, sodio, potasio, calcio, magnesio, capacidad de cambio de cationes y tanto de saturación del complejo adsorbente.

Observación de la presencia/ausencia de micorrizas de T. melanosporum.
En contra de lo que se piensa, las comunidades de hongos ectomicorrícicos que viven en las raíces de las plantas productoras de trufa son muy diversas. Esta diversidad aumenta con la edad de los árboles, hasta estar formadas, en las plantaciones maduras, por más de 100 especies de hongos distintas (Águeda *et al.,* 2005).
En este trabajo únicamente se pretende detectar la presencia de micorrizas de *T. melanosporum*, por lo que se analizan los ápices radicales extraídos en los cilindros. Las muestras se conservan congeladas a -28º C hasta su limpieza y posterior estudio.
Bajo la lupa binocular se realiza la selección de los ápices de cada muestra, diferenciando entre los no micorrizados y los micorrizados, y de entre éstos, los micorrizados con *T. melanosporum* y con otros hongos. La identificación morfológica se realizará según la metodología de Agerer (1987-2002, 1991) y Agerer & Rambold (2004-2005).

Tratamiento de la información.
Mediante técnicas de análisis de datos de tipo descriptivo se examinará la variabilidad existente para el conjunto de los parámetros, lo que permitirá establecer los posibles límites de aptitud ecológica de las plantaciones de encina productora de trufa negra. Asimismo, se utilizarán técnicas de análisis multivariante para descubrir posibles patrones de comportamiento de los datos y los parámetros asociados a la producción y/o micorrización. Se espera que los resultados proporcionados por este trabajo nos permitan aportar información útil para el buen desarrollo de las plantaciones truferas.

Estado actual del trabajo.
Debido al limitado periodo de muestreo y al amplio número de parcelas y parámetros a estudiar, este trabajo se concluirá en 2010. En la actualidad se ha superado el 30%.

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THE PRINCIPLE OF PRECAUTION IN TRUFFLE CULTIVATION

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Abstract
Truffle cultivation started in France at the beginning of the 19th century with exceptional results at the end of that century and the beginning of the next. The fall in truffle production of *Tuber melanosporum* which started after the first or second world wars - according to location - motivated a programme of revitalization from the 1970's onwards. Mycorrhizal inoculation of plants starting in 1974 plus the experimental work of the 1980's slowed the decline and saved truffle production in France. Nevertheless, truffle cultivation comes up against some difficulties which impede the complete success of its revival. We have identified the strong and weak points by comparing production conditions and the results obtained in different regions of France and other countries visited in Europe and the rest of the world. Amongst the adverse points, we have noted so far which seem to be unfavourable to truffle production: 1) wooded environments which create pressure by contamination with forest habitat fungi (*Tuber brumale*), 2) previous intensive cultivation using chemical products (pesticides and fertilizers), 3) poor management of the growth and density of mycorrhiza–inoculated trees with regard to the nature of the soil, 4) working the soil with drawn cultivators, 5) using herbicidal products which decrease biodiversity. Amongst the favourable points are: 1) the use of tested mycorrhiza–inoculated plants, 2) light cultivation techniques which respect biodiversity identical to that of natural truffle grounds, 3) planting in open, lightly-wooded countryside. In southern Italy and Spain, where the industrialisation of agriculture arrived later than in France, the difficulties of truffle production seem to be less limitative. In Australia, truffle production seems to be easier with the accompanying arboriculture because the environment is free from other contaminants of the genus *Tuber*. These observations lead us to propose that truffle production in France should be subjected to certain precautions. These precautionary measures for truffle production imply giving special attention to the following points: 1) the choice of previous methods of culture corresponding to fallow land, 2) a technical cycle which favours a phase of installation of the fungus after taking control of the tree, 3) an upkeep of the soil which maintains a certain biodiversity, 4) regulation of water for the fungus without stimulating the growth of the trees, 5) pruning the trees according to the extent of the burnt zones and the space available for the fungus to occupy, 6) introducing spores and various other practices. Work is in hand relating especially to the usefulness of the presence of certain animals within the boundaries of truffle plantations.
Key words: *Tuber melanosporum* Vittad., *Tuber brumale* Vittad., contamination pressure, preceding crop, biodiversity.

Problématique et histoire
La trufficulture en France a débuté au début du 19ème siècle avec des résultats exceptionnels à la fin du 19ème et au début du 20ème siècle. Le principe de précaution appliqué à la trufficulture a pour objet de retrouver et restaurer petit à petit l'ensemble des bonnes conditions du retour de la production. Dans cette perspective, il est proposé unes stratégie qui consiste à ajouter par petites touches les précautions connues à ce jour, de façon à remonter le courant du déclin de la production, tout en évitant de se perdre en considérations qui ne conduisent nulle part.
Pour comprendre la démarche à l'origine de ce principe de prudence, il convient de résumer le contexte de la relance trufficole en France. En effet, le plant mycorhizé est apparu au début des années 1970 comme une voie de salut de la trufficulture dont la production ne cessait de décliner. Il a permis de maintenir celle-ci et de l'introduire avec des succès remarquables dans certaines régions ou sur des plantations particulières. Cependant, ce plant contrôlé à la pépinière n'a pas suffi à retrouver les niveaux du début du XIXe siècle.

Photo 1 pépinière de chênes truffiers en 1983 avant la vulgarisation du plant mycorhizé

Les travaux de l'expérimentation ont également contribué à améliorer les techniques culturales en matière d'entretien du sol, de maîtrise de l'eau ou de la taille des arbres. Comme les plants mycorhizés, ils n'ont pas donné lieu à un décollage fort de la production truffière. Les travaux scientifiques sur la croissance des truffes, les sols, la sélection des meilleurs clones d'arbres truffiers, la génétique des *Tuber* n'ont pas encore abouti à un sursaut de la production en raison de la difficulté de la tâche.

La constante de la virulence

Si la production n'arrive pas à décoller vigoureusement, à l'exception de certaines régions du sud de l'Italie et de l'Espagne ou de certaines plantations en France, c'est donc qu'il y a un ou des problèmes mal identifiés. En comparant les situations où «ça marche» avec celles plus nombreuses où «ça ne marche pas très bien», aussi bien à l'échelle d'un pays, d'une région que d'une plantation, on observe des phénomènes constants dans la production truffière. La principale régularité est la virulence de la truffe se traduisant par un brûlé débordant par rapport à la frondaison de l'arbre ainsi que d'autres conditions, en particulier la production truffière. En se basant sur cette virulence (dont la dénomination peut être discutée) et les conditions qui l'accompagnent, il est possible de limiter les risques d'échec et d'améliorer la production par l'application du principe de précaution ou de prudence, surtout dans les situations où «ça ne marche pas très bien».

Photo 2 plantation en pelouse calcicole, sans travail du sol entre les arbre, remarquable par le niveau de sa production avec *Tuber melanosporum* (Lascabanes, Lot, France). Observer les brûlés débordants qui témoignent du phénomène de virulence.

La virulence, définie principalement à partir du brûlé (si celui-ci a effectivement pour origine le *Tuber melanosporum)* et de sa dynamique, présente cet avantage d'offrir une lecture rapide et pratique du potentiel truffier à l'état naturel ou en phase d'expérimentation. Parmi les symptômes accompagnant la virulence de la truffe, les observations conduites en France, Espagne et Italie ont permis d'en identifier quatre: 1) l'absence de récolte du *Tuber brumale* dans le milieu naturel et dans les plantations, 2) une bonne résistance à la sécheresse, 3) une précocité d'entrée en production des jeunes plantations établies avec des plants mycorhizés, 4) la pérennité de la production constatée aussi bien en truffière naturelle que dans les plantations. L'étude de ces symptômes, notamment du point de vue de la quantité de mycélium (biomasse mycélienne) colonisant le sol, est en cours.

Photo 3 la récolte du *Tuber brumale* (à gauche) apparaît comme un indice d'un milieu dans lequel *Tuber melanosporum* (à droite) manque de force ou virulence.

Nous avons identifié les points forts et les points faibles de la trufficulture en comparant les conditions de sa production en fonction des résultats observés dans différentes régions françaises et dans des pays visités en Europe et dans le monde. Il ne sera pas possible de présenter dans cette synthèse l'ensemble des données à la base de ce travail de repérage ou d'identification. Nous en donnons seulement les grandes lignes en guise de points de repère. On se réfèrera pour plus d'éléments au document édité sous le même nom (72 pages) fin 2008 et présenté dans la bibliographie.

Les points faibles de la trufficulture actuelle

Parmi les points faibles de la trufficulture repérés à ce jour et qui semblent pénaliser la trufficulture, on citera: 1) un environnement boisé qui exerce une pression de contamination par des champignons à écologie forestière (*Tuber brumale*), 2) les précédents culturaux de grandes cultures où sont employés des produits chimiques (pesticides, engrais), 3) une auvaise gestion de la croissance et de la densité des arbres mycorhizés en fonction de la nature des sols, 4) l'entretien du sol avec les outils tractés, 5) l'usage de produits herbicides limitant la biodiversité.

Photo 4 plantations truffières dans un environnement boisé d'anciennes plantations (Lalbenque, Lot).

L'environnement boisé est le facteur le plus souvent avancé pour expliquer la difficulté à produire le *Tuber melanosporum*. Ce point peut être facilement illustré par la comparaison des résultats de production entre les zones fermées des vieux bassins trufficoles (Quercy, etc.) et celles ouvertes des nouveaux bassins (Touraine, etc.). Il a aussi pour corollaire la différence de résultats entre une méthode de trufficulture basée sur l'arboriculture et celle sur la pelouse calcicole. Dans le cas de l'arboriculture truffière (système «Pallier»), un travail du sol fréquent a pour conséquence, non seulement d'appauvrir la biodiversité, mais également de faciliter la propagation des espèces indésirables telles que *Tuber brumale*. En revanche, avec la pelouse calcicole (écosystème des truffières naturelles), les contaminations sont rares avec une domination du *Tuber melanosporum* si les plants étaient bien mycorhizés par cette espèce. L'inconvénient de la pelouse calcicole est une croissance plus lente des arbres qui peut être compensée par un travail du sol manuel autour des jeunes arbres.

Les autres points faibles tiennent surtout à la pratique d'une agriculture moderne qui a été transposée vers la trufficulture avec d'autres éléments liés aux nouveaux moyens de cette agriculture, notamment l'utilisation des produits chimiques. L'usage des herbicides[1] et autres pesticides réduit la biodiversité nécessaire à la nutrition et à la croissance des truffes. On se rend compte que certains précédents culturaux sont à l'origine d'un blocage du processus de fructification de la truffe malgré l'existence de brûlés nets et la présence de mycorhizes du *Tuber melanosporum* sous les arbres truffiers. On observe que les mêmes arbres, plantés la même année sur des sols semblables d'une même exploitation agricole, produisent plus tôt et régulièrement sur un précédent jachère ou friche que sur un précédent grande culture (maïs, tournesol, blé) avec utilisation d'une fertilisation chimique. Cette constatation conduit certains trufficulteurs à rechercher des terres «reposées» pour planter des arbres truffiers.

[1] L'usage systématique du glyphosate sous des chênes improductifs dans une plantation du causse du Lot, en vue de faire de faux brûlés pour distraire les braconniers, a permis de récolter à la troisième année *Tuber brumale* sous environ trois quarts des arbres traités.

Photo 5 plantation truffière (Lot, France) dont le travail mécanique sur sol superficiel appauvrit la biodiversité et contribue à favoriser les compétitions fongiques.

Diverses observations semblent indiquer qu'une terre reposée, c'est-à-dire avec une biodiversité naturelle rétablie (vers de terre et insectes abondants dans le sol), favorise l'extension du *Tuber melanosporum* à partir des plants mycorhizés, en dépit de la pression de contamination. Ces cas sont observés sur des plantations conduites en arboriculture truffière sensibles aux contaminations dans un environnement boisé (Escamps, Lot). Le défaut de cultures pendant plusieurs années contribue au repos de la terre. De même, l'absence d'un environnement boisé (ou de pression de contamination, en particulier à partir des chênes de bordure) semble autoriser la production en arboriculture truffière sur des sols non reposés, c'est-à-dire plantés aussitôt après les grandes cultures.

Cette balance entre la pression de contamination et le repos du sol mérite plus d'approfondissements, notamment du point de vue des qualités du sol laissé à l'abandon pendant au moins cinq années. Dans l'évolution d'un milieu calcaire, depuis la terre labourée jusqu'au climax, les stades de la pelouse calcicole à graminées et de la lande à genévriers correspondent à cet état de repos de la terre. Ces stades sont remarquables par le fait que les truffières naturelles apparaissent dans ces conditions de végétation autrefois pérennisées par le pastoralisme (bloquant l'évolution). La parade, qui consistait à installer une pelouse en milieu contaminé par les champignons mycorhiziens forestiers, afin de protéger les jeunes plants mycorhizés par le *Tuber melanosporum*, peut être envisagée du point de vue de l'utilisation de sols reposés.

Les points forts actuels de la trufficulture

La présentation de ces points forts prolonge et complète la discussion sur les points faibles. Les éléments, qui ont permis de faire avancer la trufficulture à la fin du 20^{e} siècle et en ce début du 21^{e}, peuvent être distingués comme suit: 1) l'emploi du plant mycorhizé contrôlé, 2) des pratiques culturales légères respectueuses de la biodiversité à l'identique des truffières naturelles, 3) la plantation dans des paysages ouverts peu boisés.

Photo 6 le plant mycorhizé contrôlé a permis de maintenir une trufficulture vivante

Sans l'invention, la commercialisation et la vulgarisation du plant mycorhizé contrôlé, destiné à assurer l'ensemencement du *Tuber melanosporum*, il est probable que la production truffière serait tombée à un niveau insignifiant. Les pratiques culturales légères font référence à une trufficulture qui favorise l'écosytème des truffières naturelles, c'est-à-dire la pelouse calcicole ou à moutons dans les régions calcaires présentant des sols superficiels. C'est la méthode Tanguy identifiée et définie à la suite de l'hiver 1993-94. Cette méthode a notamment montré que le noisetier[2], essence présentant une forte affinité avec le *Tuber brumale*, pouvait conserver la mycorhization de base avec le *Tuber melanosporum* et produire cette espèce si, après la reprise de l'arbre, une phase d'abandon relatif de la plantation permettait l'installation d'un écosystème de pelouse. Enfin, l'introduction des plants mycorhizés dans des paysages ouverts, comme ceux de la Touraine, a révélé l'importance d'une pression de contamination faible ou inexistante en l'absence d'enfermement dans des massifs forestiers à base d'essences mycorhiziennes (chênes, charmes, noisetiers, etc.) avec les champignons susceptibles d'entrer en compétition avec le *Tuber melanosporum*.

Photo 7 paysage ouvert avec plantation truffière en Touraine (Marigny-Marmande, Indre et Loire, France)

[2] Par précaution ou prudence, il vaut tout de même mieux éviter de planter le noisetier commun qui reste malgré tout affecté par son affinité avec le *Tuber brumale*.

Au-delà de ces points forts, il convient d'ajouter quelques observations effectuées hors de France. Dans le sud de l'Italie et de l'Espagne, où l'agriculture s'est industrialisée plus tardivement qu'en France, les difficultés à produire les truffes semblent plus limitées. En Italie, les frères Angellozzi à Roccafluvione (Ascoli Piceno, Marches) (voir la communication «trufficulture en France et Italie») obtiennent des résultats exceptionnels à partir de plantations réalisées au début des années 1990, dès lors que le déclin de la production naturelle a été observée. Or, les frères Angellozzi témoignent d'une abondance de truffes naturelles dans les années 1980 due à une forte déprise agricole (en raison de l'industrialisation nécessitant la main d'œuvre des campagnes). Les résultats remarquables des plantations ont été obtenus dans un contexte de plantations juste au moment où les truffières naturelles commençaient à décliner avec le reboisement naturel qui fermait le milieu. En Espagne, dans la région de Sarrion, (province de Teruel, Aragon), le succès des jeunes plantations d'arbres mycorhizés est observé dans un paysage où les chênes sont rares (excepté le chêne kermès) et où les genévriers sabines (*Juniperus sabina*) sont abondants. La céréaliculture peu utilisatrice d'intrants (blé et orge avec rendements faibles) est en cours de reconversion vers la trufficulture. Dans les deux cas, *Tuber brumale* est absent du milieu naturel; en Espagne *Tuber aestivum* est toutefois présent et certains redoutent déjà sa compétition future à l'égard du *Tuber melanosporum*.

Photo 8 plantation sur le plateau de Teruel (région de Sarrion, Espagne) dans un environnement où la végétation arborescente est exclusivement représentée par *Juniperus sabina*

En Australie, il apparaît que la production truffière soit plus facile avec l'arboriculture truffière en raison d'un environnement indemne de contaminants du genre *Tuber*. A Manjimup (Australie de l'Ouest), Nick Malajczuk obtient des résultats intéressants et prometteurs à partir de plantations principalement à base de noisetiers mycorhizés par le *Tuber melanosporum*. Il explique[3] les difficultés actuelles à produire la truffe noire en Europe à cause d'une forte compétition fongique. Il a obtenu en pépinière la mycorhization par *Tuber melanosporum* de plants d'eucalyptus indigènes. Or, il a observé que ceux-ci, dès leur introduction sur le terrain, perdent leur mycorhizes du *Tuber melanosporum* tandis que les noisetiers communs (*Corylus avellana*) conservent celle-ci, contrairement aux dérives observées en France avec principalement *Tuber brumale*. La question des affinités avec les champignons mycorhiziens indigènes (résultat d'une co-évolution) est intéressante à analyser à partir de ce constat. En France, ce sont le chêne pubescent et le chêne vert qui ont le plus de fidélité (ou d'affinité) avec le *Tuber melanosporum*; pour le noisetier commun, l'affinité est avec le *Tuber brumale*.

[3] Communication personnelle de Nick Malajczuk en novembre 2007.

En Australie, en l'absence de *Tuber brumale* et de tout autre *Tuber* que *Tuber melanosporum*, le noisetier commun entretient des relations pérennes avec le *Tuber melanosporum*. On peut considérer qu'il présente une certaine répulsion à s'associer avec les champignons mycorhiziens des eucalyptus.

Photo 9 nettoyage d'une parcelle occupée par la forêt d'eucalyptus en vue de planter des noisetiers communs et chênes pédonculés mycorhizés par *Tuber melanosporum* (Manjimup, Australie de l'Ouest)

Les analyses effectuées sur des observations réalisées en Italie, en Espagne et en Australie témoignent de l'importance de la pression de contamination dans le principe de précaution à appliquer à la trufficulture en France.

Les solutions proposées en terme de précaution

Ces constats conduisent à proposer en France une trufficulture qui privilégie certaines précautions. Le principe de précaution appliqué à la trufficulture implique une attention particulière aux points suivants: 1) le choix de précédents culturaux correspondant à des terres reposées, 2) un itinéraire technique qui favorise une phase d'installation du champignon après la reprise de l'arbre, 3) un entretien du sol qui préserve une certaine biodiversité, 4) une maîtrise de l'eau pour le champignon sans stimuler la croissance des arbres, 5) la taille des arbres en fonction de l'étendue des brûlés et de l'espace de conquête disponible pour le champignon, 6) des apports de spores et diverses autres pratiques. Des travaux sont en cours qui portent notamment sur l'utilité de certains animaux à l'intérieur des plantations truffières.

S'il n'est pas nécessaire de revenir sur le choix du précédent cultural, celui de l'itinéraire technique de précaution peut faire l'objet d'un développement, voire d'un rappel. Celui-ci peut être décliné en trois étapes:

- Etape 1: il s'agit d'assurer la meilleure reprise possible du plant mycorhizé par *Tuber melanosporum* la première voire la deuxième année de plantation.
- Etape 2: il ne faut pas favoriser la pousse des arbres mycorhizés pour éviter les contaminations par divers champignons; l'installation de la pelouse calcicole observée autour des truffières naturelles sera favorisée pendant la phase de formation des brûlés.
- Etape 3: après le déclenchement de la fructification du champignon, on cherchera à obtenir avec les techniques culturales habituelles une production en quantité et qualité aussi longtemps que possible (pérennité).

L'entretien du sol (travail manuel ou mécanique), la maîtrise de l'eau (arrosage et paillage),

la taille et l'élagage, relèvent des techniques culturales classiques en trufficulture. La taille et l'élagage peuvent être aujourd'hui envisagés à partir du concept de virulence qui consiste à créer un équilibre entre la vigueur de l'arbre et celle de la truffe qui s'exprime dans une relation entre le rayon de la frondaison (Rf) et le rayon du brûlé (Rb), soit Rb ≥ 1,5 Rf, ou plus concrètement un brûlé assez débordant par rapport à la frondaison de l'arbre.

Photo 10 la taille et l'élagage, même en été comme cela a été montré en Italie, aide à maintenir l'équilibre entre la force de l'arbre et celle de la truffe désignée sous le terme de virulence.

La question des apports de spores semble retrouver toute son importance à partir de résultats récents de l'expérimentation et des observations conduites par des trufficulteurs soucieux d'utiliser judicieusement leurs truffes gelées sous forme d'inoculum. En fait, si les vieux arbres ont donné lieu dans le passé à des résultats négatifs, il n'en est pas de même lorsque les apports de spores ou les inoculations sont appliquées dans la zone de racines jeunes, aussi bien avec de jeunes arbres qu'avec des arbres plus âgés. Avec ces derniers, l'inoculation devra avoir lieu dans la partie racinaire qui déborde largement la frondaison de l'arbre et non sous l'arbre lui-même où se situent les racines peu ou pas réceptives à la présence de mycorhizes du *Tuber melanosporum*.

Photo 11 le pastoralisme a accompagné la production truffière dans sa période d'abondance.

Enfin, d'autres aspects ou pratiques peuvent être envisagés en termes de renforcement de la virulence de la truffe à l'origine de sa production. La présence d'animaux à certaines périodes de l'année peut contribuer à l'effet recherché. Les chevaux et les moutons sont généralement à la base d'un retour ou d'un maintien de la virulence sur des sites truffiers. Le pastoralisme a accompagné la trufficulture à l'époque où celle-ci produisait des résultats exceptionnels. D'autres éléments de biodiversité peuvent être pris en compte, en particulier la présence d'escargots (*Cernuella virgata*) nommés limaçons en Provence mais également observés dans le Sud-Ouest. La flore d'accompagnement de la truffe peut également être considérée à partir de différentes plantes: fétuque ovine, prunellier, vigne, lavande, thym, etc. Des études sont en cours pour rechercher en biologie moléculaire la présence du *Tuber melanosporum* dans les tissus racinaires de ces plantes, susceptibles d'apparaître comme des réservoirs de biomasse mycélienne.

Photo 12 la lavande est une plante favorisante de la truffe au même titre que la vigne.

Conclusion

Sur le plan pratique, on ne peut garantir une production truffière avec 100% des arbres producteurs lorsqu'une plantation est réalisée dans des conditions apparemment satisfaisantes. Par contre, si un certain nombre de précautions sont prises, en particulier par rapport à un environnement boisé ou le choix d'un sol reposé, il est possible d'espérer une production techniquement et économiquement satisfaisante. Le choix d'un itinéraire technique de précaution s'impose d'autant plus que l'on est incertain du pouvoir de contamination du sol. L'itinéraire technique classique de l'arboriculture truffière peut toutefois présenter une moindre sensibilité aux contaminations lorsque la plantation est installée sur une terre reposée, c'est-à-dire sans culture depuis plusieurs années.

Sur le plan théorique, il reste à expliquer principalement les raisons des points faibles de la trufficulture actuelle. Parmi ces points faibles, il semble essentiel de comprendre pourquoi les pratiques culturales modernes (utilisant des intrants chimiques) contribuent à bloquer le déclenchement de la fructification du *Tuber melanosporum*. L'hypothèse la plus probable est

que ces pratiques diminuent la capacité de la biomasse mycélienne du *Tuber melanosporum* à se propager dans le sol avec pour corollaire un déficit de la sexualité du champignon. Il conviendra également d'expliquer comment la faune (macro et micro) et la flore favorisante interviennent dans le développement et la stimulation de cette biomasse mycélienne. Autant de questions qui attendent des réponses de la part de la science en espérant qu'elle puisse disposer des moyens nécessaires.

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NUOVE PROSPETTIVE NEL CONTROLLO DELLE PIANTE MICORRIZATE CON TARTUFO

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Abstract: New perspectives in the quality control of *Tuber* infected plants
The production of seedlings infected with the *Tuber* spp. is the first important step in truffle cultivation. For this reason over the past several morphological methods for the evaluation of the degree of ectomycorrhizal infection of plants inoculated with *Tuber* spp. have been proposed. All methods developed (mainly in Italy and in Spain) are time consuming, requiring the direct count of a great number of tips for plant. In fact these methods are based of quantitative scale (counts or percentages). Instead, the method adopted by the French, is less time consuming because it avoids the direct counting of mycorrhized root tips, by providing an estimate of the extent of mycorrhization directly using an ordinal scale. The scale is defined according to the quantity of mycorrhizal tips present, but this method is quite subjective because it is not supported by a statistical validation of the scale and of the inspector. In this work we applied ROC curves for the evaluation of the sensibility and specificity of inspectors who have to estimate a *Tuber* infected plants using a binomial response.
Key words: ectomycorrhiza, estimation, ROC curve, truffle infection. *Tuber* infected plants.

Introduzione

La coltivazione dei tartufi si realizza attraverso la messa a dimora, in un sito idoneo, di piante preventivamente micorrizate con specie pregiate del genere *Tuber.* A questo scopo da numerosi anni, non solo in Italia, Francia e Spagna ma anche in alcuni paesi extraeuropei si producono piante micorrizate con *Tuber melanosporum* Vittad., *T. aestivum* Vittad., *T. borchii* Vittad., *T.brumale* Vittad. destinate alla tartuficoltura. (Gregori, 1991; Zambonelli e Di Munno, 1992; Chevalier, 2001; Hall *et al.,* 2007; Reyna, 2007).
Benchè sia riconosciuta da tempo l'importanza di utilizzare solo piantine con un elevato grado di micorrizazione con tartufo, il loro controllo su larga scala rappresenta un problema non completamente risolto. Restano infatti ancora da definire i sistemi e le procedure più adatte per ottenere una corretta stima dell'entità di micorrizazione dei lotti di piante forestali destinate alla tartuficoltura.
In generale, un buon metodo per il controllo di piantine micorrizate prodotte in vivaio deve soddisfare alcuni criteri fondamentali: deve essere preciso, riproducibile, ma soprattutto deve essere facilmente applicabile e veloce. Il controllo sistematico del grado di micorrizazione di tutte le piantine di un lotto, seppure preciso, è estremamente laborioso ed è praticamente inapplicabile: occorrerebbe svasare tutte le piante e contare tutti gli apici radicali micorrizati con tartufo, quelli micorrizati con altre specie di funghi ectomicorrizici e quelli non micorrizati.

Anche un approccio che si realizzi nel conteggio di tutti gli apici radicali di un campione casuale di piantine di uno specifico lotto, risulta ugualmente molto gravoso, in quanto il conteggio degli apici di una singola pianta, che ammontano ad alcune migliaia, richiede tempi estremamente lunghi, dell'ordine di 1 o 2 giornate. Per questo motivo in passato sono stati perfezionati in Italia e Spagna metodi di controllo che prevedono il prelievo di campioni di radici dalle piante sottoposte al controllo e il conteggio degli apici solo delle porzioni di radici campionate (Bencivenga *et al.,* 1987; Govi *et al.,* 1995; Fischer e Colinas, 1996; Palazon *et al.,* 2001; Reyna *et al*., 2002). Queste metodologie, tuttavia, seppure semplificate risultano ugualmente lunghe e laboriose (Donnini, 2005).
In Francia viene applicato un metodo di controllo molto più semplice che non prevede il conteggio degli apici, ma una stima visuale dell'entità di micorrizazione delle piantine campionate, tramite l'utilizzo di una scala ordinale da 0 a 5 in funzione della quantità di micorrize presenti (Chevalier e Grente, 1979; Giraud, 1988; Ricard, 2001). La precisione di questa metodologia dipende ovviamente dalla affidabilità dell'operatore che deve essere in grado di stimare in modo accurato e preciso il grado di micorrizazione delle piantine.
Le curve ROC *(Receiver Operating Characteristic)* sono utilizzate da tempo per valutare statisticamente l'accuratezza di un operatore addetto ad effettuare stime. Il primo utilizzo delle curve ROC risale alla II Guerra mondiale quando sono state impiegate per definire l'accuratezza degli operatori nell'analisi delle immagini radar e nello studio del rapporto segnale/disturbo. Esse sono poi state applicate in altri campi della tecnica e, a partire dagli anni '70, anche in campo medico (Lusted, 1971) inizialmente allo scopo di quantificare l'attendibilità dei responsi di immagini radiografiche interpretate da operatori diversi (Goodenough *et al.,* 1974; Hanley e Mcneil, 1982, 1983). In tempi più recenti, l'utilizzo delle curve ROC è diventato relativamente comune per la valutazione non solo delle immagini, ma anche dei più svariati test come per esempio nel settore medico (con particolare riguardo per i test clinici di laboratorio) (Erdrich, 1981; Henderson, 1993). In questo lavoro attraverso le curve ROC ci si propone di valutare la capacità di un operatore di discriminare tra piante ben micorrizate e non sufficientemente micorrizate, utilizzando una stima visuale dell'entità di colonizzazione radicale.
A titolo esemplificativo riportiamo la metodologia applicata alla valutazione del grado di micorrizazione da parte di due operatori esperti e di due operatori inesperti.

Valutazione del grado di micorrizazione delle piantine
Sono state esaminate 50 piantine appartenenti a un lotto di 1000 piantine di *Quercus pubescens* Willd. micorrizate con *Tuber melanosporum* Vittad. prodotte in un vivaio della Regione Marche (Sant'Angelo in Vado in provincia di Pesaro - Urbino), secondo le metodiche messe a punto dal Centro sperimentale di tartuficoltura (Gregori, 1988).
Tutte queste piante sono state analizzate da due operatori (A e B) con una lunga esperienza e da due operatori inesperti (B e C). Il criterio di valutazione è stato quello individuato da alcuni Istituti di Ricerca (Govi *et al.,* 1995), secondo cui una pianta, (per essere considerata valida ai fini della tartuficoltura) oltre a possedere tutta una serie di requisiti (innanzi tutto che faccia parte di un lotto omogeneo, cioè prodotto nello stesso giorno, con lo stessa procedura ed allevato nelle medesime condizioni; e che sia sana, robusta e senza difetti dal punto di vista forestale) deve presentare contemporaneamente: una percentuale di micorrize del tartufo dichiarato pari o superiore a 30%; una percentuale di micorrize di altri funghi diversi dal tartufo dichiarato non superiore al 15%; una differenza tra la percentuale di apici micorrizati con il tartufo dichiarato ed apici micorrizati da altri funghi, pari o superiore a 20%.
Ciascuna piantina, dopo essere stata estratta con cautela dal proprio contenitore, ed averne delicatamente lavato l'apparato radicale, è esaminata al microscopio stereoscopico per individuare la presenza di micorrize di *Tuber melanosporum* Vittad.. L'identità di tali micorrize è stata confermata tramite osservazioni al microscopio ottico, per controllarne le caratteristiche tipiche anatomo-morfologiche (Zambonelli *et al*., 1993).
Successivamente ciascun operatore ha giudicato ciascuna piantina come valida o non-valida, attraverso una stima visuale del grado di micorrizazione osservando l'intero apparato radicale

al microscopio ottico. Il livello di micorrizazione e la validità delle stesse piantine è stata valutata anche applicando la metodologia di Govi *et al*. (1995), che si basa sul conteggio, senza alcuna esclusione, dei primi 50 apici radicali (separandoli in micorrizati con specie di tartufo dichiarata, con altre specie fungine, o non micorrizati) in almeno 4 porzioni di radici prelevate casualmente in ciascuna metà (prossimale e distale) dell'apparato radicale.
Nella tabella 1, si riporta la percentuale di micorrizazione (colonna 1) e la validità di ciascuna piantina (colonna 2) ricavata applicando la metodologia di Govi *et al.,* (1995), ed i giudizi dei 4 diversi operatori effettuati con stima visuale (A, B, C, D). Con 0 si rappresentano le piante ritenute non valide, con 1 le piante ritenute valide da ciascun operatore.

Tab. 1 Percentuale di micorrizazione, e giudizio di validità di ciascuna piantina ricavato applicando la metodologia di Govi *et al.,* (1995), e mediante stima visuale da quattro operatori.

% mic piante	Metodo di Govi et al., 1995	Giudizio operatori			
		A	B	C	D
8	Non valida	0	0	0	0
13	Non valida	0	0	0	0
15	Non valida	0	0	0	1
19	Non valida	0	0	0	0
20	Non valida	0	0	0	1
21	Non valida	0	0	0	1
23	Non valida	0	0	0	1
24	Non valida	0	1	0	0
25	Non valida	1	0	0	1
27	Non valida	0	1	0	0
35	Valida	0	1	0	0
36	Valida	1	1	0	0
36	Valida	0	1	0	0
37	Valida	1	1	1	0
38	Valida	1	1	0	0
38	Valida	0	0	0	0
39	Valida	1	1	0	1
40	Valida	1	1	0	0
40	Valida	0	0	0	0
40	Valida	1	1	1	1
40	Valida	1	1	1	0
40	Valida	1	1	0	1
41	Valida	0	1	1	0
42	Valida	1	1	1	0
42	Valida	1	0	1	0
42	Valida	1	1	1	0
42	Valida	1	1	1	1
45	Valida	1	1	0	0
45	Valida	1	1	1	1
45	Valida	1	1	0	1
45	Valida	1	1	1	0
47	Valida	1	1	0	0
48	Valida	1	1	1	0
48	Valida	1	1	1	1
48	Valida	1	1	1	0
48	Valida	1	1	1	1
49	Valida	1	1	1	0
50	Valida	1	1	1	1
55	Valida	1	1	0	1

56	Valida	1	1	1	1
56	Valida	1	1	1	1
60	Valida	1	1	1	1
63	Valida	1	1	1	1
65	Valida	1	1	1	1
67	Valida	1	1	1	1
70	Valida	1	1	1	1
76	Valida	1	1	1	1
77	Valida	1	1	1	1
79	Valida	1	1	1	1
81	Valida	1	1	1	1

Sensibilità, specificità ed accuratezza

L'accuratezza di stima di ciascun operatore dipendono dalla sua sensibilità e specificità.
La sensibilità è la capacità dell'operatore di identificare correttamente come piante "valide" le piante effettivamente valide, ossia aventi un grado di micorrizazione pari o superiore al valore soglia (30% applicando il criterio di Govi *et al.,* 1995). La sensibilità è un rapporto che varia tra 0 (l'operatore non è sensibile e non riconosce nessuna pianta in realtà valida) ed 1 (l'operatore riconosce correttamente tutte le piante in realtà valide).
La sensibilità si calcola come:

$$\text{Sens} = \frac{VP}{VP+FN}$$

dove:
VP = vero positivo (l'operatore riconosce correttamente una pianta valida),
FN = falso negativo (l'operatore classifica come non valida una pianta valida).

La specificità invece è la capacità di identificare correttamente "non valide" le piante non valide, ossia aventi un grado di micorrizazione inferiore al valore soglia (30% applicando il criterio di Govi *et al.,* 1995). Anch'essa è un rapporto, variabile tra 0 (l'operatore non è specifico e non riconosce nessuna pianta non valida come non valida) ed 1 (l'operatore riconosce tutte le piante non valide come non valide) .
La specificità si calcola come:

$$\text{Sens} = \frac{VN}{VN+PF}$$

dove:
VN = vero negativo (l'operatore riconosce correttamente una pianta non valida)
PF = falso positivo (l'operatore classifica come valida una pianta non valida)
L'accuratezza di classificazione complessiva di un operatore che tiene conto della specificità e della sensibilità si calcola quindi con la seguente formula.

$$\text{Sens} = \frac{VP+VN}{VP+VN+FP+FN}$$

In tabella 2 riportiamo la sensibilità, la specificità e l'accuratezza dei quattro operatori (A, B, C, D) calcolata con le formule sopra riportate. Tutti gli operatori ed in particolare gli operatori C e D risultano più sensibili che specifici, ossia tendono a valutare piante valide come non valide.

Tab. 2 - Sensibilità, specificità e accuratezza dei quattro operatori (A, B, C, D).

	A	B	C	D
Sensibilità	97,2	92,3	85,2	80,7
Specificità	71,5	72,3	30,5	25
Accuratezza	95,4	92,5	76,6	73,9

Valutazione dell'accuratezza tramite le curve ROC

L'accuratezza di un operatore, può essere raffigurata graficamente tramite le curve ROC dove in ordinata è riportata la sensibilità (la proporzione di veri positivi) ed in ascissa la specificità (la proporzione di falsi positivi, fig. 1).

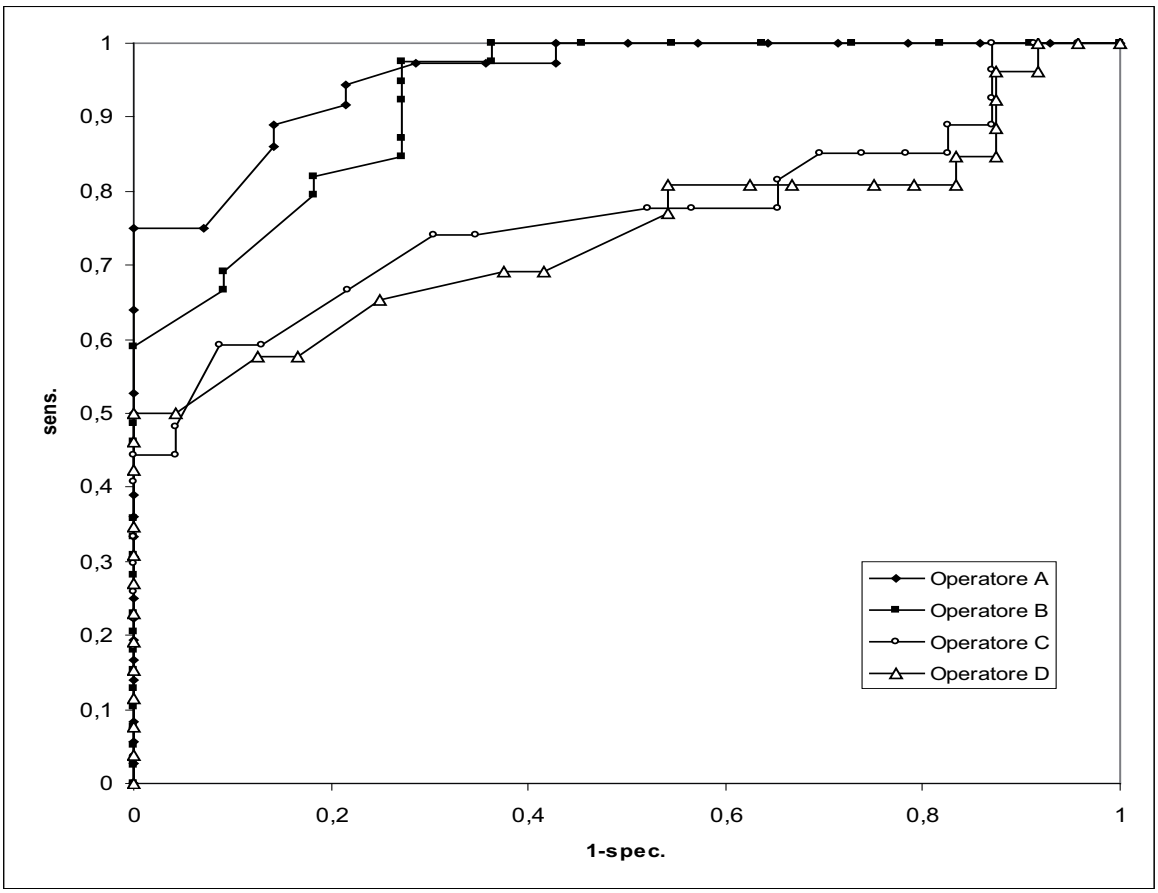

Fig. 1 Curve ROC relative alle stime dei quattro operatori A, B, C e D.

L'accuratezza è graficamente espressa come l'area (AUC = Area Under the Curve) al di sotto della curva ROC e permette una valutazione globale dell'accuratezza della classificazione, indipendentemente dal valore soglia definito a priori (nel nostro caso, almeno il 30% degli apici micorrizati con *Tuber* sp.)

Di seguito sono riportate le classi di merito in cui può rientrare l'AUC rispetto ai suoi valori secondo quanto riportato in letteratura (Siegel e Castellani, 1992):

- 0.90-1 = eccellente
- 0.80-0.90 = buona
- 0.70-0.80 = sufficiente
- 0.60-0.70 = povera
- 0.50-.060 = nulla

Per cui, come riportato in tab. 2, l'accuratezza degli operatori A e B è eccellente, mentre quella degli operatori C e D è solo sufficiente.
Tramite le curve ROC l'operatore può inoltre valutare il tipo e l'entità dell'errore che sta commettendo. Ad esempio nel paragrafo precedente abbiamo mostrato che l'operatore A e B sono molto più sensibili che specifici, cioè che tendono a valutare correttamente le piante valide, ma talora giudicano come non valide piante con un grado di micorrizazione pari o superiore al 30%, ma non siamo stati in grado di valutare l'entità dell'errore. Dalla curva ROC si può ricavare la soglia (Cut-off) dell'operatore, ossia il valore del grado di micorrizazione con cui giudica la piantina valida, mediante stima visuale e confrontarlo con quello prestabilito (30%). IL cut-off viene definito geometricamente, come la distanza minima tra il punto (0;1) - cioè dove è massima la sensibilità e non si inserisce alcun falso positivo - e i punti della curva ROC. A questo punto l'operatore considerando questa sua tendenza a sovrastimare o sottostimare il grado di micorrizazione potrà correggersi fino a quando il suo cut-off sarà prossimo o uguale a quello prestabilito. Infatti sarà possibile confrontare il proprio cut-off (ottenibile, come già detto dall'analisi della curva ROC) e quello teorico, cioè quello definito dal metodo quantitativo di riferimento.

Confronti fra stime
Il confronto fra le stime si rende necessario quando lo stesso operatore vuole monitorare la sua accuratezza nel tempo e quando si desidera confrontare l'accuratezza di diversi operatori. Il grado di concordanza tra due stime effettuate sullo stesso campione si può valutare con il coefficiente K:

$$k = \frac{p_0 - p_e}{1 - p_e}$$

$$s_k = \sqrt{\frac{p_o(1-p_o)}{N(1-p_e)^2}}$$

dove:
- k indica il coefficiente di affidabilità fra le stime
- P_o la proporzione di valutazioni concordanti tra le stime
- P_e la proporzione di giudizi casualmente concordanti
- σ la deviazione standard di k
- N il numero totale dei campioni

Essendo il valore di k compreso tra 0 e 1, il massimo accordo possibile tra due stime corrisponde con k = 1, mentre l'accordo è nullo con k = 0.
Per ottenere una interpretazione univoca di K come stima di accordo, si utilizza la seguente scala di giudizio (Siegel e Castellani, 1992):

K	**Grado di concordanza**
< 0,00	nulla
0,00-0,20	debole
0,21-0,40	appena sufficiente
0,41-0,60	moderata
0,61-0,80	buona
0,81-1,00	ottima

Tab. 3 - Confronto fra i quattro operatori (A, B, C, D) utilizzando il coefficiente Kappa di Choen (1960)

	a	b	c
a			
b	0,67		
c	0,54	0,41	
d	0,27	0,06	0,24

In tabella 3 sono riportati i valori di concordanza fra i quattro operatori. Come si vede la concordanza fra i due operatori esperti 1 e 2 è buona (K = 0,68), mentre tra gli operatori esperti e inesperti la concordanza è molto più bassa.
Nel caso di stime concordanti come ad esempio nel caso degli operatori 1 e 2 è possibile, utilizzando il K di Cohen (1960) (grado di concordanza), validare uno dei due operatori posto che l'altro abbia ottenuto una AUC elevata, superiore a 0,8.

Valutazione dell'intervallo di confidenza del lotto
Una volta definito se l'operatore è accurato nelle sue stime si può calcolare l'intervallo di confidenza (al 95%) relativo al lotto, mediante il metodo esatto di Miettinen per proporzioni (1970).
Il limite inferiore (L_1) e il limite superiore (L_2) dell'intervallo di confidenza del lotto si calcolano come

$$L_1 = \frac{X}{X + (n - X + 1)F_{a\,/\,2,v1,v2}}$$

$$L_2 = \frac{(X+1)F_{a\,/\,2,v1',v2'}}{n - X + (X+1)F_{a\,/\,2,v1',v2'}}$$

dove:

$v_1=2(n-X+1)$ $\quad$ $v1'=2(X+1)$
$v_2=2X$ $\quad$ $v2'=2(n-X)$
$\alpha/2=(1-0.95)/2$ $\quad$ $\alpha/2=(1-0.95)/2$

dove:
X=numero di piante valide del campione
n=numero totale di piante del campione
N=numero totale di piante del lotto
$F_{\forall/2,v1,v2}$ è il valore della distribuzione $F_{0.025}$ a v_1 e v_2 gradi di libertà.
Il lotto preso in considerazione ha i seguenti intervalli di confidenza sulla base delle stime effettate dall'operatore 1 e dall'operatore 2 (i soli due operatori accurati, vale a dire che hanno una capacità di discriminazione, cioè l'AUC, maggiore 0,90, tabella 2):
Operatore 1: proporzione campionaria: 0,72 (I.C.; lim. sup = 0,82; lim. inf = 0,57)
Operatore 2: proporzione campionaria: 0,76 (I.C.; lim. sup = 0,85; lim. inf = 0,61)
L'intervallo di confidenza della proporzione di piantine valide definisce in quale intervallo ricade (nel 95% dei casi) la proporzione di piantine valide del lotto (in questo caso formato da 1000 piante). Ovviamente, più piantine si campionano, minore sarà l'incertezza della stima di piantine valide. Tale incertezza dipende anche dalla proporzione vera di piantine valide; essa sarà massima se la proporzione si aggira intorno al 50%,mentre sarà significativamente minore quando la proporzione sarà vicina allo 0 o al 100%. Al fine di utilizzare correttamente

l'intervallo di confidenza, occorrerà definire a priori il numero di piantine da controllare per lotto. L'intervallo di confidenza potrà essere utilizzato al fine di definire se un lotto sarà valido o meno; infatti se l'intervallo di confidenza racchiude la percentuale minima (da definire a priori) di piante valide, il lotto sarà validato; in caso contrario il lotto non sarà considerato valido ai fini della tartuficoltura.

Conclusioni

Gli strumenti statistici descritti (curve ROC) forniscono un nuovo approccio per la stima della validità di un campione di piante micorrizate con tartufo mediante una valutazione visuale. Essi infatti sono in grado di misurare l'abilità del validatore misurandone l'accuratezza mediante l'applicazione delle curve ROC che prevedono il confronto tra le valutazioni "ad occhio" e quelle ottenute dall'applicazione del metodo di riferimento (basato su conteggi degli apici). L'operatore, una volta validato, sarà quindi in grado di eseguire ulteriori stime con il solo metodo visuale. Inoltre sulla base delle stime effettuate su di un campione limitato di piantine è possibile definire l'intervallo di confidenza della proporzione di piante valide del lotto, riguardo il loro grado di micorrizazione. Il metodo visuale proposto offre una riduzione considerevole dei tempi di controllo, superando le limitazioni temporali proprie dei metodi basati su conteggi degli apici radicali, mantenendo altresì il rigore statistico.

Questo metodo serve a validare se un operatore è in grado di effettuare una corretta valutazione visuale, indipendentemente dal metodo quantitativo di riferimento utilizzato e pertanto può essere impiegato per validare piantine giudicate idonee alla tartuficoltura sulla base dei criteri di uno qualsiasi degli altri metodi già utilizzati senza concretamente applicarli (Bencivenga *et al.,* 1987; Govi *et al.,* 1995; Fischer e Colinas, 1996; Palazon *et al*., 2001; Reyna *et al.,* 2002).

Inoltre questo metodo può essere utilizzato per il controllo di piantine micorrizate con qualsiasi combinazione *Tuber* spp.- pianta ospite. Tuttavia al variare delle specie (fungina o vegetale) sarà necessario rieseguire la validazione del valutatore sulla base delle nuove condizioni.

La stima visuale con il metodo qui riportato è già utilizzata dai vivai A.S.S.A.M. (Agenzia per i servizi nel settore agroalimentare della Regione Marche) ed è stato approvato con decreto n. 139 del 06-12-2004 dell'Amministatore Unico. Questa metodologia basata sull'uso delle curve ROC per la sua semplicità operativa e rigore scientifico potrebbe trovare in futuro una più ampia utilizzazione sia in Italia sia in altri paesi europei ed extraeuropei nel controllo delle piante micorrizate con tartufo.

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KEEPING THE EARTH EGGS IN DIFFERENT BASKETS: THE FUTURE OF DESERT TRUFFLES IN QATAR

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Abstract
The State of Qatar has undergone rapid societal changes over the last 60 years due to the discovery of vast on- and off- shore oil reserves. The changes wrought by this new-found wealth have had significant effects on the nation's economy, health, and environment. The impacts of industrialization on Qatari traditional culture have not been thoroughly documented in a scholarly way – although one can imagine the changes resulting from the shift from an economy based on camel herding, pearl diving, and fishing to an economy based on oil and gas.
One Qatari cultural practice that has remained intact is hunting for desert truffles. Telephone interviews of Qatar Nationals revealed that almost 71% of the population hunts for desert truffles. 88% of this sample goes truffle hunting as a family activity. Desert truffles, belonging to the genera *Terfezia* and *Tirmania*, are native to Qatar, and grow in association with the roots of the desert sunflower, *Helianthemum spp*. This research also suggests that *Phaeangium lefebvrei*, previously undocumented here, also grows in Qatar. The desert truffle's geographic range extends across the Mediterranean to the Middle East. Their management and associated cultural knowledge constitutes a Traditional Ecological Knowledge (TEK) system that has been managed for food and medicine since time immemorial in Qatar.
As plant symbionts adapted to a hypogeous habit, as a food source for desert animals in a nutrient poor habitat, and most importantly their role as a fulcrum for the transmission of Qatari TEK, they are ideal candidates for the restoration of both the desert ecosystem and to reinforce the Qatari's threatened cultural relationships to this damaged and fragile landscape.
Key words: desert truffles, Traditional Ecological Knowledge (TEK), natural resource management, desertification.

Introduction: overview of Qatar's biogeography, economy, and culture

Qatar is a peninsular country with an area of 11,437 km². Qatar is an extremely hot and arid landscape, with July temperatures consistently above 45˚C and receiving an average annual precipitation of 81mm (Richer, 2008). Qatar achieved independence in 1971, prior to that it was a British protectorate. Qatar's economy was based on pearl-diving, fishing, livestock and trade prior to 1949 (Gotting, 2006) at which point oil exports increased steady to the point that Qatar is now one of the world's oil exporters and has the world's highest per capita income in the world at 67,000 USD. According to the World Bank, Qatar's 2006 population was around 828,000 people, roughly 20% of which are nationals, the remainder are ex-patriate workers (Ratha and Xiu, 2006).
The development of Qatar's oil industry has had significant impacts on the traditional way of life of both Qatar's nomadic *Bedouin* and non-nomadic peoples. Many middle aged contemporary Qatari's remember growing up in tents in the desert. Now, although families make trips to the desert and may camp for weeks at a time, they are mostly settled in Qatar's capital city, Doha. An anthropologist visiting Qatar in 1984 reported that there were Sudani nomads (of distant Gulf origin) living in tents near the town of Al-Khor, north of Doha. He also reported that Qatar's Bedouin families still retained a connection with their nomadic cousins in Saudi Arabia (Ingham, 1999-2000). Indeed, the primary author (KW) has evidence, through informal conversations with local Bedouin people, that they often spend periods of time with nomadic cousins in Saudi Arabia enjoying the desert (hunting, collecting truffles, camping, looking after

camels and sheep). The extent to which industrialization has affected Qatari culture has not been explored in a scholarly manner.

Qatar has at least 9 inland habitat types and at least 5 coastal habitat types based on composition of plant communities and site physical characteristics (Abulfatih *et al*., 2002). Of the 9 identified inland habitat types, 5 support the growth of *Helianthemum* spp. (*H. lippii* and *H. kahiricum*) a known host of desert truffle species. Qatar's National Biodiversity Strategy and Action Plan published in 2004 indicate that Qatar hosts 371 species of plants and 142 species of fungi. The confirmed desert truffle species growing in Qatar include *Tirmania nivalis,* locally known as *Zubaidi* and *Terfezia clavery,* locally known as *Kholasi* (Abulfatih *et al.,* 2001). Desert truffles, their host plants, and many other members of Qatar's terrestrial ecosystem are under threat from the effects of desertification.

Desertification, or the degradation of arid lands, affects slightly less than 100% of Qatar's total area (Shakhatra, 1987). Desertification is characterized by loss of soil structure, reduction in soil fertility and in extreme cases the loss of topsoil itself. These problems are compounded by loss of biodiversity and pollution due to air and waterborne particulates. Once degraded, desert ecosystems can take hundreds of years to recover. A recent review by Renee Richer (2008) identifies some of the causes of desertification in Qatar including: pressure from overgrazing, 4x4 off-road recreation, increasing population demands, and industrial development. Climate change could also be added to this list.

Methodology and Results: Desert Truffles in Qatari culture

Sampling Methodology

Telephone interviews were conducted during April-June 2008 in order to study local knowledge of desert truffles among adults 18 years old or older living in Qatar. Interviews were scheduled either in the morning hours (9am-12 noon) or in the evening hours (4pm-9pm) seven days a week. Up to six 'call-backs' were used to reach any one household in the sample. The interviewers were students from Qatar University and College of North Atlantic-Qatar, who were trained on how to conduct the interviews. A structured Arabic/English questionnaire was developed and read over the phone to the respondent. The interview duration ranges between 15-25 minutes.

The 2007 phonebook was used as a frame, which was the most recent phone directory issued at the start of the data collection phase. To recover our frame from the possible missing telephone numbers such as private (secret) and new numbers, the "plus 1 method" (Landon and Banks, 1977) is used to sample from this X-directory. If one of the multiple-phone-line households which are listed under the same family member name was sampled, just one line is randomly selected. It is assumed that the percentage of multiple-phone-line households under different family member names for each line is negligible. In addition, it is assumed that non-telephone-households percentage in Qatar is negligible.

Two samples were selected from the phonebook. The first sample is a systematic sample from all the households in Qatar (n_1=300, margin of error~7%), while the second sample is a simple random sample of the Qatari households in the phonebook (n_2=60, margin of error~15.5%). Sample size and margin of error determination was calculated assuming the 5% significance level, maximum variance of the response in the population, and an estimated level of response rate of 66% (in light of a pretest). Under the assumption of 48% of the population are having private or new numbers, n_1 is split into 157 phone numbers from the phone book directory and 143 elements from the X-directory. Table 1 provides the AAPOR's (American Association for Public Opinion Research) response, cooperation, refusal and contact rates (category 3) for the two samples.

Table 1: American Association for Public Opinion Research Rates for Sample 1 and Sample 2

Rate (category 3)	Sample 1 - Residents and Qatari	Sample 2 - Qatari
Response Rate	0,263	0,438
Cooperation Rate	0,517	0,774
Refusal Rate	0,436	0,219
Contact Rate	0,985	0,969

In the contacted household, once an adult is reached on the phone, the interviewers used a modified version of the YMOF - Youngest Male or Oldest Female (Gaziano, 2005) method to select the respondent with whom the interview will be performed. The YMOF is one of the respondent selection methods available in the literature which helps in the representation of the males in the sample. The method picks the youngest male 18 years or older who is home at the time of the call, if this person is not home, the oldest female 18 years or older is chosen. If neither is at home the interview is rescheduled. During the pretest it became clear that this method is not suitable for a conservative population like the Qatari one. It was unacceptable for an interviewer to ask about an opposite-gender respondent. Therefore, a slight modification has been done to the original YMOF method. The interviewer read the following selection phrase at once "I would like to speak to the **youngest** male, 18 years or older, who is now at home, or the **oldest** female 18 years or older, who is now at home**".** In the original method, the interviewer would read the first part first then if the male is not home the second part is read**.**

Results

Demographics of the samples

Sample 1: The age of the respondents was between 18 and 55 with median around 33 and inter-quartile-range (IQR) of 12 years. Female proportion is 53.4%. Qatari nationality is almost 47%. Bedouin proportion is 40%. Almost 85% of the sample has at least secondary education.

Sample 2: The age of the respondents was between 18 and 60 (outlier at 70) with median around 35 and IQR of 17 years. Female proportion is 72%. Bedouins proportion is 41.7%. Almost 87% of the sample has at least secondary education. 61% of the respondents have income level of at least 12,000 QR (3,400 USD) per month.

It became obvious that truffle hunting is a Qatari hobby. In Sample 1 the percentage of trufflers was almost 35%, while in Sample 2, the Qatari sample, it was almost 71%. Most of the respondents reported that the Zubaidi truffle grows in Qatar (~92% in Sample 1 and 100% in Sample 2). The Kholasi truffle was mentioned as the second type of truffle with ~68% in Sample 1 and 11% in Sample 2. Houbri and Amlasi are two other types that were mentioned but with very low percentages (3% and 2% respectively in Sample 1, and 6% and 2% respectively in Sample 2). Truffles are collectively known in Qatar as fag'aa – *that which emerges from the earth*, or less commonly bayd al ard - *earth eggs*.

Zubaidi is also the most hunted truffle type as reported by respondents (~86% in Sample 1 and 75% in Sample 2), while Kholasi is the second hunted truffle type (~45% in Sample 1 and 65% in Sample 2).

It seems that truffle hunting is a recreational and cultural activity and not an economic endeavor. Only one respondent among the 22 hunters found in our Sample 1 agreed that he sells the Zubaidi truffles that he gathered. Similarly, in Sample 2, only one respondent out of the 17 hunters reported that he sells the Zubaidi and Houbri truffles that he gathers. Reporting Houbri here as a sold type was strange since all respondents agreed that this type is not usually eaten by humans. Zubaidi is the most popular type eaten by people (82% in Sample 1, ~88% in Sample 2). Kholasi ranked second in popularity for food (55% in Sample 1, ~65% in Sample 2). Overall, Zubaidi is the most preferred type for food (~86% in Sample 1, 82% in Sample 2). for its good taste (88.8% in Sample 1, ~86% in Sample 2), cleanness (11.1% in Sample 1), smell (5.5% in Sample 1, ~21% in Sample 2), size (5.5% in Sample 1) and color (7% in Sample 2).

Truffle hunting is typically a family activity (68% in Sample 1, 88% in Sample 2). However, some respondents indicate that they hunt for truffles primarily with men of the family (36% in Sample 1). Finally, few people hunt truffles with their friends (only 27% in Sample 1 and 18% in Sample 2).
The "Hima" system of land management was practiced across the Middle East for over a thousand years. It involved herders moving their animals from one place to another so that good grass will grow again (Kilani *et al*., 2007). 10% of the respondent in Sample 1 believed that truffles were part of a "Hima" system in Qatar, while 40% disagreed with this. In Sample 2, only 23.53% reported that truffles were part of a "Hima" system, while 35.29% disagreed with this. The remaining proportion did not know this information.

Discussion: how truffles can address Qatar's challenges
Qatar is a signatory party to both the UN Convention to Combat Desertification (UNCCD) and the UN Convention on Biodiversity (CBD) and has developed national strategies to cope with the latter. Two of these strategies, identified in the *National Biodiversity Action Plan*, include: the identification and establishment of protected areas capable of generating economic return for rural development, and the conservation of agro-biodiversity and the promotion of sustainable development in rural areas as a means to combat desertification (Scenr, 2004).
Qatar's General Secretariat for Development Planning (2008) published *Qatar National Vision 2030*. This document outlines the strategy for economic and social development in Qatar for the next 20+ years. The National Vision identifies several important directions, two of which include: the integration of modern life with the preservation of local culture and traditions, and sustainable economic development that includes the protection of the environment for present and future generations.
Three areas of national priority can be identified from the *National Biodiversity Action Plan* (Scenr, 2004) and the *Qatar National Vision 2030* (2008); these include:

1. Threats to traditional culture and values from globalization.
2. Desertification and the loss of biodiversity.
3. Sustainable economic growth.

Qatari society has shifted, over the last sixty years, from a primarily nomadic way of life of material poverty and hardship to a settled existence in a post-industrial context due to the exploitation of extensive oil and gas reserves. This shift has affected Qatari society and culture in many ways, of particular relevance is Qatari people interacted with the desert landscape as a matter of necessity prior to the development of the oil industry. Food, medicine, shelter, some clothing, and more were obtained from the natural environment. Contemporary interactions with the desert landscape are limited in scope and frequency to primarily recreational activities such as camping, off-road recreation, truffle collecting, and falconry. Of these activities truffle hunting and camping are the only family activities, the remaining two are exclusive male domains. According to the *Qatar National Vision 2030* (2008), traditional relationships based on trust and 'deep-rooted' social values are being challenged by economic and social progress. These values are augmented across Qatari society by the participation in traditional family-based activities, such as truffle hunting (68% of Sample 1 and 88% of Sample 2 hunt truffles with their families). Conservation of existing, and restoration of historical, wild truffle habitat would serve as the basis for the protection of Qatari traditional desert knowledge and practices.

Desertification is characterized by loss of soil structure, reduction in soil fertility and in extreme cases the loss of topsoil itself (Bainbridge, 2007). These problems are compounded by loss of biodiversity and pollution due to air and waterborne particulates. Once degraded, desert ecosystems can take hundreds of years to recover. As mycorrhizal fungi, truffles play an important role in the development of ecosystem structure and maintenance of healthy ecosystem function (Smith and Read, 1997). Several studies have demonstrated that some mycorrhizal fungi help to improve soil physical properties such as aggregation and porosity, (Caeser-TonThat, 2002; Rillig and Mummey, 2006) and influence soil fertility (Smith and

Read, 1997). Truffle spores are disseminated by animal vectors. In some cases, truffle biomass constitutes the primary component of some animals diets (Trappe *et al.*, 2007). Our interviews are the first to suggest that, *Phaeangium lefebvrei,* a small truffle found in Kuwait, is also present in Qatar. This truffle, known regionally as *Houbri* (Mandeel and Al-Laith, 2006) has been identified as a source of food for eleven species of birds in Kuwait (Alsheikh and Trappe, 1983). In these ways and probably more, truffles are keystone species in the maintenance of ecosystem health. There is no published research on the effects of desert truffle mycorrhizae on degraded desert soils, and very limited field ecology research on the role of desert truffles in the ecosystem. Our preliminary results suggest the possible existence of two new truffle records for Qatar - *Phaeangium lefebvrei* (known as *Houbri* in Qatari dialect Arabic) and the unknown *Amlasi;* which could be a new species, a new record, or a synonym for an already documented species in Qatar. These findings should be explored further.

Oil and natural gas comprise 62% of Qatar's economy (Ratha and Xiu, 2006). According to Khan (2002), Qatar has the world's third largest natural gas reserves and the most rapidly growing economy in the Middle East with most recent estimates of annual GDP growth at 21%. *Qatar's National Vision 2030* (2008, p8) emphasizes the importance of economic development that does not compromise the ecological integrity of this region: "*Economic development and protection of the environment are two demands neither of which should be sacrificed for the sake of the other*". This goal could be achieved through the employment of environmentally friendly technologies in the oil and gas industry, but also through the development of a sustainable agricultural industry based on local, desert-adapted crops, such as desert truffles.

The Hima system of land management is a community-participatory conservation framework that could address all three of the aforementioned national priorities. The Hima pre-dates Islam, has been practiced for over 1400 years in the Arabian Peninsula, and is the possibly the oldest recorded system of natural resource conservation on the planet (Llewellyn, 2003). It is community managed conservation area that regulates activities such as grazing, hunting, etc. so that range animals can be sustainably pastured on a rotational basis, local people can collect medicinal plants, and local beekeepers have sufficient flora for honey production. Himas across the Arab world have fallen from favor since the 1930s due to the centralization of government and subsequent loss of local control of resources. The Hima is experiencing a revival, however, most notably in Lebanon. The Hima Kfar-Zabad has been established in the West Bekaa Valley, in an area noted for its rich avian biodiversity (Kilani *et al.,* 2007). The Hima Kfar-Zabad provides benefit to local farmers through availability of managed local forages, tourism opportunities, and more. Our results indicate that 10% of the Sample 1 respondents, and 23.53% of the Sample 2 respondents believed that truffles were part of a Hima system in Qatar. Although the Hima is actively practiced in Saudi Arabia, this is little evidence that it was practiced in Qatar (Serhal, 2008). These results are exciting and need to be verified and explored futher.

The protection of existing wild truffle beds under the auspices of a Hima system, the restoration of degraded truffle habitat through outplantings of inoculated host seedlings as well as concerted efforts to encourage the transmission of truffle cultural knowledge through field education programmes could serve to reverse the effects of desertification, to stimulate a new agro-industry and agro-tourism, and to protect existing Qatari cultural knowledge and practices associated with the desert.

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LA COLTIVAZIONE DI *TUBER MACROSPORUM* VITTAD.

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Abstract: *Tuber macrosporum* Vittad. cultivation
In 1996, at Roè Volciano (Brescia, Italy), a truffle plantation was made using plants mycorrhized with *Tuber macrosporum* Vittad.; after five years, in 2001, some plants have started producing.
Fruit-bodies were non collected until 2007, when 70% of the plants had become productive, and the plantation had achieved a total shading, a condition required by this species. The truffle harvest started in the middle of June and ended in December. In 2008, part of plantation was tilled, to favour the formation of bigger fruit bodies as compared to those initially produced.
Key words: truffle production, harvest period, *Tuber macrosporum* Vittad.

Premessa

Tuber macrosporum Vittad. (Fig.1) è una specie di tartufo poco diffusa in Italia, poco conosciuta dai consumatori, ma di ottimo valore gastronomico. I corpi fruttiferi sono generalmente di modeste dimensioni con un profumo che richiama in tono minore quello del tartufo bianco pregiato, inoltre, sono dotati di un buon grado di conservabilità che li mantiene integri per lungo tempo.

Fig. 1 – Corpi fruttiferi di *Tuber macrosporum* Vittad.

Allo stato naturale vive negli ambienti tipici del tartufo bianco, pur non disdegnando ambienti con terreni argillosi bruno-rossastri, con limitata presenza di carbonati, a condizione che sia garantito un discreto grado di umidità ed un buon ombreggiamento.
Le tartufaie naturali sono site principalmente nei fondovalle in terreni con esposizione prevalente

a nord. E' stato raccolto in simbiosi con *Corylus avellana* L., *Ostrya carpinifolia* Scop., *Quercus robur* L. *e Carpinus betulus* L. ad una altitudine che oscilla dai 95 ai 900 mt. s.l.m. Fino ad ora non era mai stata tentata la sua coltivazione.

Materiali e metodi

Nella primavera del 1996, piante di *Corylus avellana*, *Ostrya carpinifolia*, e *Carpinus betulus* sono state micorrizate da *Tuber macrosporum* utilizzando sporocarpi autoctoni e le comuni tecniche di micorrizazione che si usano per la produzione delle piante micorrizate con altre specie di tartufo.

Le micorrize sono state ottenute con qualche difficoltà per la scarsa germinabilità delle spore; queste avevano i caratteri descritti in precedenza da vari autori (Giovannetti e Fontana, 1980; Zambonelli *et al*., 1993).

Nel mese di novembre 1996 è stata realizzata una piantagione sperimentale in località Tormini di Roè Volciano, Provincia di Brescia, in un terreno posto ad una altitudine di mt. 180 s.l.m. situato in un fondovalle con esposizione Nord - Est.

Tab. 1 – Caratteri del terreno dove è stata realizzata la tartufaia

Parametri	*Unità di misura*	**Risultato**
Argilla	**%**	23,70
Limo	**%**	17,70
Sabbia	**%**	58,60
Scheletro	**%**	8,70
Calcare totale	**%**	21,00
Calcare attivo	**%**	5,50
PH		7,70
Sostanza organica	**%**	3,80

Fig. 2 – Noccioli con accenno di pianello

Fig. 3 – Tartufaia a 10 anni di età.

L'impianto sperimentale è stato eseguito su una superficie di 500 mq. con 70 piante di essenze miste come descritto in precedenza. Al fine di creare un buon ombreggiamento, l'impianto è stato realizzato su tre filari orientati da est verso ovest, con una distanza di interfila di mt. 3 ed una distanza tra le piante di m 2.
Prima della messa a dimora delle piante, il terreno è stato arato ad una profondità di cm 25. Negli anni successivi all'impianto, le cure colturali sono state limitate a due sfalci annuali dell'erba e a leggere potature di orientamento.
Dopo due anni dalla messa a dimora, alla fine di novembre, sono state prelevate, in tutte le piante, porzioni di radici a 40 cm. dal fusto, per l'esame della micorrizazione. Questo per verificare la permanenza delle micorrize della specie inoculata e l'eventuale presenza di micorrize di altri funghi simbionti.
Tutti i campioni analizzati presentavano micorrize tipiche di *Tuber macrosporum*, esenti da inquinamenti.

Risultati
Già al terzo anno, si notava, attorno alle piante, una stentata crescita della vegetazione erbacea (Fig. 2) che, successivamente, ha dato origine ad un autentico pianello.
La produzione è iniziata dopo 5 anni dalla messa a dimora, (Vezzola, 2005). I primi corpi fruttiferi sono stati raccolti sotto *Ostrya carpinifolia*, successivamente sotto *Carpinus betulus* ed infine sotto *Corylus avellana*.
All'inizio, la produzione era alquanto limitata, riservata principalmente alle piante meglio sviluppate. Negli anni successivi venivano coinvolte, nella produzione, un numero sempre maggiore di piante. Si trattava tuttavia di un numero limitato di sporocarpi, quasi sempre affioranti, che venivano lasciati degradare nella tartufaia per una distribuzione naturale delle spore.
Nel 2006, dopo 10 anni, l'ombreggiamento aveva raggiunto la quasi totalità dell'impianto (Fig. 3) e l'anno successivo veniva raggiunta una copertura totale nelle interfile e parziale nei lati esterni.
Agli inizi del mese di giugno del 2007, si potevano notare gruppi di corpi fruttiferi affioranti di colore rossastro (Fig. 4) sparsi ovunque all'interno delle interfile, mentre nelle parti esterne, ai bordi dell'impianto, la produzione era del tutto inesistente.

Fig. 4 - Corpi fruttiferi immaturi di *Tuber macrosporum* Vittad.

I carpofori erano distribuiti in forma isolata o in gruppi, alcuni anche in numero di dieci, generalmente dalle dimensioni di due – tre cm di diametro, uguali a quelli raccolti in natura. Di dimensioni mediamente superiori erano quelli presenti sotto *Carpinus betulus*. La raccolta nelle interfile, iniziata il 15 di giugno 2007, è proseguita ininterrottamente a cadenza quindicinale fino a dicembre inoltrato). Nel mese di novembre, la formazione dei corpi fruttiferi si era estesa anche nella parte esterna della tartufaia, quella meno ombreggiata.
Considerato che le fruttificazioni erano quasi esclusivamente affioranti per la compattezza del terreno, nel mese di maggio 2008 si è deciso di sottoporre una parte dell'impianto ad una leggera lavorazione meccanizzata ad una profondità massima di otto- dieci centimetri.
Ciò ha causato, nella parte lavorata, un ritardo della produzione: invece di iniziare in giugno è cominciata ai primi di settembre, con una raccolta di corpi fruttiferi in numero minore, ma con dimensioni decisamente superiori, alcuni avevano un diametro di oltre cinque centimetri. Alla fine di dicembre 2008 la raccolta è stata sospesa ed aveva interessato il 70 per cento delle piante con una produzione di oltre 3.500 grammi.

Conclusioni

Questo risultato mette in evidenza l'interesse commerciale che potrebbe derivare dalla coltivazione di questa specie, la quale pur essendo poco conosciuta, è facilmente conservabile e di ottimo valore gastronomico.
Un dato interessante è il periodo di produzione, che va dal mese giugno fino a dicembre inoltrato.
Una considerazione particolare va fatta nei riguardi dei terreni dove cresce che, pur essendo quelli tipici del tartufo bianco, si estende anche ai terreni argillosi bruno rossastri, a condizione che sia garantito un buon grado di umidità e un buon ombreggiamento. Ciò consente di utilizzare molti terreni ecologicamente idonei al tartufo bianco ma con caratteri chimici che ne impediscono la crescita come ad esempio una elevata presenza di ferro.
Si auspica venga valorizzata detta specie in modo da farla conoscere e apprezzare dai consumatori.

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WIRELESS SENSORS NETWORK E LORO APPLICAZIONI IN TARTUFICOLTURA

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Abstract: Wireless sensors network and their application on truffle cultivation
A relevant aspect of the modern truffle cultivation is the opportunity to practice the irrigation in a controlled way. The evolution of climatic conditions plays a determinant role on the correct vegetative development of the symbionts with a direct influence on production in terms of quantity and quality. The possibility of locally monitoring, archiving, analysing and controlling some of the most important ambient parameters such as humidity and temperature both of the air and of the groud, atmospheric pressure and precipitation, wind speed and direction, etc. can be an important instrument to study the relations between them and the optimal conditions of development of the symbionts. The current technological state of the art permits the displacement of interconnected ambient sensors, even on huge areas. Periodically they acquire in situ data and transmit them through a wireless communication to a point devoted to gather, archive and area control. The possibility of visualization, analysis, interpretation and sharing of data and the remote control of the system can now benefit from the widespread and pervasive connection to Internet. Thanks to the relative simplicity of use and implementation the application adapts to a wider diffusion, not only in the academic and in the scientific community, thereby it has the possibility to access to large databases.
Key words: cultivation, wireless sensors network, ambient parameters, irrigation control.

Introduzione

La produzione naturale di tartufi è in costante diminuzione mentre la domanda da parte del mercato è in continuo aumento. La sopravvivenza di questi preziosi funghi è demandata alla tartuficoltura con l'opportunità di realizzare positivi risultati economici.
Dal punto di vista scientifico sono stati studiati i legami esistenti tra le micorrize e la pianta simbionte, così come gli aspetti pedoclimatici in relazione alla specie messa a dimora. Ciò ha reso possibile l'ottenimento di piantine micorrizate su larga scala di eccellente qualità e l'individuazione di siti di impianto ottimali per il tipo di tartufo coltivato. Di pari importanza è stata l'identificazione di pratiche agronomiche tese a favorirne lo sviluppo, la produzione ed il suo incremento nel tempo quali ad esempio la sarchiatura, la pacciamatura, la potatura e l'irrigazione. Il corretto riferimento temporale per l'esecuzione di tali interventi è dato dal ciclo biologico del tartufo, tutt'ora oggetto di studio, fondamentalmente suddiviso nelle due fasi simbiotica e saprofitica. I fattori che inducono alla fruttificazione durante la prima fase non sono ancora del tutto noti mentre si è potuto osservare che all'inizio della fase saprofitica il numero di carpofori presenti nel terreno sono in numero molto più elevato di quelli che effettivamente giungono a maturazione. Durante tutta questa fase il contenuto di umidità e sostanze nutrienti nel terreno determinano la percentuale dei tartufi che giungeranno a maturazione, quindi la quantità e qualità del raccolto.

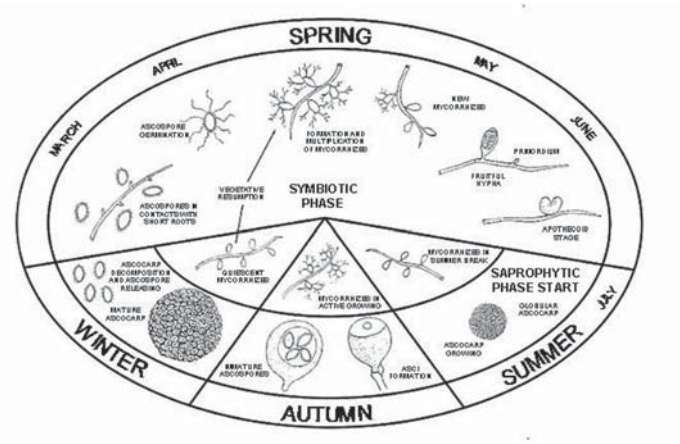

Fig. 1 Ciclo biologico di *Tuber melanosporum* Vittad. (da Granetti *et al.,* 2005)

Intervenire in questa fase con il giusto apporto idrico sia nel tempo che nella misura, come avviene per qualsiasi pratica colturale, equivale a stabilizzare la produzione su livelli ottimi e svincolarsi, almeno in parte, dai sempre più frequenti cambiamenti climatici.
Il fine di questo lavoro consiste nel descrivere uno strumento di monitoraggio e misura di alcuni parametri ambientali quali temperatura ed umidità dell'aria e del suolo, quantità di pioggia, direzione ed intensità del vento, utile all'indagine scientifica e alla conduzione della tartufaia al fine di analizzare i legami che intercorrono tra essi ed i valori di produzione così da poter praticare un'irrigazione controllata.

Materiali e metodi: Wireless sensors network
Una rete di sensori può essere vista come un'insieme di trasduttori, dislocati all'interno di una certa area, in grado di comunicare fra loro e con un'unità centrale che fa da collettore per le informazioni da essi trasmessi. Più specificamente nel caso di sensori ambientali, essi sono dislocati nell'area che si intende sottoporre a monitoraggio e inviano informazioni relative ai parametri ambientali (temperatura, umidità, pressione, quantità di pioggia, etc.) che sono in grado di misurare.

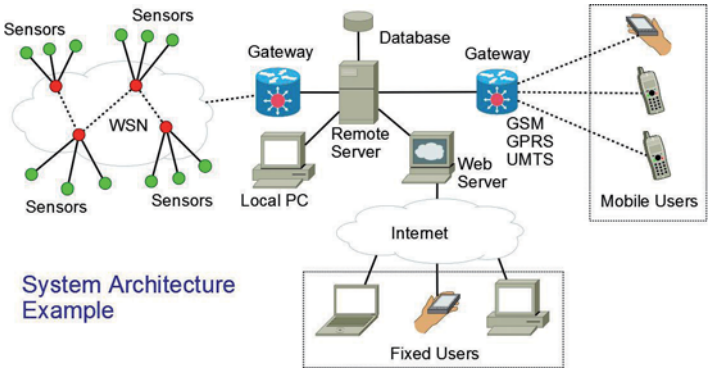

Fig. 2 Architettura di sistema WSN

All'interno del sistema si individuano i seguenti tipi di dispositivi: i sensori, i nodi ed i gateway. I sensori sono specifici per ogni parametro ambientale in esame e sono direttamente collegati ai nodi. Questi ultimi raccolgono le informazioni provenienti da un insieme di sensori e periodicamente le trasmettono con una comunicazione senza fili o ad altri nodi vicini o direttamente al gateway a seconda della configurazione. Il gateway è il collettore finale di tutte le informazioni provenienti dai nodi e rende disponibili i dati organizzati per lo scopo dell'utente finale (archiviazione, visualizzazione, trasmissione, applicazioni web, etc).
Il sistema, attraverso opportuni protocolli è in grado di configurarsi da solo, così la sua

espansione o potenziamento non richiede interventi particolari: ogni nuovo nodo entra a far parte della rete in maniera automatica e inizierà da solo a scambiare i dati.

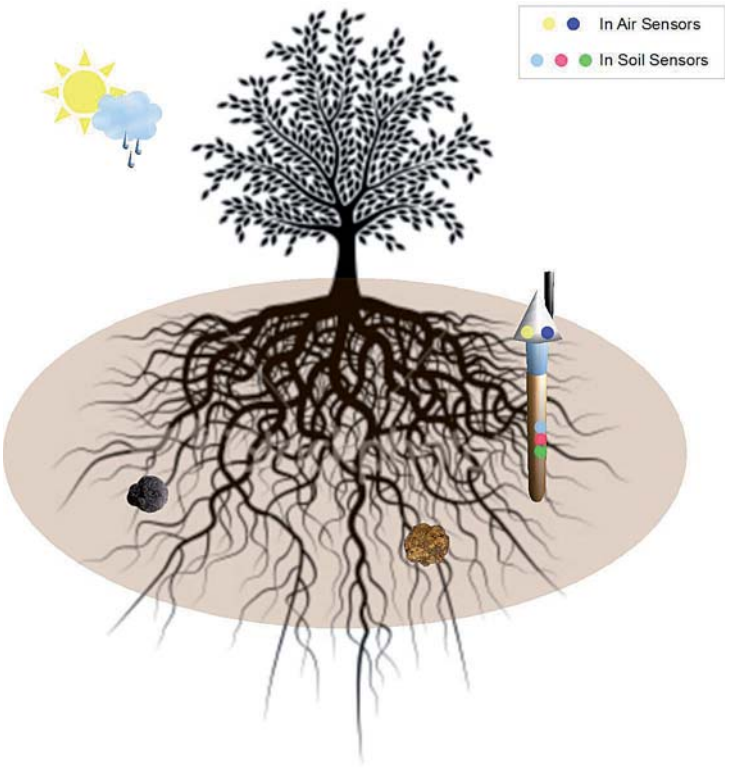

Fig. 3 Schematizzazione di monitoraggio

I vantaggi di una simile applicazione sono riassumibili nella elevata facilità di installazione, espandibilità e praticità di uso, nonché la possibilità di monitoraggio accurato di aree estese. Pur trattandosi di un prodotto di recente commercializzazione e all'avanguardia della tecnologia è economicamente vantaggioso se paragonato ad altre applicazioni.

Fig. 4 Schematizzazione di istallazione

Come esempio di utilizzo di tale tecnologia è stata condotta una sperimentazione presso una tartufaia coltivata e produttiva di *Tuber aestivum* Vittad.. L'impianto, di circa venti anni di età,

ha una estensione di 1 ha con sesto di 5m x 5m; le piante messe a dimora erano dichiarate micorrizate da *Tuber melanosporum* Vittad.. Attualmente circa il 20% delle piante è produttivo di *T. aestivum*. Il controllo della micorrizazione sulle restanti piante non produttive ha dimostrato la colonizzazione da parte di *T. aestivum* e, in alcuni casi, di specie fungine ectomicorriziche non appartenenti al genere *Tuber.*
Nella tartufaia è stata collocata una centralina munita di misuratore di precipitazioni ed è stata registrata la quantità di pioggia giornaliera dal 1 gennaio 2008.
I dati della produzione sono stati verificati mediante sopralluoghi settimanali, con un cane opportunamente addestrato, dal 1 giugno 2008, data di inizio della raccolta, fino al 30 luglio 2008 data di chiusura della medesima. Infine sono stati ricavati i grafici di tali grandezze che mostrano gli andamenti delle misure nel tempo.

Risultati
Nel grafico riportato in figura 5 sono rappresentati i dati di piovosità relativi al periodo Gen – Giu 2008 espressi in mm di pioggia ed i dati della raccolta di carpofori di *T. aestivum* espressi in gr. Questi ultimi sono stati traslati temporalmente di un offset variabile per cercare la migliore correlazione con i dati di pioggia.

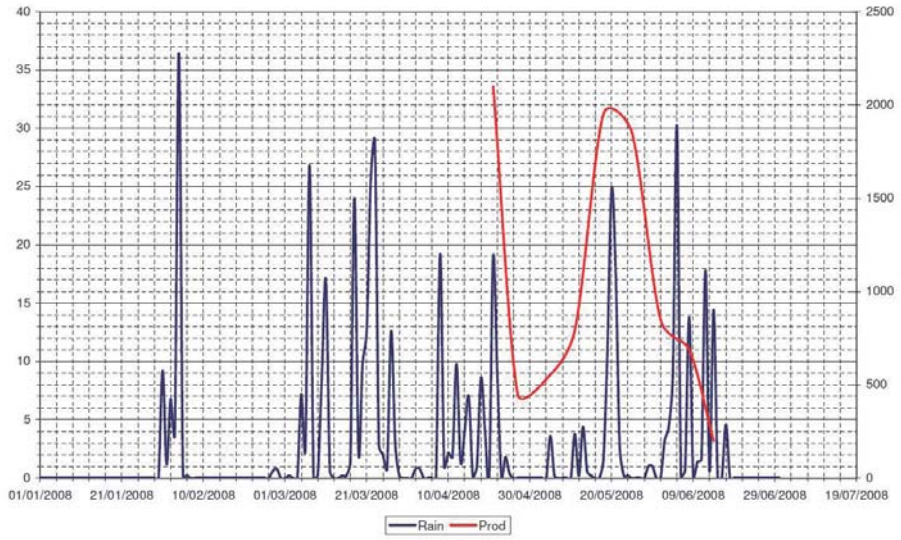

Fig. 5 Grafico temporale piovosità-produzione

Per la situazione particolare in oggetto si può notare una buona relazione tra i grafici in corrispondenza ad un offset di circa 40 giorni. Con questa ipotesi infatti ad un'iniziale picco della quantità di carpofori raccolta, dovuto probabilmente al favorevole andamento della pioggia nel periodo invernale ed inizio primaverile, seguono alcune osservazioni di minimo relativo in corrispondenza della scarsità di precipitazioni del periodo che intercorre tra la fine di aprile e metà maggio 2008. Si registra invece un massimo relativo della raccolta in corrispondenza dell'evento atmosferico di rilievo registrato il 20 maggio 2008 per poi decrescere piuttosto rapidamente in corrispondenza del termine del periodo di maturazione. L'aumento delle osservazioni nel tempo, la misura di ulteriori parametri ambientali localizzati nei punti produttivi, quali la temperatura ed umidità dell'aria e del suolo e l'accesso a dati relativi a molteplici impianti con situazioni pedoclimatiche differenti potrebbero fornire utili indicazioni sul periodo e quantità degli apporti idrici corretti per ogni specie. Nel complesso panorama tecnologico attuale gli sviluppi implementabili nel breve-medio periodo possono essere individuati nell'automatizzazione dell'impianto di irrigazione, la telesorveglianza per allarmi anti-intrusione, l'integrazione di nuovi sensori che si renderanno disponibili e la creazione di data base contenenti dati condivisi sia dalla comunità scientifica che dai tartuficoltori.

Conclusioni

L'impiego delle reti di sensori può essere di aiuto sia alla ricerca scientifica che ai tartuficoltori nel monitoraggio ed interpretazione dei parametri ambientali e del terreno, valutare, avere l'opportunità di mantenere e prevedibilmente aumentare il rendimento della coltura, gestire al meglio le risorse idriche con possibilità di automatizzazione e aumentare così l'efficienza del lavoro.

La possibilità di visualizzazione, analisi, interpretazione, condivisione dei dati e la remotizzazione del controllo del sistema può giovarsi della ormai diffusa e capillare connessione ad internet.

Data la relativa semplicità di uso e messa in opera, l'applicazione si adatta ad una diffusione non esclusivamente dedicata alla comunità scientifica, ampliando in questo modo la possibilità di accesso da parte di quest'ultima a basi di dati molto estesi ed ad ampio spettro.

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COLTIVAZIONE E PRODUZIONE IN UNA TARTUFAIA DI *TUBER AESTIVUM* VITTAD. DI 5 ANNI

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Abstract: Truffle cultivation and production in a 5-year old T*uber aestivum* Vittad. plantation
Up to the middle of the eighties, the truffle cultivation focused on plants mycorrhized with the most valuable truffle species, *Tuber magnatum* Pico and *Tuber melanosporum* Vittad.; no technical analyses of the land truffle production potentialities were undertaken. Over the last decades, both the planning and the cultivation of a truffle plantation have evolved, thanks to the scientific research results on the truffle ecology and on their cultivation.
The present work highlights a truffle cultivation of *Tuber aestivum* Vittad. located in Umbria (Terni) and made in 2003. Before carrying out the truffle plantation, the land owner has contacted a technical expert, who has analysed the area, to choose the right plant-truffle combination to cultivate and to identify the better cultivation techniques to be applied before and after bedding out the plantlets. The truffle plantation comprises 94 truffle-producing plants: *Quercus pubescens* Willd., *Quercus ilex* L., *Quercus cerris* L., *Ostrya carpinifolia* Scop. and *Corylus avellana* L., mycorrhized with *T. aestivum* and certified. Over the years, plants have been watered and pruned, while the soil around them has been weeded, covered on the surface, tilled, and inoculated with *T. aestivum* spores. The truffle plantation has started producing in 2007, and it has also produced in 2008, with a production of about 5 kg of truffle.
It is necessary that the new truffle plantations will be plant after a careful technical analysis, in order to favour the truffle production of the cultivated truffle species.
Key words: Cultivation, *Tuber aestivum*, production results.

Introduzione

Dagli anni ’80, grazie all’utilizzo di piante micorrizate prodotte da vivai specializzati, in Italia, sono state impiantate numerose tartufaie. I tartufi maggiormente coltivati fino alla metà degli anni ‘90 sono stati il *Tuber melanosporum* Vittad. ed il *Tuber magnatum* Pico. Molte di queste tartufaie furono realizzate senza fare indagini tecniche, ottenendo talvolta scarsi risultati.
Sulle basi delle esperienze precedenti in tartuficoltura, dagli anni 90 numerosi studi sono stati condotti per capire meglio la scelta del sito d’impianto (Bencivenga *et al*., 1990; Lulli, 1995; Olivier *et al*., 2002), la scelta della specie forestale e del sesto d’impianto (Baciarelli Falini *et al*., 2000) e le tecniche colturali da applicare alle tartufaie (Chevalier, 1990; Baciarelli Falini e Bencivenga, 2002). Di conseguenza in questi ultimi anni, si stanno realizzando tartufaie tenendo conto della vocazionalità tartufigena del sito d’impianto al fine di coltivare la giusta combinazione pianta – tartufo ed applicare le idonee tecniche colturali alle nuove piantagioni durante il corso degli anni.
In questo lavoro è riportata la storia di una tartufaia coltivata a *T. aestivum*, ubicata in Italia nella regione Umbria in provincia di Terni, impiantata nel 2003, partendo dalle indagini tecniche prima dell’impianto, alla preparazione del terreno, alle cure colturali post – impianto, fino alle prime produzioni.

Indagine tecnica e descrizione dell’appezzamento

L’indagine tecnica, volta a realizzare e coltivare al meglio la tartufaia, è stata condotta da un esperto in tartuficoltura e ha riguardato diversi elementi rilevando dati fondamentali per una più sicura produzione di tartufi.
L’appezzamento è ubicato in Italia nella regione Umbria in provincia di Terni, ha una superficie

di circa 3000 m^2, una altitudine media di 430 m s.l.m., una esposizione ad Ovest ed una inclinazione media di 18°.
Nella zona gravita un clima sub-mediterraneo con piogge frequenti tra l'autunno e la primavera, inverni piuttosto miti o non troppo rigidi e le estati calde e abbastanza siccitose (fig. 1).
In vicinanza dell'appezzamento, la vegetazione arborea prevalente risulta composta da querceti misti con *Quercus pubescens* Willd., *Quercus cerris* L., *Ostrya carpinifolia* Scop., *Fraxinus ornus* L. ecc. e da rimboschimenti a *Pinus nigra* L..
L'appezzamento negli anni passati era stato coltivato a cereali. Nella zona, naturalmente, si raccoglie il *T. aestivum* e *Tuber borchii* Vittad..
Le caratteristiche fisico – chimiche del terreno (tab. 1) evidenziano che si tratta di un suolo con tessitura franco – argillosa con abbondante scheletro e valori del limo e dell'argilla rispettivamente di 46% e 30%. Il pH è sub-basico, basso è il valore percentuale del $CaCO_3$, assente il $CaCO_3$ attivo. Il terreno, inoltre, è mediamente dotato di sostanza organica.

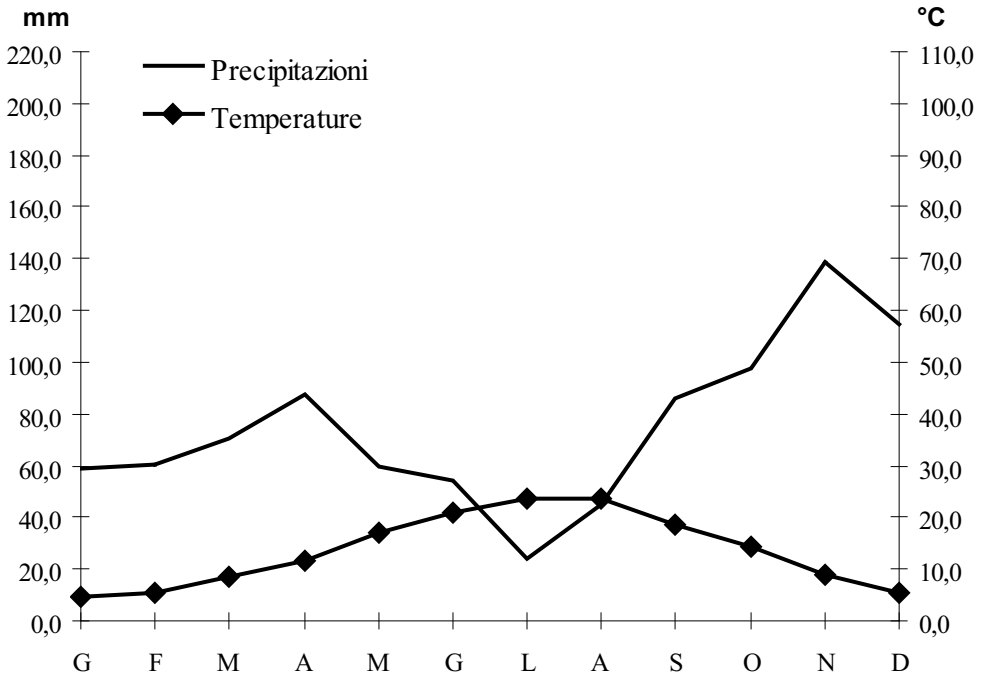

Fig. 1 Diagramma termopluviometrico della zona studiata.

Tab. 1 Analisi fisico-chimiche dell'appezzamento studiato.

Scheletro	42 %
Terra fine	58 %
Sabbia grossa	12 %
Sabbia fine	12 %
Limo	46 %
Argilla	30 %
pH	8,3
Calcare totale	9 %
Calcare attivo	–
Sostanza organica	2,19 %

Realizzazione della tartufaia e pratiche colturali

Dalla elaborazione dei dati raccolti come: clima della zona, natura del terreno (tessitura e mancanza di carbonato di calcio attivo), tartufo raccolto nelle vicinanze ecc., l'appezzamento risulta idoneo alla coltivazione del *T. aestivum* (Bencivenga *et al*., 1990; Tanfulli *et al*., 2001). Le pratiche colturali consigliate prima dell'impianto e dopo la messa a dimora delle piante sono state effettuate dopo una attenta sintesi di numerose esperienze disponibili in letteratura (Ferrara *et al*., 2001; Vinay e Pirazzi, 2001; Baciarelli Falini e Bencivenga, 2002; Granetti *et al.,* 2005).

Prima di realizzare la piantagione, a terreno asciutto, è stata effettuata una aratura ad una profondità di circa 40 cm ed una affinatura delle zolle, per garantire un migliore attecchimento delle piantine tartufigene.

Nel novembre del 2003, sono state messe a dimora 90 piante simbionti micorrizate con il *T. aestivum* e certificate: 42 *Q. pubescens*, 21 *O. carpinifolia*, 14 *Corylus avellana* L., 7 *Quercus ilex* L. e 6 *Q. cerris*, utilizzando un sesto d'impianto 5m x 4m (Fig. 2). Durante il corso degli anni sono state praticate le seguenti cure colturali:

- irrigazione con acqua di pozzo in ragione di 3-5 litri al m^2 ogni 20 – 25 giorni seguendo le piovosità naturali. I primi due anni fu utilizzato un impianto a goccia, mentre negli anni successivi l'irrigazione fu fatta con tubo a mano;
- pacciamatura delle piantine tartufigene con circa 1 m^2 di telo nero plastificato, forato a mano e ricoperto con uno strato di terra di 2-3 cm. Il telo pacciamante fu rimosso dopo 3 anni;
- taglio della vegetazione erbacea 2 – 4 volte l'anno. Tra le file, il taglio dell'erba, fu fatto con decespugliatore, mentre, in vicinanza delle piante venne sradicata a mano;
- una erpicatura tra le file utilizzando un trattore leggero ad una profondità di circa 7 cm ed alla distanza di 1m dalle piante tartufigene nel periodo invernale;
- una sarchiatura manuale intorno alle piante tartufigene con piccola zappa (dopo tolto il telo pacciamante) alla profondità di 3 – 5 cm nel periodo invernale;
- potature leggere di alcuni rami a partire dal secondo anno per ottenere un giusto portamento delle piante;
- inoculo sporale con una soluzione di acqua e spore di *T. aestivum* a partire dal primo anno in vicinanza delle piante. Per ciascuna pianta ad ogni somministrazione, è stata aggiunta una soluzione di 1 litro di acqua e 40 - 50g di *T. aestivum*.

Fig. 2 Tartufaia studiata a 5 anni dalla messa a dimora.

Risultati

Dopo tre anni dall'impianto sono iniziati a formarsi i primi pianelli intorno alle piante simbionti. La piantagione ha iniziato a produrre i primi tartufi estivi nel 2007, a quattro anni dall'impianto; le specie simbionti che hanno prodotto i tartufi sono stati un *Q. ilex* e due *Q. pubescens*. La produzione di *T. aestivum* è aumentata notevolmente l'anno successivo (2008) raggiungendo un quantitativo di circa 5kg di tartufo con esemplari di oltre 100 g.

La crescita delle piante simbionti (tab. 2), dopo 5 anni dalla piantagione, risulta molto vigorosa, *Q. pubescens* presenta un'altezza media di 199 cm, una larghezza media della chioma di 149 cm ed un diametro medio del fusto di 6,4 cm; *O. carpinifolia* ha un'altezza media di 320 cm, una larghezza media della chioma di 214 cm ed un diametro medio del fusto di 9,6 cm. Questa vigorosità è dovuta sia dal terreno fertile, sia dalle pratiche colturali apportate periodicamente con molta attenzione.

Il 70% delle piante simbionti presenta un pianello abbastanza evidente distribuito in maniera eguale su tutte le specie tartufigene presenti.

Per verificare il grado di micorrizazione della tartufaia, ed osservare se nelle radici delle piante tartufigene sono entrati in competizione altri funghi, sono stati prelevati 3 campioni di radici sottoposti ad analisi della micorrizazione (tab. 3). Le micorrize presenti negli apparati radicali delle piante simbionti campionate sono state identificate utilizzando pubblicazioni di vari autori, Zambonelli e Govi, 1990; Zambonelli *et al*., 1993; Granetti, 1995; Granetti *et al.*, 2005. In tab. 3, emerge che in tutte le piante campionate c'è la presenza di micorrize di *T. aestivum*, inoltre sono assenti gli altri funghi. Da questi risultati si evidenzia il buon grado di micorrizazione delle piante campionate ed il buono stato della tartufaia.

Tab. 2 Valori medi dell'altezza, della larghezza della chioma e del diametro del fuasto del 30% delle piante simbionti.

Specie simbionte	Altezza cm	Larghezza della chioma cm	Diametro del fusto cm
Q. pubescens	199	149	6,4
Q.ilex	206	126	6,0
Q. cerris	249	138	6,6
O. carpinifolia	320	214	9,6
C. avellana	182	143	—

Tab. 3 Analisi della micorizzazione di 3 campioni di radici

Numero pianta	Specie Simbionte	% di Micorrize di:			Apici vivi non micorrizati	Micorrize ed apici secchi
		Tuber aestivum	Altri *Tuber*	Altri Funghi		
1	*Q. pubescens*	55	-	-	30	15
2	*O. carpinifolia*	50	-	-	40	10
3	*C. avellana*	45	-	-	35	20

Conclusioni

La tartufaia, realizzata seguendo i consigli forniti da un esperto in tartuficoltura, dopo 5 anni dall'impianto si trova in ottimo stato: piante simbionti vigorose, elevata percentuale di pianelli e produzioni soddisfacenti.

Probabilmente molti degli insuccessi che si verificano nelle piantagioni di tartufo sono dovuti sia alla carenza di risultati scientifici in materia, sia all'errore commesso da molti tartuficoltori

che realizzano le loro piantagioni senza l'aiuto di esperti del settore.
Nel futuro prossimo, per evitare errori di realizzazione e conduzione delle tartufaie, è indispensabile che le nuove piantagioni siano impiantate dopo una attenta indagine tecnica necessaria a facilitare la produzione della specie di tartufo coltivata. Inoltre è fondamentale che la ricerca scientifica venga condotta in campo per concentrare gli studi sulle coltivazioni delle tartufaie e sul comportamento dei simbionti nei diversi contesti pedoclimatici.

Ringraziamenti
Si ringrazia il Sig. Giannetti per aver dato la possibilità di effettuare lo studio sulla sua piantagione.

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LA DISTRIBUZIONE POTENZIALE DEI TARTUFI NEL VALLO DI DIANO (SALERNO)

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Abstract: The potential presence of truffles in the "Vallo di Diano" area (Salerno)

It is usually considered that the presence of truffles in Campania is reduced only to *Tuber mesentericum* Vittad., commonly harvested and eaten in the area of Bagnoli Irpino. This theory is due to the lacking of both scientific and popular papers about truffles of this region. However, it is well known that also the southern regions of Italy are great suppliers of large amounts of truffles for ages. The presence of different species of truffles checked by collectors and mycologists in the "Vallo di Diano" area (Salerno), could represent an important chance of rural development for these territories. In this area it was performed a cartographic survey with GIS software approach to better understand the potential distribution of truffle species in wild environments as well as to find the most appropriate areas for cultivation. The thematic maps of Vallo di Diano have been studied, with reference to the geolitological map, the Corine Land Cover map (CLC 2000) and the Digital Elevation Model map (DEM). The areas with appropriate ecological features for the different truffle species have been delimited in each map collected. Then these maps have been overlaid to estimate the areas where there was the concomitance of the appropriate environmental factors for each species.

The result of the research gave the chance to build *potential truffles' presence map* for every truffle species considered. In these maps is possible to notice that *T. aestivum* Vittad. is the most present and cultivable species followed by the *T. aestivum* Vittad. forma *uncinatum* (Chatin) and the *T. mesentericum* Vittad.. On the contrary *T. borchii* Vittad., *T. magnatum* Pico and *T. melanosporum* Vittad. are localized in smaller areas.

This research shows that "Vallo di Diano" area can be considered as a potential truffles presence area both for wild production and cultivated truffle purposes. Further field investigations will be undertaken with the intent to verify the actual presence of these fungi in the identified wild production areas.

Key words: Truffles, Vallo di Diano, *Tuber mesentericum,* GIS.

Introduzione

I funghi del genere *Tuber* appartengono alla classe Ascomycetes, e tutti vivono in simbiosi ectomicorrizica (Agerer, 1986-97; Agerer *et al*., 1996-01) con specie arboree ed arbustive, principalmente angiosperme e gimnosperme, appartenenti a vari gruppi tassonomici (Norris *et al*., 1994; Hall *et al.,* 2007). Alcune specie producono ascomi commestibili, comunemente conosciuti come tartufi, caratterizzati dal particolare aroma e sapore, molto ricercati e considerati prodotti d'elite della cucina internazionale.

La coltivazione del tartufo è possibile grazie alla produzione, da parte di vivai specializzati, e la messa a dimora di piante micorrizate su terreni idonei in base alle esigenze ecologiche specifiche per la combinazione pianta-tartufo (Granetti *et al.,* 2005).

Ad oggi alcune specie di tartufi sono ampiamente coltivate in ragione di compensare il declino della produzione naturale in Europa (Hall *et al.,* 2001; Hall *et al*., 2003).

Si ritiene, comunemente, che in Campania la presenza dei tartufi sia ridotta al solo *Tuber mesentericum* Vittad. raccolto e consumato soprattutto nella zona di Bagnoli Irpino in provincia di Avellino (Marotta e Varricchio, 2007). Questa opinione nasce dalla carenza di studi scientifici e divulgativi sulla distribuzione di altre specie nel territorio campano. Gli addetti ai lavori, però, sanno che anche le regioni meridionali del nostro paese sono, da molti anni, fornitrici di

grossi quantitativi di tartufi. La presenza di diverse specie del genere *Tuber*, ormai accertata da raccoglitori e micologi anche nell'area del Vallo di Diano in provincia di Salerno (Fig. 1), può rappresentare per queste zone un'importante occasione di sviluppo rurale (De Roman *et al.,* 2006). Nell'area in esame è stata condotta un'indagine cartografica mediante software di elaborazione GIS (Geographical Information System) con l'obiettivo di individuare gli ambienti forestali di produzione naturale e localizzare zone idonee per la coltivazione delle più importanti specie di tartufo commercializzate.

Fig.1 Vallo di Diano, Campania.

Materiale e metodi

- Sono state consultate le normative relative alla raccolta, commercializzazione e coltivazione delle diverse specie di tartufo, in particolare la Legge quadro nazionale n. 752/1985; la Legge Regionale campana n. 13/2006 e relativo regolamento attuativo n. 3/2007. Per la possibilità di ottenere finanziamenti sono stati consultati il Regolamento Comunitario n. 1698/2005 e il Programma di Sviluppo Rurale della Regione Campania 2007 - 2013.
- E' stato condotto uno studio sulla ecologia delle principali specie di tartufo commercializzate in Italia ai sensi della normativa vigente.
- Sono state studiate le carte tematiche del Vallo di Diano, con particolare riferimento alla Carta geolitologica, alla Carta della copertura del suolo (CORINE LAND COVER 2000 di 4° livello), alla Carta delle fasce altimetriche (Digital Elevation Model) ed alle Ortofoto dell'area esaminata.
- Per l'elaborazione cartografica sono stati utilizzati software open - source che operano in ambiente GIS quali: GRASS GIS e Quantum GIS (QGIS) (GRASS, 2008; QGis, 2008).

Risultati

Il processo di elaborazione GIS, condotto attraverso la procedura di overlay grafico dei tre tematismi (Fig. 2) ha reso possibile la realizzazione di un'unica mappa contenente contemporaneamente i parametri geolitologici, vegetazionali e altimetrici dell'area oggetto di studio.

Il file vettoriale ottenuto tramite l'overlay è stato esportato da GRASS GIS (Fig. 3)(GRASS Development Team, 2008) ed elaborato con QGIS per realizzare delle *query*, ossia delle ricerche, tramite il linguaggio Structured Query Language (SQL), all'interno del database ad esso legato (Fig. 4) (Clerici, 2007; Quantum GIS Development Team, 2008).

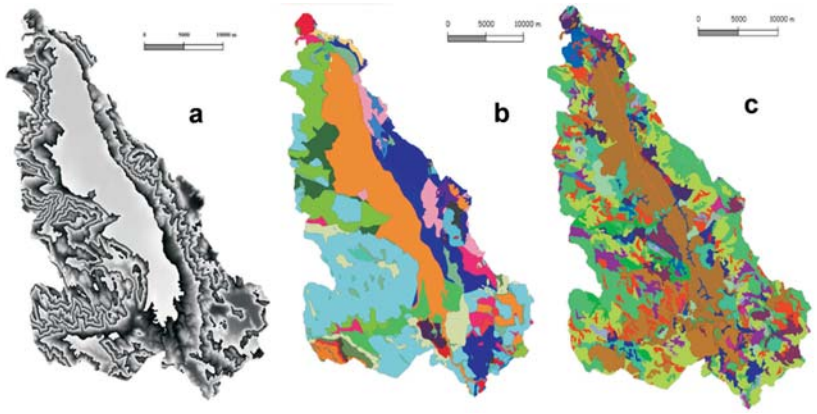

Fig. 2 (a) Digital Elevation Model (DEM) dell'area del Vallo di Diano; (b) Carta Geolitologica dell'area del Vallo di Diano; (c) Carta della copertura del suolo (CLC 2000 – 4° livello) dell'area del Vallo di Diano.

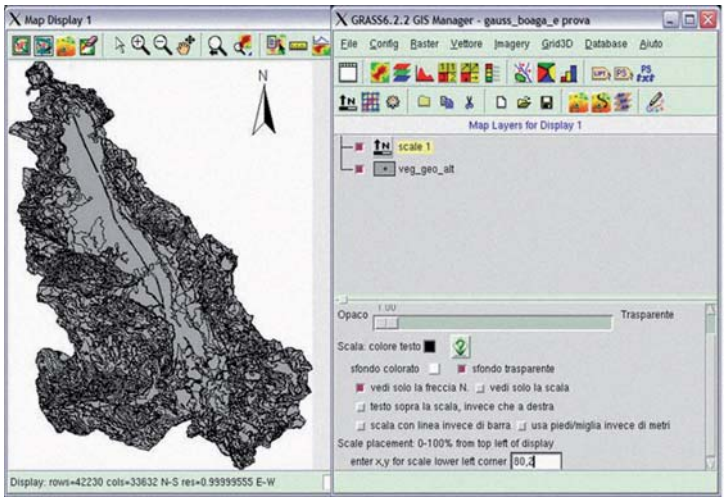

Fig. 3 Overlay del DEM, della Carta Geolitologica e della Carta della copertura del suolo.

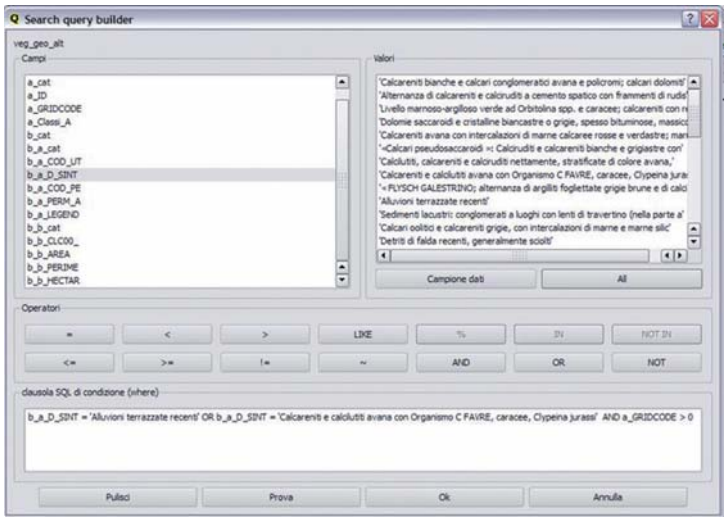

Fig. 4 Realizzazione delle *query* tramite il Search query builder del software QGIS.

Le *query* hanno reso possibile l'individuazione di aree caratterizzate dalla concomitanza dei parametri geolitologici, vegetazionali e altimetrici necessari alla produzione naturale ed alla coltivazione delle diverse specie di tartufo. Tramite questo procedimento sono state ottenute delle *Carte della vocazione tartufigena potenziale* che esprimono, per ciascuna specie, la distribuzione dei potenziali siti di produzione naturale e delle aree maggiormente idonee per la coltivazione (Figg. 5; 6; 7; 8).

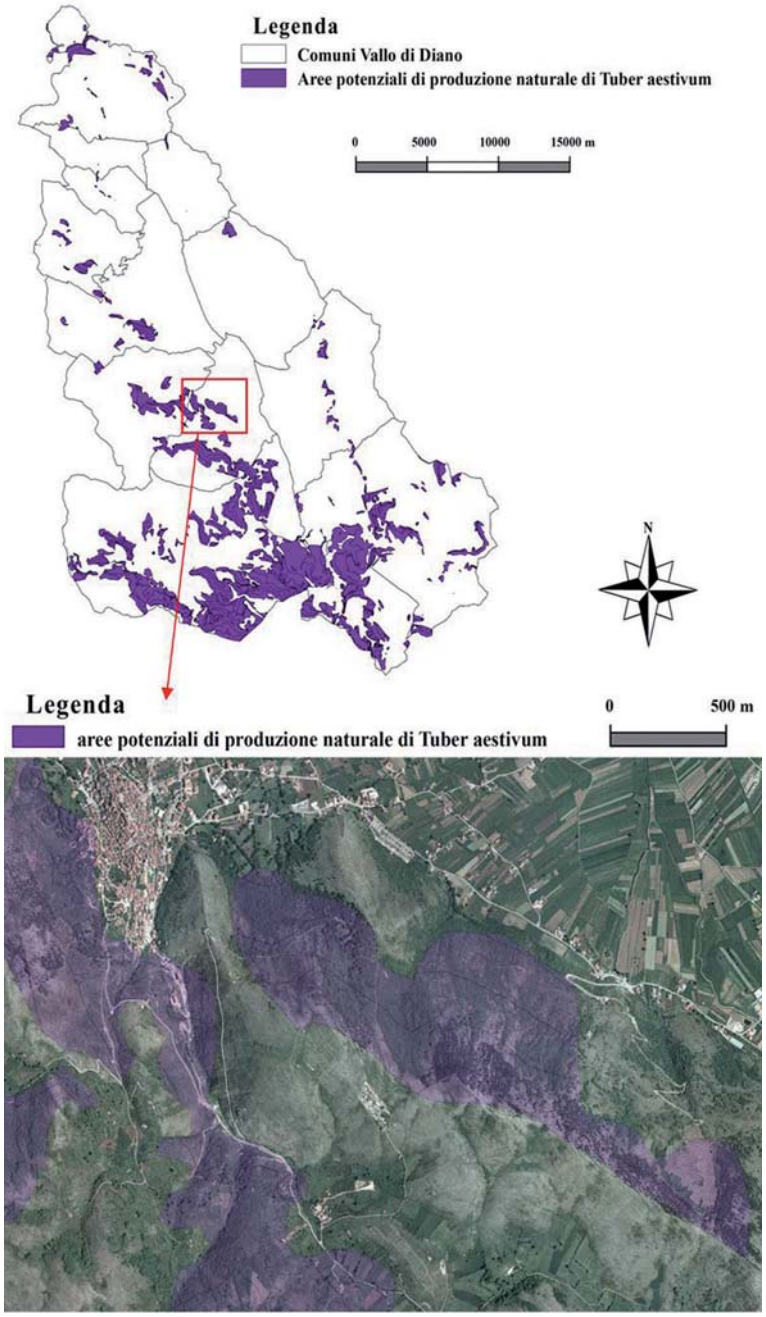

Fig. 5 Aree potenziali di produzione naturale di *Tuber aestivum*.

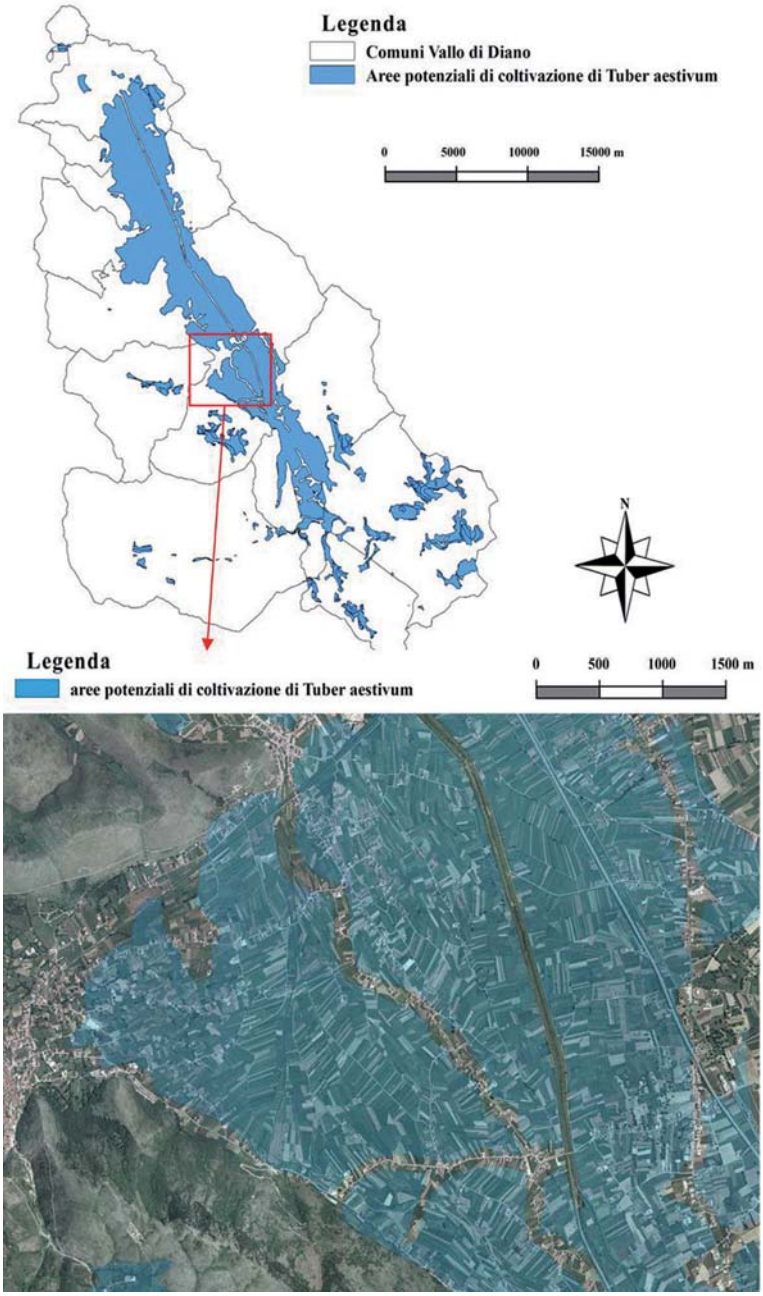

Fig. 6 Aree potenziali di coltivazione di *Tuber aestivum*.

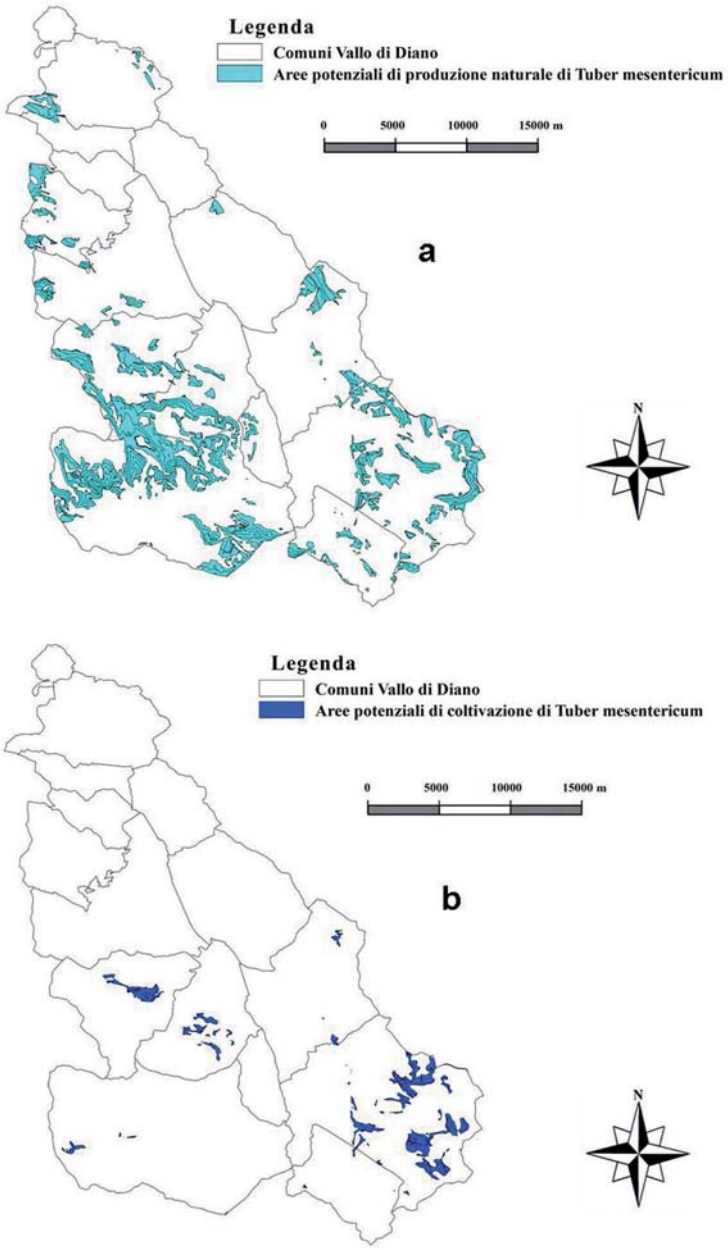

Fig. 7 (a) Aree potenziali di produzione naturale di *Tuber mesentericum*; (b) aree potenziali di coltivazione di *Tuber mesentericum*.

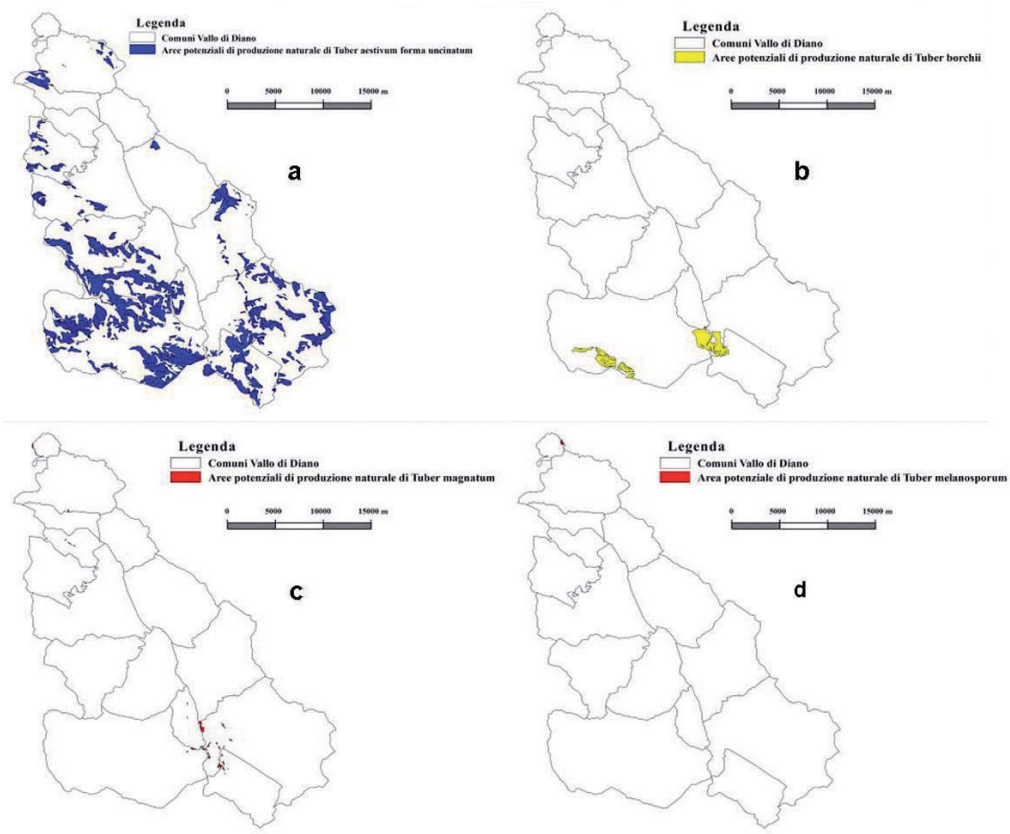

Fig. 8 (a) Aree potenziali di produzione naturale di *Tuber aestivum* forma *uncinatum*; (b) *Tuber borchii*; (c) *Tuber magnatum* e (d) *Tuber melanosporum*.

Conclusioni

Grazie all'indagine cartografica è possibile considerare il Vallo di Diano un territorio a potenziale vocazione tartufigena dove è ormai certa la presenza di alcune specie di tartufo. Infatti, i risultati ottenuti sono stati confermati da interviste a raccoglitori e micologi del posto, i quali affermano che le specie prevalentemente raccolte sono il tartufo estivo (*Tuber aestivum* Vittad.), il mesenterico (*Tuber mesentericum* Vittad.) e lo scorzone d'inverno (*Tuber aestivum* Vittad. forma *uncinatum* Chatin). Con molta probabilità è presente il tartufo bianchetto (*Tuber borchii* Vittad.), mentre non si hanno notizie sulla presenza delle due specie più pregiate (*Tuber magnatum* Pico e *T. melanosporum* Vittad.).

Incentivando lo sviluppo della tartuficoltura nel Vallo di Diano, grazie anche ad eventuali finanziamenti previsti dalle normative vigenti, è possibile garantire il rilancio, attraverso attività di tipo integrato, delle aree rurali, non solo dal punto di vista ambientale ma soprattutto in termini di livello dei redditi e di qualità della vita.

Ulteriori indagini in campo saranno intraprese con l'intento di verificare l'effettiva presenza di questi funghi nelle aree naturali di produzione individuate attraverso questo studio.

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PROGRESS TOWARD BURGUNDY TRUFFLE CULTIVATION IN MISSOURI, USA: DISCOVERY OF NATIVE *TUBER* SPP.

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Abstract
In a greenhouse inoculation trial, *T. aestivum* mycorrhiza production with hybrid *Quercus robur* x *Q. bicolor* seedlings was compared under two greenhouse seedling production systems: 1) the 'Root Production Method' (RPM®), used to produce seedlings with very large root systems in bark-based media; and 2) a more typical system employing a peat/sand-based medium in book planters. The latter system supported far greater production of *T. aestivum* mycorrhizae than did the RPM® system. Two years after outplanting, *Tuber aestivum* remained the most abundant mycorrhizal fungus on outplanted seedlings grown under the more typical system. Among the indigenous ectomycorrhizal fungi detected as ITS sequences from seedling mycorrhizae sampled in the field were four *Tuber* spp. previously unknown from Missouri: *T. whetstonense, T. maculatum*, *T. rufum*, and *T. lyonii*, in order of decreasing frequency of detection. To date, only *T. lyonii* has been found fruiting in Missouri, twice in a contiguous plantation of hybrid *Corylus* seedlings initially inoculated with *T. melanosporum*. While no hypogeous fruiting has yet been detected in the immediately adjacent forest, a *T. rufum* mycorrhiza collected beneath *Q. alba* yielded an ITS sequence identical to that of a mycorrhiza collected from a plantation seedling, and a mycorrhiza collected beneath *Q. rubra* provided an apparent *Genea cazaresii* sequence.
Key words: Burgundy truffle cultivation, *Corylus*, ectomycorrhizal community, Missouri, *Quercus*, *Tuber aestivum* syn. *T. uncinatum*, *T. lyonii*, *T. maculatum*, *T. rufum, T. whetstonense.*

Introduction
We are studying the biology and ecology of *Tuber aestivum* syn. *T. uncinatum* and *T. melanosporum*, from the perspective of their eventual cultivation in the central United States. Our efforts have focused on: 1) development of a seedling production system that emphasizes lateral root development for greater root density; and 2) analysis of mycorrhizal community development following plantation establishment. In the course of these studies, we have discovered the existence of several native *Tuber* spp. previously not known to exist in Missouri.

Materials and Methods
Our first truffle cultivation study involved establishment in 1999 of a small plantation of hybrid *Corylus* spp. inoculated in the greenhouse with *T. melanosporum*. The plantation site, located in Howard County, Missouri, USA, in the University of Missouri Horticulture and Agroforestry Research Center (GPS coordinates 39 ºN, 93 ºW), is a well-drained "Renfro silt loam" soil of loess origin on a ridge top, with a surface soil pH (water method) of 7.5-7.8 after adjustment with crushed dolomitic lime. The site supported an unmanaged weedy stand of alfalfa (*Medicago sativa* L.) for several decades prior to plantation establishment. Angiosperm forests occupy the north and south side slopes of the ridge (Fig. 1).
Our first *T. aestivum* study evaluated the effects of several treatments on *T. aestivum* mycorrhizae development on *Q. robur* root systems in the greenhouse: 1) two types of lime; 2) two root dip inoculation techniques; 3) two potting mix infestation methods; and 4) two sources of *T. aestivum* truffle inoculum. Seedlings were grown in the greenhouse in a 2:1:1 mixture of field soil, vermiculite, and perlite (v:v:v). High-Ca pelletized quick release Pel-Lime® was compared with a natural crushed dolomitic agricultural lime. The two *T. aestivum* sources were provided by Dr. Gérard Chevalier and Dr. Eric Danell. Soil infestation was accomplished by mixing a

aqueous slurry of ascospores into the potting mix, but the slurry used to infest half of the pots was amended with the gel-based compound Stockosorb®. Each seedling also received a root dip inoculation: either 1) water; 2) water + ascospores; or 3) water + ascospores + Stockosorb®. Details are published in Pruett *et al*. (2008a).

Our second *T. aestivum* study compared the effects of two very different seedling production systems on *Q. robur* x *Q. bicolor* seedling development and *T. aestivum* colonization levels. The 'Root Production Method' (RPM®) system is based on a ground bark potting medium and involves three potting events culminating in 8-litre pots at outplanting. In contrast, the more typical system involves growing seedlings in much smaller book planters containing a peat/sand-based medium. Methods and materials for this first stage of the study are detailed in Pruett *et al*., (2009). We then evaluated the fate of *T. aestivum* in relationship with the development of ectomycorrhizal communities for two years following outplanting in 2005 to a location contiguous to the *Corylus* plantation described above (Fig. 1). Ectomycorrhizal community development was evaluated on the basis of species identity, species richness, Shannon diversity index, and non-metric multidimensional scaling (see Pruett *et al*., 2008b).

Both the *Corylus* plantation mentioned above and the forests immediately adjacent to the planted ridge have been searched repeatedly for hypogeous fruiting bodies. Mycorrhizae have been sampled in conjunction with focused raking of the forest floor, and the ITS regions of fungal DNA extracted from *Tuber*-like mycorrhizae have been sequenced and blasted against the GenBank and Unite databases. In addition, the historical collection records of the Missouri Mycological Society have been searched for observations of hypogeous species, and articles have been published in trade journals soliciting information on observations of truffle fungi in Missouri and surrounding states (Bruhn, 2007a, b).

Fig. 1 Young *Q. robur* x *Q. bicolor* Burgundy truffle plantation (left), and adjacent 6-year-old hybrid *Corylus* Perigord black truffle plantation (right), indicating the location of *T. lyonii* ascocarps in September 2005.

Results

The type of lime used to raise the potting mix pH affected *Q. robur* seedling colonization by *T. aestivum* sources differentially. Overall, the dolomitic lime provided better results than did Pel-lime®. Also, root dip inoculation did not increase infection levels beyond those achieved by potting mix infestation with ascospores, and the hygroscopic polymer Stockosorb® was ineffective (Pruett *et al*., 2008a).

Seedlings grown in the more typical system were much smaller yet their roots were much more densely colonized by *T. aestivum* than were roots of seedlings produced by the RPM® system. Seedlings grown in book planters developed more root tips l^{-1} than did seedlings grown in RPM® containers regardless of which potting medium was used, and the peat/sand-based medium supported development of more *T. aestivum* root tips than did the bark-based RPM® medium regardless of the container method used (Pruett *et al*., 2009). At the time of outplanting, seedlings grown under the more conventional system contained a higher percentage of *T.*

aestivum ectomycorrhizae and a lower percentage of competitor ectomycorrhizae than did seedlings grown under the RPM® system (Pruett *et al*., 2008b).

Only two competing mycorrhiza species developed in the greenhouse, and one of these (unidentified) could no longer be detected two years after outplanting. Ectomycorrhizal species diversity increased to a greater extent on RPM® seedlings than on 'typical' seedlings. Over the three years of sampling, twice as many species were detected on RPM® seedlings as were detected on 'typical' seedlings (34 vs. 15). Among the competitors were four *Tuber* spp. with affinities to *T. whetstonense*, *T. maculatum*, *T. rufum*, and *T. lyonii* (in order of decreasing frequency of detection) (Pruett *et al*., 2008b). *Tuber rufum* was only detected on RPM® seedlings.

In September 2005, we found six *T. lyonii* truffles clustered 0-2-cm beneath the soil surface beside a six-yr-old *Corylus* seedling (Fig. 1). This was the first record of a *Tuber* sp. in Missouri, and a new host record for *T. lyonii* (Bruhn *et al*. 2006). The truffles were identified microscopically as *T. lyonii* (Trappe *et al*., 1996), and representatives have been deposited in the Oregon State University herbarium. The ITS sequence of one of these truffles was identical to that obtained from underlying mycorrhizae (Fig. 2). In July 2007, another 12 *T. lyonii* truffles were collected beneath a different *Corylus* seedling *ca*. 100-m distant from the first collection. ITS sequences from our two truffle collections were 97% similar to one another, and were 99% and 98% similar to that of a *T. lyonii* truffle collected in 2007 in a pecan tree (*Carya illinoinensis*) orchard in Calhoun County, GA (provided by Dr. Tim Brenneman).

Soils from the adjacent *Corylus* and *Quercus* plantings are compared in Table 1.

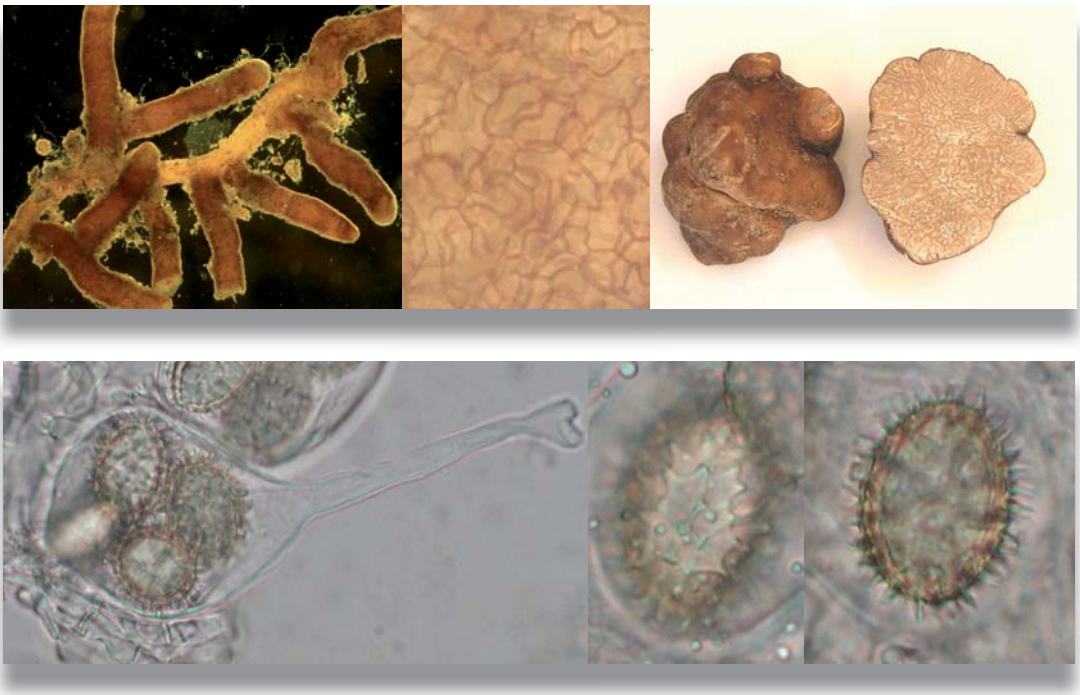

Fig. 2 Clockwise from upper left, *T. lyonii* mycorrhizae, mycorrhizal mantle structure, ascocarp, ascospores, and ascus.

Tab. 1 Soil characteristics under *Corylus* seedlings associated with *T. lyonii* fruiting, and under *Quercus* seedlings infected by *T. aestivum*.

Parameter	Host species	
	Corylus	*Quercus*
Clay < 2µm %	17.5	10-15
Silt 2-50 µm %	67.5	65-70
Sand 50-2000 µm %	15	17.5-22.5
Soil pH (water method)	7.5-7.8	7.7-8
Exchangeable calcium (Ca; ppm)	2700-3200	1200-2200
Assimilable phosphorus (P_2O_5; ppm)	25	10-23
Exchangeable magnesium (Mg; ppm)	200-270	80-110
Exchangeable potassium (K; ppm)	250-370	130-180
Ca : Mg	11.7-13.4	15-22.7
K : Mg	1.3	1.5-2.3
Organic matter (ppm)	40 000-77 000	25 000-36 000
Organic carbon (ppm)	23 000-45 000	14 600-21 000
Organic nitrogen (ppm)	2600	2000-2600
C : N	8.8-17.3	6.3-9.5

We have found no previous records of hypogeous ascomycete collections from Missouri. And despite repeated searches, both in the Ozark Mountains and around our plantation site, we have detected no other fruiting of hypogeous ascomycetes in Missouri. However, *T. rufum* mycorrhizae collected beneath a nearby *Q. alba* provided an ITS sequence identical to that obtained from our *Quercus* plantation seedlings, and mycorrhizae collected beneath a nearby *Q. rubra* provided an apparent *Genea cazaresii* sequence. Searches have been handicapped by the lack of a trained dog.

Discussion

It's not surprising that different forms of lime can affect the levels of seedling infection by various provenances of *T. aestivum*, but it is useful to note that both strains performed equally well with our dolomitic lime source, even though its Ca and Mg contents were lower and higher, respectively, than those of Pel-lime® (Pruett *et al.,* 2008a). Because of both the less refined nature of dolomitic lime and its physical proximity to many greenhouses and plantation sites in the central USA, both the retail and transportation costs of dolomitic lime argue in favor of its use.

We observed an increase in mycorrhizal community diversity during the second year in the field, regardless of the greenhouse production system employed (Pruett *et al*., 2008b). We suggest two possible explanations. First, removal of the water-permeable weed barrier fabric from seedlings after their first year in the field may have favored development of indigenous mycorrhizal species (Zambonelli *et al*., 2005). Second, the prolonged heavy freeze that took place in early April 2007, after a warm winter, may have influenced the ectomycorrhizal populations sampled 1.5 months later, when the seedlings had been two years in the field.

The detection of ectomycorrhizae representing *T. whetstonense* (Frank *et al*., 2006), *T. maculatum*, *T. rufum*, and *T. lyonii* (Trappe *et al*., 1996) in our *T. aestivum* plantation was interesting, both because of the old-field nature of the plantation site, and because there were

no records of *Tuber* spp. occurrence in Missouri until *T. lyonii* was found fruiting under six-year-old *Corylus* shrubs in 2005 and 2007, at least 20-m away. Native *Tuber* spp. ectomycorrhizae were much more frequently encountered on RPM® seedlings than on seedlings produced using the more typical system. It is interesting to note that *T. lyonii* is the *Tuber* sp. least frequently observed on the roots of our *Quercus* seedlings, yet it is the only *Tuber* sp. known to have fruited at our study site to date (albeit on *Corylus*).

In her meticulous studies of *T. lyonii* syn *T. texense* in Texas and New Mexico, Taber (1990) did not observe formation of a well-developed *T. lyonii* mantle on pecan tree roots in the greenhouse or the field, and suggested that the corresponding mycorrhizal association (with pecan) might be "fragile". In contrast, we have observed well-developed ectomycorrhizal morphology and mantle anatomy very characteristic of *Tuber* spp. on both *Corylus* and *Quercus* root systems. Taber's (1990) observations that the numerous specimens she had received from Texas and New Mexico had all been collected near pecan trees of improved cultivars growing in yards or orchards are intriguing. They parallel our own failure to observe *T. lyonii* fruiting outside our plantations. Yet collection records for *T. lyonii* from eastern North America indicate a much broader host (and geographic) range, indicative of a species complex. A list of suggested host genera now includes *Carya*, *Corylus*, *Crataegus*, *Quercus*, and *Tilia* (Bruhn, 2005; Bruhn *et al*., 2006).

Inasmuch as truffle fungi depend upon mycophagy for spore dispersal, Taber (1990) reported that pet dogs, squirrels and armadillos have drawn homeowners' attention to fruitings of *T. lyonii* in Texas. We have noted extensive rodent burrowing in our own plantations, and we are aware that deer have long traversed the ridge upon which our plantings are located. Thus, it is not surprising to find that our plantation site has been furnished with a generous bank of *Tuber* spp. inoculum (*e.g*., Ashkannejhad and Horton, 2006). To date, the only evidence of *Tuber* spp. we have found in the adjacent forest has been a collection of *T. rufum* mycorrhizae with an ITS sequence identical to that obtained from our *Quercus* seedlings.

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FIELD RESPONSE OF *QUERCUS ILEX* SEEDLINGS INOCULATED WITH *TUBER MELANOSPORUM* PRODUCED BY THREE DIFFERENT COMMERCIAL NURSERIES

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Abstract

The objective of this work was to evaluate the field response of *Quercus ilex* (holm oak) seedlings with various levels of *Tuber melanosporum* mycorrhizae after 18 months in 3 different plantation sites. The seedlings, produced by 3 different commercial nurseries, were evaluated prior to outplanting to determine the numbers of total fine roots, non-mycorrhizal roots, *T. melanosporum* mycorrhizae, and non-*T. melanosporum* mycorrhizae. After 18 months in the field, plants from each treatment were carefully dug-up from each plantation and the roots were divided into 2 groups: old roots from the initial nursery plug and new roots, which had developed outside the plug. We measured root length of the taproot, root length of new roots, total fine roots, mycorrhizal levels, and plant height. Seedlings from Nursery A had significantly greater numbers of *T. melanosporum* mycorrhizae prior to ouplanting than seedlings from the other 2 nurseries. Eighteen months later we observed no differences in the number of truffle mycorrhizae in the original root plug based on nursery source. Seedlings from Nursery C had developed greater numbers of truffle mycorrhizae as well as greater numbers of fine roots in the field-developed new roots. Mycorrhizae colonization level is important for successful truffle plantations but not the only prognostic factor for successful truffle plantation establishment.

Key words: Mycorrhizal colonization level, fine roots, truffle cultivation.

NEW DATA ON IMPACT OF EARTHWORMS ACTIVITY ON BLACK TRUFFLE SOILS

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Abstract
Among soil invertebrates, the earthworms (Anellida, Oligochaeta) are unique in their ability to integrate physical, chemical and biological processes in the soil ecosystem (Edwards and Bohlen, 1996; Shuster *et al*., 2002). Several authors have outstanding the research on earthworm feeding ecology and its implications for nutrient cycling and soil fertility. However Curry and Schmitd (2007) remarked that many aspects of the interactions between earthworms and soil mineral constituents are still poorly understood.
The amount of carbonates in soils depends on soil forming factors such as relief, biological factors, human and natural disturbances, time and climate (Jenny, 1941; Breemen & Buurman, 1998). Chan (2003) explained that soil carbonate mobility is related with the direct action of earthworms and other soil fauna, and demonstrated the earthworm ability to incorporate lime into subsoil in the calcareous amendments applying to ameliorated acid soils where the incorporation by tillage is not feasible.
On truffle research, many authors pointed that black truffle (*Tuber melanosporum* Vittad.) production is closely linked to calcium carbonate soil availability and mobility on overall soil profile. Callot (1999) empathises the currently study on the relationship of cause and effect among earthworms activity, pH of the soil and development of black truffle. Lulli *et al.*, (1999) and Castrignanò *et al.,* (1999) also point out that black truffle require considerable soil porosity originated by biological activity, above all, by earthworms and ants.
The objective of the present study is to analyse the pH, carbonate fractions and abundance of total organic carbon (TOC) in a representative number of soil samples from *Tuber melanosporum* burns and earthworm cast samples, to establish statistical patterns on these soils parameters and to analyse the impact of earthworms activity on black truffle soils.
Key words: Truffle ecology, lime movement, earthworm casts, truffle culture.

OPTIMAL METHOD FOR CULTIVATING *TUBER MELANOSPORUM*

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Abstract
The results of cultivating *Tuber melanosporum* in France are recognized to be variable. A few plantations created and managed with exceptional care show that it is possible to specify an optimal method for cultivating *Tuber melanosporum.*
The reference plantation for this study is situated in the département of the Tarn. It consists of 57 ever green oaks mycorrhized with *Tuber melanosporum* and planted in 1995. It is compared with other plantations established in similar conditions but with different methods of management. The outstanding method includes progressive working of the land, mowing the grass, watering, pruning the trees and disseminating spores. Each operation is carried out with hand tools or light equipment, with a constant concern to avoid compacting the soil or disturbing the habitat.
The burnt areas appeared from the third year onwards, and production started in the fifth: production which started under 10% of the trees and can be seen under more than 90% in the twelfth year, varying between 15 and 45 kilos per hectare.
These results show that if the maintenance of truffle plantations (working the soil, watering for recovery and production, pruning the trees) is carried out diligently, constantly and regularly, production generally starts early and increases steadily. New generations of mycorrhized plantings with carefully managed cultivation, re-uniting traditional practices and recent progress, allow us to look to the future with confidence.
Key words: *Tuber melanosporum* Vittad., soil, mycorrhized trees, watering, pruning.

Photo 1 jeunes plantations truffières (Tarn, France)

Introduction
Une enquête sur le devenir de jeunes plantations truffières avait mis en lumière qu'un choix judicieux du terrain, associé à une conduite culturale adaptée, permettait d'obtenir une production satisfaisante de truffes noires *Tuber melanosporum*.
Après avoir présenté de façon synthétique les résultats de cette enquête, on développera un itinéraire technique qui tendrait à favoriser la production à l'optimum du *Tuber melanosporum*. Cet itinéraire technique sera illustré par le cas concret d'une parcelle dont le propriétaire

trufficulteur semble avoir accompli, avec réussite, la synthèse des techniques ancestrales et des avancées modernes, notamment avec le plant mycorhizé.

I- Enquête sur le devenir des plantations truffières tarnaises

A partir de la fin des années 80, le syndicat des trufficulteurs du Tarn a relancé la dynamique de plantation truffière.

Si le Tarn est depuis toujours un département riche en truffières naturelles, il n'y avait pas eu de tentatives de création de truffières par plantation sinon au début des années 70 où quelques parcelles furent plantées avec des plants traditionnels du Lot. Par manque d'entretien ou à cause de la mauvaise qualité initiale des plants, ces parcelles n'ont pas donné de résultats.

Sous l'impulsion du syndicat, les plantations ont redémarré à partir des années 1988-89 et se poursuivent aujourd'hui au rythme annuel de 10 à 20 hectares.

Réalisées avec des plants mycorhizés contrôlés, suivies sur le plan technique, il était intéressant d'en connaître le devenir. C'est pourquoi il a été décidé de réaliser une enquête auprès des trufficulteurs Tarnais.

I-1 Objectifs de l'enquête

L'enquête a pour objectif principal de connaître l'état de production des jeunes plantations truffières tarnaises et de mettre en évidence les causes principales de réussite ou d'échec et notamment de vérifier si l'incidence de l'origine des plants truffiers (pépiniéristes) est forte, comme cela est souvent avancé par les trufficulteurs.

I-2 Présentation

L'enquête a été réalisée en novembre 2006 auprès d'un échantillon de 34 trufficulteurs regroupant 58 parcelles ou plantations pour une surface de 41 ha.

Toutes ces plantations ont été mises en place à partir de l'hiver 1988/89 et jusqu'à l'hiver1999/2000. Les plantations plus jeunes n'ont pas été retenues car peu susceptibles d'être entrées en production.

Un questionnaire a été établi pour renseigner les critères d'étude, à savoir:

- l'origine des plants,
- le précédent cultural,
- le type de sol,
- la conduite culturale.

Ces critères ont été choisis car ils sont susceptibles d'avoir une incidence sur le comportement des plantations et sont stables dans le temps et indépendants du climat ou du trufficulteur. Ils se prêtent donc bien à une analyse groupée.

Les interventions comme la taille, l'arrosage, etc., n'ont pas été prises en compte car elles n'ont pas une influence directe sur l'entrée en production ou non d'une plantation mais plutôt sur le rendement et la pérennité de la production, ce qui n'est pas l'objet de l'enquête.

I-3 Aperçu du contexte pédoclimatique

Les plantations enquêtées sont essentiellement situées dans le Nord-Ouest du département du Tarn (43 sur 58), c'est-à-dire dans le triangle Cordes-Albi-Gaillac; 7 le sont dans la partie centrale (Graulhet-Lautrec) et 8 dans le Sud du département (Causse de Castres-Labruguière).

Chaque zone traditionnellement truffière du département du Tarn est donc représentée.

Au point de vue du climat, celui des zones truffières est assez homogène et qualifié de subméditerranéen à tendance océanique, bien que le caractère méditerranéen s'affirme de plus en plus au travers de sécheresses estivales marquées et répétées.

L'altitude où se situent les parcelles enquêtées varie peu, entre 200 et 300 mètres environ.

Cette unité se retrouve au niveau géologique, où le calcaire lacustre oligocène domine très largement (calcaires de Cordes, Albi, etc.):

- calcaire lacustre oligocène: 41 parcelles,
- calcaire éocène: 8 parcelles,

- poudingue éocène: 2 parcelles,
- marne ou molasse oligocène: 5 parcelles,
- calcaire jurassique (Lias dolomitique): 2 parcelles.

Ces formations calcaires donnent le plus généralement des sols assez superficiels, caillouteux et calcaires qualifiés de «rendosols» ou des sols plus profonds et argileux lorsque l'on se situe sur marne ou molasse, on a alors affaire à des «calcosols».
Tous les sols sont bien pourvus en calcaire, voire très riches en cet élément.

Photo 2 coupe de sol sur calcaire jurassique

I-4 Résultats

I-4.1 Effet de l'année de plantation

L'enquête met en évidence que:

- la plupart des parcelles entrent en production avant 15 ans, souvent avant 10 ans, les premières truffes étant généralement récoltées sous chênes verts 5 à 8 ans après plantation, qu'il s'agisse de *Tuber melanosporum* ou de *Tuber brumale*.
- La qualité de la production s'est très nettement améliorée au cours du temps, notamment à partir de la campagne de plantation 1995/1996. Les plantations postérieures à cet hiver ne sont presque plus concernées par la récolte de *Tuber brumale*: 17% des parcelles entrées en production contre 70% pour les parcelles plantées avant 1995/96.
- Le niveau de production (on hésite à parler de «rendement») a progressé. A partir de 1995/96, 70% des parcelles productrices de *Tuber melanosporum* ont un niveau de production bon ou un début prometteur, contre 25% auparavant.

On ne peut encore définir les causes de cette progression, sans doute est-elle due à l'amélioration de la qualité des plants ou à une meilleure maîtrise des techniques culturales ou encore au choix plus approprié des sites d'implantation.
Cette évolution à partir de 1995/96 est un élément majeur et va conditionner l'approche et l'interprétation des différents résultats de l'enquête. Pour chaque critère retenu, il sera procédé à une analyse globale puis à une analyse restreinte aux hivers de plantation postérieurs à 1994/95.

I-4.2 Effet de l'origine des plants

Parmi la douzaine de fournisseurs de plants recensés, 3 pépiniéristes dominent et, à eux seuls, représentent les trois quarts des implantations (en nombre de plantations, pas en nombre de plants). Pour préserver leur anonymat, ils ont été désignés par les lettres A, B et C.

Le pépiniériste A, le plus représenté avec 32 plantations, a des résultats de production partagés entre *Tuber melanosporum* et *Tuber brumale*, contrairement aux pépiniéristes B et surtout C qui sont peu concernés. Les plantations non encore en production sont pour la quasi-totalité des parcelles jeunes, sans lien avec la pépinière d'origine des plants.
Si on peut relever un effet de l'origine des plants sur la qualité de la production, on ne peut le faire en termes de précocité et de quantité.

I-4.3 Effet de l'origine des plants à partir de 1995/96
Le changement est flagrant: la préoccupation causée par *Tuber brumale* n'en est plus une, le comportement des différentes origines de plants devient alors sans effet sur la production, autant en termes de qualité que de quantité. Un palier a été franchi et on peut affirmer aujourd'hui que la qualité des plants mycorhizés est sécurisée par les différents contrôles auxquels ils sont soumis (truffe, mycorhization, développement, etc.)

I-4.4 Effet du type de sol
Les sols recensés peuvent être regroupés en trois catégories ou types:
- sols peu ou pas caillouteux à texture argileuse,
- sols caillouteux à texture argileuse,
- sols caillouteux à texture équilibrée ou légère.

Avec l'examen des données relatives aux plantations effectuées après l'hiver 1994/95, la tendance entrevue sur l'ensemble des résultats s'affirme et se clarifie. Il apparaît que:
- un sol argileux peu ou pas caillouteux est très peu favorable à la production truffière: le taux de parcelles improductives est le plus élevé (50%), il y a encore récolte de *Tuber brumale* dans 25% des cas et il n'y a pas de parcelle à bon niveau de production de *Tuber melanosporum*;
- un sol à texture argileuse dominante mais caillouteux ou *a fortiori* un sol à texture équilibrée ou légère et caillouteux est un bon support d'une production truffière de qualité. Ces sols correspondent aux terres de causse. Ils sont en général de faible épaisseur, bien structurés et drainants, développés sur des calcaires fissurés. Sur ce type de sol, le risque de production de *Tuber brumale* semble écarté.

En outre, selon les données recueillies lors de l'enquête, plus le sol semble «maigre» et caillouteux, mieux la plantation truffière se comporte.

I-4.5 Effet du précédent cultural
Il a été retenu trois classes de précédents culturaux, bien représentés au sein de l'échantillon de l'enquête:
- la vigne, culture pérenne, traditionnellement associée à la truffe et considérée comme un précédent éminemment favorable (14 plantations),
- les terres de culture, au sens large, souvent des céréales ou des prairies temporaires ou artificielles ou des jachères incluses dans une rotation (20 plantations),
- des friches, landes, pacages, vignes abandonnées, prairies et jachères anciennes, terres délaissées, qui ont en commun de ne pas avoir été cultivés de longue date et parfois même jamais (30 plantations).

Au vu des résultats, une constatation s'impose et étonne: il s'agit du comportement des plantations réalisées après vignes. Que l'on considère l'échantillon global ou à partir de 1995/96, la vigne apparaît clairement comme un précédent défavorable entraînant le plus souvent une production de *Tuber brumale* ou l'absence de récolte. Il est entendu que ce sont des vignes arrachées depuis peu, ayant donc connu les méthodes de culture modernes, notamment les intrants: engrais chimiques et pesticides.
Pour les autres précédents culturaux, terres cultivées ou non, il n'apparaît pas de différences significatives, sinon une entrée en production plus hâtive après terre cultivée qui peut s'expliquer par une implantation plus facile des arbres et un développement accéléré.

I-4.6 Effet de la conduite culturale

La conduite culturale de la jeune plantation, et notamment le travail du sol, sont réputés influencer le devenir des jeunes plantations. Les parcelles ont été classées selon l'itinéraire retenu, de la simple préparation des trous destinés à recevoir les plants, au travail complet du champ sans interruption depuis la plantation.

Les résultats mettent en évidence essentiellement que:

- la conduite culturale n'influence pas la production truffière en termes de qualité, c'est-à-dire que la récolte de *Tuber melanosporum* ou de *Tuber brumale* n'est pas liée à l'itinéraire cultural retenu.
- Une préparation limitée du sol retarde (et peut-être parfois compromet) la production truffière.
- Le travail du sol avant plantation continué les premières années (1 à 5 ans) semble être le bon compromis, autant au niveau de la précocité d'entrée en production que des quantités récoltées. Ce travail du sol ne doit toutefois pas être exécuté à proximité immédiate du plant, pour ne pas léser le jeune système racinaire de surface.

I-5 Synthèse des résultats et conclusion de l'enquête

Pour tenter d'établir une synthèse de l'ensemble des résultats, il était naturel de se limiter aux parcelles qui ne présentaient pas de critères défavorables.

Ces critères défavorables sont:

- la plantation avant 1995/96,
- un sol argileux peu ou pas caillouteux,
- le précédent cultural vigne,
- les conduites culturales les moins indiquées.

A partir de l'échantillon initial de 58 parcelles et après élimination de celles présentant un ou plusieurs critères défavorables, il reste 14 parcelles. Ces parcelles ont donc en commun d'avoir été plantées à partir de 1995/96, dans un sol caillouteux à texture argileuse ou équilibrée, après culture ou friche et avec préparation du sol puis travail les premières années seulement.

Sur ces 14 parcelles, 12 sont entrées en production au plus tard à leur dixième année et seules deux restent encore improductives mais commencent à brûler (plantées en 1998/99 et 1999/00).

Pour 10 parcelles, le niveau de production est prometteur à bon, soit pour 71% de l'échantillon. Cette valeur est à comparer à celle de l'ensemble des parcelles retenues pour l'étude soit 24% ou à celle des plantations effectuées à partir de 1995/96 soit 33%.

Il apparaît alors clairement que le choix du site d'implantation d'une truffière (sol), son passé cultural et l'itinéraire technique conditionnent directement la réussite de l'opération.

On peut donc admettre, dans le contexte tarnais, que la plantation truffière peut être entreprise avec un maximum de chances de réussite dès lors que l'on ne s'éloigne pas des critères favorables bien identifiés au travers de cette enquête. La trufficulture restera possible au delà mais la production de truffes noires plus aléatoire, voire compromise.

II- Un itinéraire technique pour la production à l'optimum du *Tuber melanosporum*

Photo 3 plantation remarquable (Les Cabanes, Tarn, France)

Une plantation de l'échantillon enquêté, remarquable par ses résultats mais surtout par la qualité du travail accompli par le propriétaire, a été retenue pour illustrer ce que pourrait être un itinéraire technique aboutissant à une production à l'optimum du *Tuber melanosporum*. Cette plantation, intégrée au réseau des truffières expérimentales de la région Midi-Pyrénées, offre tous les critères définis comme favorables dans l'enquête présentée ci-dessus. De plus, les techniques et moyens mis en œuvre par le trufficulteur peuvent être accessibles à tout un chacun.

II-1 Présentation de la parcelle

La plantation a été effectuée en novembre 1995. D'une superficie de 1850 m², elle comprend 57 chênes verts contrôlés mycorhizés issus de la pépinière Agri-Truffe. Les écartements sont de 8 m entre les rangs et de 4 m entre les arbres sur le rang soit une densité de 312 arbres par hectare. La plantation a été établie après une friche qui succédait depuis de nombreuses années à une ancienne vigne.

Le sol, développé sur des dolomies grises en plaquettes de l'Hettangien (Lias = Jurassique inférieur), est très épais. En effet, l'horizon A, de 40 à 70 cm d'épaisseur, calcaire, caillouteux, de texture limono-argileuse, dont le pH eau est de 8,3, surmonte un important horizon C de roche fragmentée et bouleversée avec un peu de terre intercalée. Ce n'est que vers 180 cm de profondeur que l'on retrouve la roche R, dolomies en plaquettes. Cette constitution physique du sol permet à la fois un excellent drainage et la conservation d'humidité en profondeur.

II-2 Préparation du sol avant plantation

Les travaux aratoires préalables à la plantation ont consisté à la suppression de la végétation herbacée et arbustive de la friche, puis à un labour assez profond. Plusieurs passages de cultivateur ont complété cette préparation et permis le bon enfouissement et le bon mélange des résidus organiques ainsi que la préparation de terre fine nécessaire pour une plantation dans les meilleures conditions de reprise.

II-3 Entretien de la truffière

II-3.1 Travail du sol

Le travail du sol au cultivateur est poursuivi dans les inter-rangs, de façon «croisée», au cours des deux ou trois premières années, en fait jusqu'aux premiers signes de «brûlé». La fréquence des interventions est dictée par le rythme de pousse de la végétation herbacée, l'objectif étant d'éviter l'enherbement de la truffière à ce stade.

Pour les mêmes motifs, un rayon de 50 cm autour des plants est maintenu «propre» par un sarclage répété 6 à 7 fois dans l'année.

Photo 4 travail du sol mécanique seulement entre les inter-rangs

Dès que les brûlés commencent à se former, le travail du sol est éloigné des arbres. Des piquets blancs sont implantés de façon à matérialiser la zone qui ne sera pas travaillée mécaniquement. Ces piquets sont écartés d'environ 20 cm par an pour suivre l'extension supposée du système racinaire. Le matériel utilisé évolue aussi. Le cultivateur attelé au tracteur est abandonné au profit d'un motoculteur équipé d'un petit cultivateur, ce qui limite le tassement du sol par le poids des outils.
Le pourtour de l'arbre n'est alors sarclé qu'une seule fois au printemps, à la «vieille lune» de mars.
Après 3 ans de plantation et selon ce mode de conduite, 100% des chênes verts de la jeune plantation attenante (effectuée en novembre 2005) présentent un brûlé.

Photo 5 motoculteur équipé de griffes (cultivateur) pour le travail du sol.

Après entrée en production, le même principe de travail du sol est conservé, autant pour les inter-rangs que les brûlés dont seule la zone d'accroissement n'est pas travaillée. Le binage printanier des brûlés est réalisé avec un outil de très petite taille pour ne pas abîmer les racines de surface.

Photo 6 bineuse manuelle à 2 dents

II-3.2 Maîtrise de la végétation herbacée

Lors des 2 ou 3 premières années, toute végétation herbacée est éliminée selon les modalités de travail du sol évoquées précédemment. Après apparition des signes de brûlé, le travail du sol n'étant effectué qu'une fois au printemps, l'herbe s'installe spontanément, mais de façon clairsemée; elle est alors régulièrement tondue ou coupée régulièrement tondue ou coupée.

Photo 7 tondeuse pour l'entretien de la végétation herbacée

II-3.3 Taille des arbres

La taille des arbres est accomplie chaque année avec le plus grand soin. Démarrée dès la deuxième année après plantation, elle est effectuée pendant l'été, à la «lune vieille» du mois d'août. Elle consiste à dégager progressivement la base des troncs et limiter le développement de la frondaison, autant en hauteur que de façon latérale, de manière très progressive, sans jamais couper de grosses branches, pour traumatiser le moins possible les arbres.

Photo 8 taille soignée des chênes verts à la cisaille

Après 13 années, les chênes verts occupent un espace très limité, de sorte que l'espace de conquête est économisé (voir Questions d'écologie appliquées à la trufficulture). L'écosystème truffier est maintenu dans un état d'équilibre très favorable à la production.

Photo 9 vue générale de la plantation avec les chênes verts taillés en cône renversé

II-3.4 Arrosage

Les jeunes arbres sont régulièrement arrosés après plantation dès que la sécheresse est installée; quelques litres d'eau (5 à 10 litres) sont apportés à chacun toutes les 2 semaines environ.

Une fois la truffière en production, l'arrosage est entrepris chaque fois qu'une période sans pluie significative est observée, sans laisser plus de 15 jours sans pluie ou arrosage. L'apport d'eau, effectué au tuyau sur les seules zones de brûlé, représente environ 15 litres/m². L'eau est pompée dans un petit lac naturel formé au fond de l'excavation d'une carrière attenante (eau de pluie collectée par ruissellement et eau de source).

La fréquence des arrosage est adaptée pour atteindre une pluviométrie mensuelle d'au moins 60 mm de mai à septembre.

II-3.5 Autres interventions

Le trufficulteur procède régulièrement à un «re-ensemencement» des brûlés. Pour cela, les truffes non commercialisables sont conservées congelées et remises en terre au printemps lors du travail du sol des brûlés, broyées et mélangées à de la vermiculite.

II-4 Production

tableau 1 évolution de la production

Saison de récolte	Quantité récoltée (grammes)
2000/01	200
2001/02	4450
2002/03	2800
2003/04	0
2004/05	3647
2005/06	5874
2006/07	2140
2007/08	9613

Les premières truffes ont été récoltées sous deux arbres après 5 ans de plantation. La production a ensuite fortement évolué et concerne aujourd'hui 55 des 57 chênes verts plantés en novembre 1995, soit plus de 96% d'arbres producteurs.
Malgré une récolte nulle liée à l'année caniculaire 2003, la plantation affiche une moyenne de 4,75 kg/an pour une surface d'à peine 1850 m², soit 25,7 kg/ha. La production est en constante progression même si elle demeure très influencée par les conditions climatiques. Une meilleure gestion des arrosages entreprise en 2007 par le raccourcissement de l'intervalle entre deux précipitations (pluie ou arrosage) semble avoir été bénéfique pour la production de l'hiver qui a suivi.

Conclusion

Une enquête, conduite sur un nombre important de jeunes plantations truffières du Tarn, a permis de mettre en évidence l'importance de certains critères pour sécuriser, autant que faire se peut, la réussite d'une plantation truffière. Le choix d'un exemple concret est venu conforter cette approche, en précisant les contours d'un itinéraire technique favorisant la production à l'optimum du *Tuber melanosporum*.
La vulgarisation de cet itinéraire technique, en l'adaptant aux contraintes de milieu, mais aussi à la superficie des truffières, et surtout à la disponibilité des trufficulteurs, peut laisser présager une sensible progression des résultats de la trufficulture tarnaise.

Photo 10 cavage avec Agathe, chienne Lagotto Romagnolo

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TUBER MELANOSPORUM VITTAD. RECUPERO DI UN IMPIANTO TARTUFICOLO ABBANDONATO ED IMPRODUTTIVO

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Abstract: **Recovery of truffle plantation by *Tuber melanosporum* abandoned and unproductive**
A truffle plantation, rather dense, (3m x 3m) of oak mycorrhized by *Tuber melanosporum* Vittad., made in 1980 in the Community of Montana and Nerone Catria that had never entered into production was subject to a number of cultural practices for groped productivity to recover after about twenty years from making homeless.
After first verifying, by taking radical, while the mycorrhized by *Tuber melanosporum* on the apparatus radical plant made homeless, it was the cleaning up of the entire surface area (about 6.000m^2) by chopping machine. Subsequently, the first Pianella of *Tuber melanosporum* has made a slight "forconatura" them, (and also gradually in subsequent years, as demonstrated such a will on other plants) while the areas under the plants and grass still remain productive of *Tuber brumale* Vittad. were scattered hydrated lime. In a second time pruning were made to raise the canopy and better illuminate the ground below. In turn a few years, the plants treated in this way have started to produce truffles, the first in a sporadic and small amounts then resulting in discrete quantities collection also excellent size.
The authors point out the positive results achieved may be considering a way, all things fairly simple and economical, groped for the recovery of many truffle plantation executed and then, for a variety of causes, abandoned and then never come into production.
Key words: truffle cultivation, *Tuber melanosporum*, ecology.

Premessa e scopo
Il problema del miglioramento e del recupero delle tartufaie coltivate di *Tuber melanosporum* Vittad. divenute improduttive o poco produttive è molto sentito dai tartuficoltori. In Italia esso è stato oggetto di ricerca da parte del Centro Sperimentale di Tartuficoltura di Sant'Angelo in Vado. Infatti nella seconda metà degli anni 90 e primi anni 2000 tale Centro ha condotto alcune ricerche e sperimentazioni in proposito (Gregori *et al.,* 2001) che hanno interessato sia la rigenerazione di vecchie tartufaie impiantate da Mannozzi–Torini nelle Marche (Mannozzi-Torini, 1958) sia i possibili interventi di recupero di tartufaie di più recente costituzione nel Piceno che, nel tempo, hanno cominciato a presentare problemi e sono divenute improduttive (Gregori, 2005).
Ma mentre nei casi enunciati si è potuto far riferimento alla Francia dove il rinnovamento delle tartufaie di nero pregiato è tecnica comune (Sourzat, 1996; Olivier *et al.,* 1994) il caso oggetto di studio rappresenta una novità per il settore ed è quindi anche per questo molto interessante.
In vero non si tratta di recuperare alla produttività la "solita" tartufaia coltivata divenuta nel tempo improduttiva per cause fisiologiche e/o colturali ("chiusura dell'ambiente", compenetrazione delle chiome, infeltrimento del cotico erboso alla superficie del terreno, etc.) ma bensì di una tartufaia impiantata e subito abbandonata, dove cioè nessuno è mai, in alcun modo, intervenuto. Infatti nell'impianto tartuficolo in questione, se pur realizzato in ambiente pedologicamente adatto, non è mai stata effettuata alcuna cura colturale né intervento agronomico ma esso è stato lasciato alla sua libera naturale evoluzione, tanto da diventare in poco tempo una vera e

propria cenosi forestale, assimilabile ad un bosco di neoformazione.
Scopo della sperimentazione quindi è stato innanzi tutto quello di verificare la possibilità di recuperare alla produzione un impianto tartuficolo mai curato ed abbandonato e poi, in caso positivo, di tracciare una prima via tecnica da utilizzare in casi analoghi.

Principali caratteri ecologici della stazione di impianto
Il substrato geologico (Carta Geologica delle Marche, 1986) della stazione di impianto è in parte quello del Bisciaro (emipelagiti calcaree) ed in parte quello della Formazione Marnoso Arenacea (torbiditi pelitico-arenacee con intercalazioni di torbiditi carbonatico –silicatiche) che hanno dato luogo rispettivamente a due tipologie di terreno: il primo abbastanza sciolto (70% terra fine; 30% scheletro), a medio impasto, (tessitura F), calcareo ($CaCO_3$ 50%) a reazione sub-alcalina (pH 7.5); il secondo è più compatto e meno drenante (90% terra fine; 10% scheletro), tendenzialmente a medio impasto (tessitura FSA), calcareo (30%), a reazione sub alcalina (pH 7.5). La zona ricade nell'ambito del Macroclima temperato della Regione Marche è più precisamente nel Piano bioclimatico sub mediterraneo che comprende la fascia collinare caratterizzata in prevalenza dai querceti caducifogli di roverella e dagli ostrieti, dove le sclerofille (in prevalenza leccio) si attestano in gole rupestri microclimaticamente condizionate. (I Tipi Forestali nelle Marche, 2001). In particolare dal punto di vista fitoclimatico, cioè climatico forestale l'ambiente è quello della fascia fitoclimatica del CASTANETUM di Pavari, con un regime pluviometrico (>900 mm) di tipo AIPE (massimo principale in autunno-inverno e massimo relativo in primavera) con moderata aridità estiva; temperatura media annua 12° C, temperatura media delle minime assolute annue > a -12° C.

L'impianto tartuficolo
L'impianto è stato effettuato nel 1980, in località Caicaccia, del Comune di Cagli (PU) su un appezzamento di circa ha 00.60.00, (a superficie declive (pendenza circa 30%) ed esposta a Nord, ubicato ad una quota variabile da 480 m.slm a 510 m. slm,) secondo un sesto di 3m.x 3m., con piante di roverella (*Quercus pubescens* Willd.) micorrizate con il tartufo nero pregiato (*Tuber melanosporum* Vittad.) provenienti dal Vivaio Valmetauro di Sant'Angelo in Vado (PU) della Regione Marche.
La tartufaia, una volta impiantata, per una serie di cause legate alle vicissitudini familiari del proprietario (espatrio) non è stata assoggettata ad alcuna pratica colturale, ma come già detto è stata abbandonata a se stessa.
Nel 1998, al momento dell'acquisto da parte di un nuovo proprietario, essa si configurava piuttosto come un bosco misto di latifoglie ("querceto degradato") ricco di alberi (ciliegio, orniello, carpino nero etc) e con fitto sottobosco di arbusti (liane, ginestre, spini neri etc.) (Fig. 1) tuttavia si notava comunque la presenza di 180 roverelle, di dimensione variabile e compresa fra 0,70 cm. e 3 m. di altezza, derivanti dalla precedente piantagione tartuficola.

Fig. 1 Visione, dall'alto, della tartufaia di *Tuber melanosporum* abbandonata ed oggetto di studio prima dei trattamenti di recupero.

Trattamenti colturali di recupero

Mentre nel caso di tartufaie coltivate che sono entrate in produzione e poi hanno smesso o diminuito tale capacità, è possibile, attraverso una appropriata indagine conoscere le ipotetiche cause che hanno determinato tale cambiamento produttivo (es. "chiusura" dell'ambiente vegetazionale della tartufaia, evoluzione negativa delle condizioni pedologiche del suolo, perdita del patrimonio micorrizico delle piante simbionti, etc.) ed anche tentare di porvi rimedio con appropriati interventi colturali (per restare negli esempi citati: potatura e/o diradamento degli alberi per ridurre la vigoria e la densità; ripristino delle migliori condizioni pedologiche con lavorazioni o ammendanti calcarei; rimozione delle condizioni sfavorevoli alla crescita delle micorrize e somministrazione di soluzioni sporali) (Gregori, 2005). Nel caso di tartufaie messe a dimora ed abbandonate, che non sono mai entrate in produzione, diventa molto più complesso cercare di recuperarle alla produzione. Inoltre trattandosi di una cosa nuova non esistono itinerari tecnici già tracciati di cui poter usufruire. Tutto viene fatto per la prima volta per cui si opera più secondo buon senso che per tecnica codificata ed è per questo che il presente caso oggetto di recupero riveste particolare interesse. Non avendo quindi la strada già tracciata, ma tenendo ben presenti i criteri e le tecniche comunemente in uso per il recupero e ripristino delle tartufaie coltivate divenute improduttive (Gregori *et al*., 2001) nonché le risposte della tartufaia in oggetto ai singoli interventi colturali, si è operato nella maniera seguente e secondo questa precisa cronologia.

Nel 1998, anno dell'acquisto, durante il periodo tardo invernale (primi di marzo), si è operata una trinciatura meccanica, (Fig. 2) con eliminazione totale di tutti gli arbusti (soprattutto ginestre, *Spartium junceum* L.), rovi (*Rubus fruticosus* L.) e vitalbe (*Clematis vitalba* L.), e di tutte le piante forestali (carpino nero, *Ostrya carpinifolia* Scop.), orniello (*Fraxinus ornus* L.), ciliegio selvatico (*Prunus avium* L.) e roverella (*Quercus pubescens*) cresciute spontaneamente nelle interfile, al fine di "liberare" completamente le singole roverelle micorrizate con il *Tuber melanosporum* messe a dimora nel 1980.

Fig. 2 Esecuzione, nella parte a monte, dei lavori di trinciatura meccanica per “aprire” la tartufaia abbandonata.

Nel 1999, durante la primavera, ed a più riprese, è stata praticata una ripulitura, con decespugliatore a filo, di tutti i ricacci della vegetazione forestale preesistente (Fig. 3). Successivamente si è proceduto alla “vangatura” manuale, con forcone a quattro denti, dell’area posta attorno le roverelle che avevano dato segni di incipiente “bruciatura”.
Nel periodo fra il 2000 ed il 2002, man mano che la situazione evolveva, tale operazione di “vangatura” manuale, durante il periodo primaverile, è continuata secondo la seguente modalità: solo sulla fascia perimetrale esterna nei pianelli già manifesti; su tutta la superficie nelle aree di accenno del pianello.

Fig. 3 Esecuzione delle operazioni di ripulitura nella parte a monte della tartufaia abbandonata, con decespugliatore a filo.

Nel 2003, con l'intento di verificare lo status micorrizico delle piante della tartufaia, sono stati prelevati campioni radicali per un controllo della micorrizazione tanto delle roverelle che presentavano pianello (n° 4 campioni) che di quelle che ne erano al momento prive (n° 3 campioni).
L'esito del controllo è stato positivo per tutti i campioni nel senso che tutti riportavano micorrize di *Tuber melanosporum*, ma è da segnalare soprattutto il fatto che nei campioni afferenti alle roverelle con pianello le micorrize si trovavano in formazioni "a glomeruli" di notevoli dimensioni.
Nel 2005 e nel 2006 a primavera, dopo la solita vangatura primaverile, con forcone a quattro denti, si è proceduto alla somministrazione di una soluzione di spore, (prodotta con i tartufi provenienti dal medesimo impianto), nelle sole aree con accenno di pianello. Mentre sulla superficie dei pianelli in produzione, e relativamente alla sola parte rimossa con le lavorazioni manuali (corona perimetrale) è stato effettuato uno spargimento di stabilizzato di frantoio (la classica ghiaia calcarea).
Nel 2006 è stata effettuata anche una potatura sia sulle piante produttive che non, per innalzare la chioma e prevenire l'eccessivo ombreggiamento della superficie del suolo.
Nel 2007-2008, l'esecuzione dei lavori di vangatura con i medesimi criteri (cioè totalmente nelle aree con accenno di pianello e limitatamente al perimetro esterno nelle aree dove il pianello risulta evidente) è continuata sotto molte altre piante che progressivamente manifestavano accenno o evidenza di pianello.

Risultati
La risposta produttiva alle operazioni colturali attuate è stata immediata, e se pur variabile, per ogni singola pianta, sia per quanto concerne l'inizio di entrata in produzione che per il quantitativo procapite di tartufo prodotto, tuttavia essa è andata via via aumentando nel tempo secondo la seguente seriazione.
Nel 1999, quindi dopo neanche un anno dall'inizio delle pratiche di ripristino, si sono avuti, durante la tarda estate, i primi accenni di formazione del "pianello" sotto qualche roverella. Già nel 2000 si manifesta, in maniera ben evidente, la comparsa dei primi 18 "pianelli" (la famosa area priva di vegetazione detta "brulé" dai francesi) sotto altrettante roverelle che viene incrementata nel 2002 con la comparsa di ulteriori 12 "pianelli". Nella stagione di raccolta 2004/2005, ha inizio la prima fruttificazione di *Tuber melanosporum*; più precisamente dei 30 pianelli precedentemente comparsi 18 pianelli sono diventati produttivi ed il quantitativo complessivo di tartufi raccolto ammonta a 1Kg.
Nella stagione di raccolta 2005/2006, pur rimanendo invariato il numero (18) dei pianelli produttivi si ha un aumento della fruttificazione; infatti il quantitativo dei tartufi raccolti sale a 3 Kg.
Nella stagione di raccolta 2006/2007 si ha un considerevole aumento della fruttificazione dovuto all'entrata in produzione di altri pianelli; su un totale di 37 pianelli presenti, quelli produttivi passano a 26 ed il quantitativo di tartufi raccolto passa a 10 Kg.
Nella stagione di raccolta 2007/2008, si ha un incremento del numero di pianelli che passano a 48 ma la fruttificazione risulta penalizzata a causa della forte aridità estiva che si è registrata in tutta Europa e che è stata molto deleteria per la raccolta del *Tuber melanosporum*. Comunque dai 26 pianelli produttivi esistenti si raccolgono 5 Kg di tartufo.
Nella stagione di raccolta 2008/2009 si ha un ulteriore incremento positivo sia nel numero dei pianelli produttivi che arrivano a 30 (su un totale di 55) sia del quantitativo di tartufo raccolto che sale a circa 15 Kg .

Discussione dei risultati e conclusioni
I risultati conseguiti appaiono molto interessanti sia dal punto di vista meramente produttivo che tecnico (tartuficoltura) che come contributo all'aumento delle conoscenze bio-ecologiche del *Tuber melanosporum*.
Se si pensa che a solo un anno di distanza dall'inizio dei trattamenti colturali la tartufaia "ha

risposto" con la creazione dell'area di fruttificazione del *Tuber melanosporum* (pianello) sotto numerose roverelle messe a dimora e che cinque anni dopo si sono raccolti i primi tartufi, il cui quantitativo complessivo è andato sensibilmente aumentando, nel tempo, fino a raggiungere la non disprezzabile quantità di 15Kg per 30 piante marcatamente produttive, si deve concludere che i risultati in questo senso (produttivo) ci sono stati e sono stati molto positivi. Inoltre, pur con tutte le avvertenze del caso ed i necessari approfondimenti sulla questione della rigenerazione produttiva delle tartufaie, tuttavia l'esempio proposto dimostra che è possibile recuperare alla produzione, mediante l'esecuzione di appropriate tecniche, anche tartufaie abbandonate di *Tuber melanosporum*, che non sono mai entrate in produzione.
Infine lo studio condotto mette in luce la grande capacità delle micorrize e del micelio di tartufo nero pregiato, normalmente considerato un tartufo di "debole virulenza" di resistere, in uno stato vegetativo latente, anche a condizioni sfavorevoli per la fruttificazione (purché inserito in un contesto di condizioni pedoambientali idonee) ed inoltre evidenzia una buona velocità di risposta della fruttificazione se la tartufaia viene "rigenerata" e gestita in maniera appropriata. Infatti se si ricreano le condizioni idonee, per la sua vita il *Tuber melanosporum* è capace, celermente, di sviluppare congruamente il proprio ciclo biologico e di procedere fino alla formazione ed alla maturazione dei corpi fruttiferi.

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TUBER MAGNATUM: ALCUNI ESEMPI PRODUTTIVI DI TARTUFAIE COLTIVATE IN ITALIA

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Abstract: Few examples of truffle-producing *Tuber magnatum* plantations in Italy
The cultivation of the white truffle (*Tuber magnatum* Pico), highly developed in the eighties, due to the great value recognized to this species, has slowly decreased and eventually stopped. The lack of productivity of the majority of these truffle plantations and the following scepticism towards the white truffle cultivation is one of the main reasons of this halt. However, a few successful plantations do exist, and even if their productivity is modest if compared to that of other truffle species (see *Tuber melanosporum*, *Tuber aestivum*, among others), they prove that the white truffle can be cultivated, despite its complexity.
The authors show a few cases, in Italy, of productive white truffle plantations, and describe both their common features and the peculiar techniques applied to each one.
The aim is to publicize these positive results and to find a possible scientific elucidation of the carpophore production by *Tuber magnatum* Pico, to promote a renewed interest and research work on this species and its cultivation.
Key words: truffle cultivation, *Tuber magnatum*, carpophore production.

Introduzione

La coltivazione intrapresa con il tartufo bianco pregiato (*Tuber magnatum* Pico), specie di grande pregio gastronomico e commerciale, ha avuto un notevole sviluppo negli anni ottanta per le grandi risorse messe in campo e, soprattutto, per la quantità e la qualità degli studiosi che si sono cimentati, ciascuno per le proprie competenze, negli studi e nelle ricerche riguardanti la biologia, l'ecologia e la coltivazione di questa preziosa specie di *Tuber*. Ciò ha comportato, in quel periodo, notevoli progressi per tutto il settore della tartuficoltura in generale e del tartufo bianco in particolare. Basterebbe scorrere i titoli delle tantissime pubblicazioni dell'epoca per rendersi conto della mole di studi e di ricerche riguardanti tale specie. A riprova di quanto asserito ci si può limitare a considerare gli Atti del 2° Congresso Internazionale sul tartufo di Spoleto (tenutosi nel 1988) dove vengono riportati lavori sul *Tuber magnatum* Pico praticamente in ogni tipo di sessione: da quella riguardante la tassonomia, la citologia e l'ultrastruttura del corpo fruttifero (Ciappelloni *et al.,* 1990*;* Fontana *et al.,* 1990*;* Tocci *et al*., 1990) a quelli concernenti le culture in vitro, le micorrize, l'ecologia e la coltivazione in pieno campo (Bencivenga e Granetti*,* 1990a, 1990b; Giovannetti, 1990; Granetti, 1990; Granetti *et al*., 1990; Gregori *et al*., 1990; Lo Bue *et al*., 1990; Pirazzi, 1990), come a quelli relativi alla composizione chimica, la conservazione e la valorizzazione (Coli *et al,* 1990; Senesi, 1990), tanto che si può tranquillamente concludere che più della metà dei lavori presentati a questo importante Congresso Internazionale da Autori italiani (negli altri Paesi il tartufo bianco non esiste o in alcuni non era ancora stato segnalato!) hanno riguardato il *Tuber magnatum*.
Ma già negli anni novanta tale coltivazione cominciava a subire un rallentamento, se non una vera e propria involuzione, e si cominciavano a manifestare le prime grandi perplessità come dimostrano i titoli delle pubblicazioni dell'epoca sull'argomento: Gregori, 1995 "P*roblematiche ed aspettative nella coltivazione del tartufo bianco*"; Bencivenga e Vinay, 1996 " *Considerazioni sulla ecologia e sulla coltivazione di* Tuber magnatum *in Italia*"; Pirazzi, 2001 "*Tuber magnatum Pico*: *un fungo micorrizogeno tardivo*".
A seguito poi, del palesarsi di alcune problematiche (Gregori, 2001) legate al comportamento non sempre rispondente della micorrizazione indotta in laboratorio, a seguito delle perplessità

avanzate dai metodi di controllo biomolecolare sulla identità di tali micorrize, unite al vertiginoso incremento di prezzo dei carpofori da impiegare per l'inoculo, la produzione vivaistica su larga scala delle piante micorrizate con tartufo bianco pregiato è andata diminuendo sensibilmente, fino a cessare completamente.
Tutte queste concause, unite anche alla mancata massiccia produzione di tartufi negli impianti realizzati con questa specie, hanno provocato un dilagante scetticismo nei confronti della tartuficoltura con il tartufo bianco tanto da decretarne, di fatto, la fine. Inoltre da allora non solo non si sono più fatte nuove piantagioni, ma anche quelle già realizzate non sono state più gestite dal punto di vista agro-colturale e sono state completamente abbandonate.
Accanto a questi esempi non riusciti, va tuttavia segnalato che, fra le numerose tartufaie realizzate con *Tuber magnatum*, si sono verificati casi di produzione estemporanea, limitata e parziale di corpi fruttiferi e, in alcuni casi, di produzione costante, duratura e significativa (Donnini *et al*., 2000). Questi rappresentano esempi riusciti di coltivazione, i quali anche se da un punto di vista meramente produttivo potrebbero essere considerati poca cosa, soprattutto se comparati al successo delle produzioni di altre specie di tartufo (vedi *Tuber melanosporum* Vittad., *Tuber aestivum* Vittad*.,* etc.), costituiscono degli importanti casi che testimoniano la possibilità che anche il tartufo bianco può essere "coltivato".
Lo scopo di questo lavoro è quello di illustrare alcuni casi di tartufaie coltivate di tartufo bianco pregiato, entrate in produzione e tuttora produttive, ubicate nel territorio di differenti regioni italiane (Veneto, Emilia-Romagna, Marche, Umbria) e di prendere in esame, anche se in maniera sintetica, le principali condizioni ecologiche dei differenti siti, l'itinerario tecnico-colturale a cui gli impianti sono stati assoggettati dopo la messa a dimora delle piante, l'età della loro entrata in produzione ed i quantitativi di tartufo prodotto, al fine di capire cosa ha determinato la riuscita della coltivazione ed evidenziare, se possibile, un sistema colturale che possa tornare utile per riprendere, in maniera più appropriata, la coltivazione di *Tuber magnatum*.

Principali caratteristiche ecologiche dei siti di alcune tartufaie coltivate di *Tuber magnatum* in produzione
Delle varie tartufaie coltivate di *Tuber magnatum* entrate in produzione (nel 2004 erano circa una ventina quelle segnalate in tutta Italia), si riportano a titolo di esempio quattro piantagioni ritenute rappresentative della situazione generale, in quanto oltre ad essere state gestite secondo un itinerario tecnico del tutto simile, sono entrate in produzione, più o meno, alla stessa età ed hanno continuato tutte, con una maggiore costanza, la produzione dei tartufi.

A - Tartufaia coltivata in provincia di Ravenna (Emilia-Romagna).
Questa tartufaia (Fig.1) è stata realizzata nel 1993, su una superficie complessiva di Ha.00.80.00, mettendo a dimora, in parte a febbraio ed in parte nel novembre successivo, con sesto a quinconce e spaziatura di 5 x 4 m., 290 tigli (*Tilia cordata* Miller; *Tilia platyphyllos* Scop.) e 80 Farnie (*Quercus robur* L.).
Il suolo, che deriva da substrati alluvionali prevalentemente di tipo sabbioso-argilloso (Principi, 1960), è pianeggiante (altitudine s.l.m. 20 m.), si presenta profondo e fresco (umidità residua 6%), abbastanza sciolto, (tessitura Franco–Sabbiosa), discretamente calcareo ($CaCO_3$ 15-25%), a reazione sub-alcalina (pH 7,5-7,8).
Il clima di questa parte dell'Emilia Romagna presenta caratteri di transizione tra un clima mediterraneo ed un clima padano continentale (Cacciamani C. *et al*., 1980). In base alla posizione, l'area dove è localizzata la tartufaia risente sensibilmente dell'influenza mitigatrice marina per cui presenta un periodo di nove mesi temperati, due aridi ed uno freddo ed umido.

La vegetazione naturale della zona, se pur limitata ai fossati ed ai corsi d'acqua, è di tipo ripariale con farnia (*Quercus robur* L.), pioppo (*Populus nigra* L.), salici (*Salix alba* L., *Salix viminalis* L.), acero campestre (*Acer campestre* L.) ed altre specie arbustive ed erbacee meso-igrofile.

Fig. 1 Tartufaia coltivata in provincia di Ravenna (Emilia-Romagna)

B - Tartufaia coltivata in provincia di Padova (Veneto).
La tartufaia (Fig. 2) è stata realizzata nell'aprile del 1992, mettendo a dimora su una superficie di Ha 01.30.00 e con un sesto di 4 x 3,5m. 250 farnie *(Quercus robur* L.), 300 roverelle *(Quercus pubescens* Willd.) e 50 pioppi bianchi (*Populus alba* L.).
Il suolo, pianeggiante (altitudine s.l.m.12 m) e di origine alluvionale, è privo di scheletro, ha una struttura glomerulare e una buona permeabilità. Ha una reazione sub-alcalina (pH 7,8-8,1), è mediamente dotato di calcare totale ($CaCO_3$ 6-11%) ed è sufficientemente umido (umidità residua 8%).
Il clima di questa porzione del Veneto appartiene al "Distretto Climatico Mediterraneo" (Poldini, 1989), ma per una serie di situazioni geomorfologiche particolari, a Padova, dove si trova la tartufaia, le condizioni climatiche sono diverse. Infatti, gli inverni sono piuttosto rigidi, le temperature medie invernali sono prossime allo zero e le minime invernali sono inferiori a zero (gennaio -1,0°C) (Gregori, 1991). Durante l'estate le temperature medie non superano i 24°C (luglio 23,6°C) e non si verifica una vera e propria stagione secca. L'umidità atmosferica annua è estremamente elevata (77,4%) e la temperatura media annua è di 12,7°C. Il regime pluviometrico della provincia di Padova, chiamato da De Marchi (1935) "regime sub-litoraneo", presenta una rilevante differenza fra il massimo primaverile e quello autunnale delle precipitazioni la cui media annua è variabile fra 900 e 1000mm.
La vegetazione della zona, se pur limitata ai bordi dei campi, è tipicamente ripariale con tigli (*Tilia platyphyllos* Scop.; *Tilia cordata* Miller; *Tilia* x *vulgaris* Hayne), olmo (*Ulmus minor* Miller), platano (*Platanus hybrida* Brot.), ontano nero (*Alnus glutinosa* (L.) Gaertner), pioppo (*Populus nigra* L.), salici (*Salix alba* L.; *Salix viminalis* L.), acero campestre (*Acer campestre* L.), nocciolo (*Corylus avellana* L.), sambuco (*Sambucus nigra* L.) e alcune specie arbustive ed erbacee meso-igrofile.

Fig. 2 Tartufaia coltivata in provincia di Padova (Veneto)

C - Tartufaia coltivata in provincia di Pesaro-Urbino (Marche)
La tartufaia (Fig. 3) è stata realizzata su un terreno leggermente declive (esposizione prevalente Nord/Nord-ovest) nel 1991, mettendo a dimora in primavera (aprile), su una superficie di Ha 01.20.00, 500 piante così suddivise: 150 tigli (*Tilia platyphyllos* Scop.), 150 pioppi bianchi (*Populus alba* L.), 100 cerri (*Quercus cerris* L.) e 100 carpini neri (*Ostrya carpinifolia* Scop.) con un sesto di 5 x 5 m. Trattandosi di una tartufaia sperimentale con funzione pilota sono stati piantati anche alberi, della stessa specie, non micorrizati con funzione di testimoni.
Il suolo, che deriva da substrati geologici dell'era terziaria (miocene) e più precisamente dalla Formazione marnoso-arenacea (Principi, 1960), è abbastanza sciolto, ha tessitura variabile dal franco-sabbioso al franco-sabbioso-argilloso, è ricco di calcare ($CaCO_3$ 22-37%), ha reazione sub-alcalina (pH 7,5-7,8) ed è abbastanza fresco (umidità residua 5%).
Il clima della zona può essere definito come sub-mediterraneo montano, cioè abbastanza piovoso (1000mm annui) e non eccessivamente arido (anche durante i mesi estivi si registrano significative precipitazioni). Il regime pluviometrico della zona è di tipo sub-equinoziale, con piogge distribuite in prevalenza nei mesi autunnali e in quelli primaverili.
Il regime termico è di tipo sub-continentale con inverni freddi (gennaio 0,2°C) ma non troppo rigidi ed estati calde (luglio 21°C) ma mai eccessivamente aride.
La vegetazione naturale della zona, essendo limitrofa a boschi cedui naturali, è quella tipica del *Castanetum* di Pavari (1966), dove le specie arboree prevalenti sono roverella (*Quercus pubescens* Willd.), cerro (*Quercus cerris* L.), carpino nero (*Ostrya carpinifolia* Scop.), orniello (*Fraxinus ornus* L), ma anche aceri (*Acer obtusatum* W. et K., *Acer campestre* L.), pioppi (*Populus alba* L., *Populus nigra* L.), salici (*Salix caprea* L., *Salix alba* L.), sorbi (*Sorbus domestica* L., *Sorbus torminalis* (L.) Crantz), ciliegio (*Prunus avium* L.), olmo (*Ulmus minor* Miller) e nocciolo (*Corylus avellana* L.).

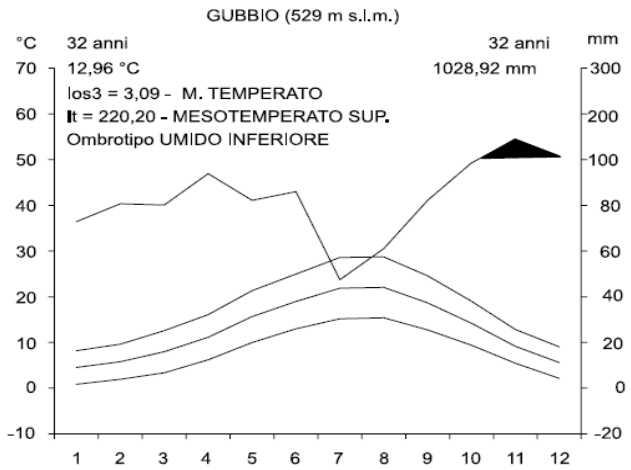
Fig. 3 Tartufaia coltivata in provincia di Pesaro-Urbino (Marche)

D - Tartufaia coltivata in provincia di Perugia (Umbria)

La tartufaia (Fig. 4) è stata realizzata nel 1986 in località Colbassano, Comune di fossato di Vico, su una superficie di circa Ha 02.00, con terreno pianeggiante situato a 300 m. di quota. La tartufaia fu realizzata mettendo a dimora, con fini sperimentali, tutte le specie simbionti che all'epoca venivano unite in simbiosi con il tartufo bianco: *Corylus avellana* L., *Quercus pubescens* Willd., *Ostrya carpinifolia* Scop., *Salix capraea* L., *Populus nigra* L., *Populus alba* L.. Con le diverse specie furono realizzate parcelle monospecifiche.

Il suolo è ricco di limo (sabbia 18,40%, limo 58,5%, argilla 23,1%), ha una reazione sub alcalina (pH 8,01), è ricco di calcare (calcare totale 20,8%, calcare attivo 11,9%) e povero di sostanza organica (2,16%).

Il clima della zona viene definito, secondo ARPA (2004), macrobioclima temperato, bioclima oceanico, termotipo mesotemoperato superiore e ombrotipo umido inferiore. La media delle precipitazioni annue (media di 32 anni) è di 1029mm. La siccità estiva è limitata a circa 45 giorni tra i mesi di giugno, luglio ed agosto.



La vegetazione della zona è quella tipica delle aree di fondovalle con prevalenza di *Populus nigra* L., *Quercus pubescens* Willd., *Quercus cerris* L., *Populus alba* L., *Salix* sp.pl. e arbusti quali *Cornus sanguinea* L., *Rubus ulmifolius* Schott, *Clematis vitalba* L., ecc.

Fig. 4 Tartufaia coltivata in provincia di Perugia (Umbria)

Itinerario tecnico-colturale

Tutte le tartufaie, riportate come esempio, sono state realizzate negli anni “90: (tartufaia D impiantata nel 1986, C nel 1991, B nel 1992, A nel 1993) mettendo a dimora piante micorrizate, prodotte mediante “inoculazione sporale”, a partire da ascomi sicuri di *Tuber magnatum*, ed ottenute secondo le metodiche e le tecniche in auge all’epoca (Palenzona e Fontana, 1978; Bencivenga, 1982; Zambonelli, 1985; Granetti, 1987; Gregori e Ciappelloni, 1990; Pirazzi *et al*., 1989).

I lavori di pre-impianto eseguiti nelle quattro tartufaie sono: aratura estiva più o meno profonda, una estirpatura autunnale ed una leggera erpicatura nella primavera successiva prima della messa a dimora delle piante simbionti

Da segnalare una bizzarra coincidenza: i terreni utilizzati nelle prime tre piantagioni, prima dell’impianto della tartufaia, sono stati coltivati a frutteto (pescheti o pereti). La tecnica di coltivazione dei frutteti prevedeva più fresature annuali con erpici a dischi durante i primi cinque anni, quindi inerbimento e solo taglio della vegetazione erbacea. Nella tartufaia di Colbassano il terreno, prima dell’impianto, veniva utilizzato per colture erbacee con una rotazione che vedeva l’alternarsi di cereali e leguminose foraggere.

La densità d’impianto è abbastanza elevata (A m 4x5; B m 4x3,5; C m 4x4; D m 4x4).

Le operazioni colturali di post-impianto sono state:

a) zappettatura manuale attorno la pianta nei primi 2-3 anni e sostituzione delle fallanze (1-2%);

b) sfalcio ripetuto dell’erba, accompagnata in alcuni casi (tartufaia B e C) dalla lavorazione primaverile del suolo con mezzo meccanico nel primo periodo di vita (fino verso il 6°-7° anno);

c) potatura dei primi rami lungo il tronco per conformare e innalzare la chioma nei primi anni di vita; in alcuni casi la potatura è stata ripetuta in età avanzata (tartufaia B, C e D) e in maniera a volte intensa e comprendente anche la cimatura delle piante più “allungate” (tartufaia B). Potatura protratta per più anni nella parcelle dei salici (tartufaia D) che tendono, anche in età avanzata, a produrre rami lungo il tronco;

d) irrigazione di soccorso, con quantitativi di acqua variabili da caso a caso, sia dopo la messa a dimora che durante il periodo produttivo;

e) pacciamatura con materiali vari (ginestre nella tartufaia C; film plastico nella tartufaia B) praticata solo nei primi 5 anni;

f) drenaggio per eliminare periodici ristagni di acqua effettuato undici anni dopo la piantagione nella tartufaia D.

Risultati

Quasi tutti gli impianti in questione hanno iniziato a produrre, in età differente (tartufaia B 6 anni dopo l’impianto; C 8 anni; A e D 10 anni). Tre di essi, per alcuni anni (per 2-3 anni tartufaia A; 3-4 anni tartufaia C; 4-5 anni tartufaia B), hanno prodotto corpi fruttiferi, a volte anche di bella

pezzatura, di aspetto simile al tartufo bianco pregiato (*Tuber magnatum*) ma con caratteristiche (profumo, spore etc.) ascrivibili a quelle del grande gruppo del tartufo bianchetto (*Tuber borchii* Vittad.) ed in particolare a una forma con caratteri morfologici esterni intermedi tra *Tuber magnatum* e *T. borchii*, indicato con il termine generico di "tartufo delle terre matildiche".
Laddove si è verificata tale produzione di carpofori strani, senza motivo apparente, è poi diminuita drasticamente (tartufaia A) o addirittura completamente cessata (tartufaie B e C) per dare luogo alla produzione di "veri" tartufi bianchi pregiati (*Tuber magnatum*) ad una età della tartufaia variabile, a seconda dei casi, ma compresa fra gli 11 ed i 12 anni. Per precisione l'inizio della produzione di *Tuber magnatum* (Fig. 5) è iniziata:
- per la tartufaia A nella stagione 2004, cioè a 11 anni dalla messa a dimora (1993).
- per la tartufaia B, nella stagione 2004, cioè dopo 12 anni dall'impianto (1992).
- per la tartufaia C, nella stagione 2002, cioè dopo 11 anni dall'impianto (1991).
La tartufaia D ha avuto un comportamento differente, perché ha prodotto solo *Tuber magnatum* iniziando dal quattordicesimo anno. La produzione è iniziata l'anno successivo all'intervento di drenaggio, che ha eliminato i periodici ristagni di acqua, e ha interessato solo la parcella investita a *Populus alba*, dove la chioma degli alberi determina una copertura totale del suolo.

Fig. 5 – Produzione di *Tuber magnatum*

Attualmente tutti gli impianti, sia pure gestiti in maniera non omogenea, sono produttivi ed i quantitativi raccolti variano nelle diverse annate da qualche etto di tartufo ad alcuni chilogrammi.

Gli andamenti produttivi nelle stagioni successive sono stati i seguenti:
La tartufaia A ha prodotto nella stagione 2004 1Kg di tartufo bianco; nella stagione 2005 circa 0,5Kg; nella stagione 2006 circa 0,8Kg; nella stagione 2007 circa 0,5Kg.; nella stagione 2008 1Kg.
La tartufaia B ha prodotto nella stagione 2004 3Kg di tartufo bianco; nella stagione 2005 circa 0,2Kg; nella stagione 2006 circa 0,5Kg; nella stagione 2007 circa 0,5Kg.; nella stagione 2008 0,5Kg.
La tartufaia C ha prodotto nella stagione 2002 5Kg di tartufo bianco; nella stagione 2003 circa 0,1Kg; nella stagione 2004 5Kg; nella stagione 2005 circa 0,8Kg; nella stagione 2006 circa 1Kg; nella stagione 2007 circa 0,5Kg.; nella stagione 2008 2,5Kg;
La tartufaia D ha prodotto mediamente ogni anno 300g. di tartufo bianco nella sola parcella investita a *Populus alba*, che ha una superficie di circa 2000 mq.

Discussione e Conclusioni
Questi esempi positivi permettono di fare alcune considerazioni conclusive.

Innanzi tutto, al di là dei quantitativi prodotti, si dimostra che anche la "coltivazione" del tartufo bianco, sia pure lunga e complessa, è possibile. Inoltre, trattandosi di piantagioni ubicate in siti dove non esiste la raccolta spontanea di tartufo bianco, si ritiene che il veicolo produttivo sia riferibile alle piante micorrizate; ciò è dimostrato anche dal fatto che quelle non micorrizate messe a dimora come testimoni nella tartufaia C, non hanno mai prodotto tartufi di nessuna specie.
L'entrata in produzione, tra l'altro avvenuta in concomitanza di stagioni molto produttive, si è verificata dopo un periodo di tempo lungo e più o meno alla stessa età (dopo 11-14 anni). Ciò indurrebbe a pensare che il *Tuber magnatum* sia una specie "climax", vale a dire che per fruttificare richiede la formazione di uno specifico corteggio biologico preparatorio, che crei idonee condizioni per la fruttificazione. L'aspetto relativo al corteggio fungino sarebbe dimostrato dalla presenza di carpofori (in antecedenza o in concomitanza con la produzione di *Tuber magnatum*) di altre specie fungine (den. *Hymenogaster*, *Balsamia*, *Laccaria, Tuber,* etc).
Sarebbe comunque interessante capire perché queste tartufaie, realizzate con piante inoculate con spore provenienti da carpofori di *Tuber magnatum*, una volta messe a dimora producono esemplari di *Tuber* assimilabili a *Tuber borchii* di cui non si comprende l'origine.
Una volta entrate in produzione, le tartufaie coltivate di *Tuber magnatum* risentono di più, rispetto alle altre specie di *Tuber*, dell'andamento stagionale più o meno favorevole, infatti, la quantità di tartufi prodotti, che dovrebbe tendere nel tempo ad aumentare, è sempre molto variabile nelle differenti stagioni, a prescindere dai trattamenti colturali eseguiti.
Per cui la conclusione non può essere che un auspicio: e cioè che, anche sulla base di questi positivi esempi di produzione, vi sia una ripresa degli studi e delle ricerche concernenti la coltivazione del *Tuber magnatum,* a partire dalla produzione su vasta scala di piante micorrizate. Il passo successivo è la messa a punto di un itinerario tecnico specifico per capire quali sono i fattori biologici e colturali che inducono la formazione dei carpofori al fine di attuare concretamente il rilancio della coltivazione del tartufo bianco, specie di grandissimo pregio e considerata il gioiello della flora micologica italiana.

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TUBER BRUMALE TESTING OF TRUFFIÈRES IN NEW ZEALAND

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Abstract
In summer 2006/2007, *Tuber brumale* mycorrhizae were discovered in New Zealand both in a nursery and in three Black truffle (*T. melanosporum*) plantations. A nationwide survey was conducted on behalf of the New Zealand Truffle Association to determine how widespread *T. brumale* is in New Zealand truffières. A protocol for collecting roots from around a sampled tree's cardinal points was sent to participating growers. In most cases, one random hazel (*Corylus avellana*) and oak (*Quercus robur*) were sampled from each truffière. Microscope and DNA analyses were used to detect and identify *Tuber* mycorrhizae. A total of 71 root samples from 30 truffières (20 and 10 from the North and South Islands, respectively) were analysed. The presence of *T. brumale* mycorrhizae was confirmed on samples from 10 truffières, 8 in the North Island, 2 in the South Island. *T. brumale* was confirmed on 11 trees (3 hazels and 8 oaks). Of these 11 trees, 7 also had *T. melanosporum* mycorrhizae present. Although our study cannot estimate the abundance of *T. brumale* in New Zealand truffières, it is the first fully documented report on the multiple occurrences of mycorrhizae of *T. brumale* in Black truffle plantations in the Southern Hemisphere. So far, *T. brumale* has only been found in its mycorrhizal form in New Zealand. *Tuber brumale* is likely to have entered New Zealand as a contaminant on trees inoculated with *T. melanosporum*. Further dissemination of *T. brumale* in New Zealand through the establishment of new truffières can largely be eliminated through the introduction of robust industry standards.
Key words: *Tuber brumale*, *Tuber melanosporum*, spiky cystidia, New Zealand, truffière.

Introduction
In November 2006, Crop & Food Research (C&FR)[1] discovered a small amount of *Tuber brumale* mycorrhizae on trees that were inoculated and mycorrhized by Périgord black truffles (*T. melanosporum*) from Europe. Since *T. brumale* was thought to be absent from New Zealand at that time, all trees were destroyed under the supervision of the Ministry of Agriculture and Forestry (MAF).
In joint work by C&FR and MAF, *T. brumale* mycorrhizae were discovered in early 2007 in three Black truffle plantations. Findings were confirmed by PCR testing (Paolocci *et al*., 2009) and sequencing of the rDNA ITS region.
Following these findings, a nationwide survey was conducted for the benefit of the New Zealand Truffle Association (NZTA) to determine how widespread *T. brumale* was in New Zealand truffières.

Methodology
A protocol for collecting roots was sent to participating growers. Roots were collected from around the sampled tree's cardinal points to increase the chances of detecting *T. brumale*. In most cases, one random tree per tree species, ie: hazel (*Corylus avellana*) or oak (*Quercus robur*) was sampled from each truffière. Microscope and DNA analyses (PCR and nested PCR) using published primers (Paolocci *et al*., 2009, Amicucci *et al*., 1998) were used to detect mycorrhizae of the genus *Tuber* and to identify them to the species level, focusing on mycorrhizae with spiky cystidia.

Results
A total of 30 truffières (20 and 10 from the North and South Islands, respectively) provided root samples. We analysed a total of 71 root samples from 32 hazels and 39 oaks (Table 1).

Tab. 1 Summary of findings on the 71 trees sampled in 30 truffières.

Location	Number of truffières tested	Tree species	Number of trees tested	Number of trees showing the following mycorrhizal types or species			
				PBT	BRU	MAC	OEF
North Island	20	hazel	19	16	3	2	14
		oak	25	14	6	3	18
South Island	10	hazel	13	5	0	2	11
		oak	14	5	2	3	14
Both Islands	30	hazel	32	21	3	4	25
		oak	39	19	8	6	32
		both	71	40	11	10*	57

Abbreviations: PBT, Périgord black truffle (*Tuber melanosporum*) mycorrhiza; BRU, brumale (*T. brumale*) mycorrhiza, MAC, maculatum (*T. maculatum*) mycorrhiza; OEF, other ectomycorrhizal fungi. The presence of *T. brumale* and *T. maculatum* mycorrhizae has been confirmed by DNA analysis.
*: The presence of *T. maculatum* was confirmed by DNA for 10 trees only but results suggest that the number of trees hosting *T. maculatum* is likely to be at least 17.

The presence of mycorrhizae of *T. brumale* was confirmed on samples from 10 of the 30 truffières involved in this survey, and was distributed in New Zealand as follows: 8 in the North Island (40% of the North Island sites), 2 in the South Island (20% of the South Island sites). *T. brumale* was confirmed on 11 trees (15.5% of the sampled trees): on 3 hazels and on 8 oaks (Tab. 1). Of the 11 trees, 7 also had *T. melanosporum* mycorrhizae present. The morphological distinction between *T. melanosporum* and *T. brumale* mycorrhizae and the similarity between *T. brumale* and *T. maculatum* mycorrhizae is shown in Figures 1-3.

Fig. 1 Comparison of the external morphology of the mycorrhizae of *Tuber melanosporum* (top + bottom left) versus *T. brumale* (top + bottom right) (dissecting microscope).

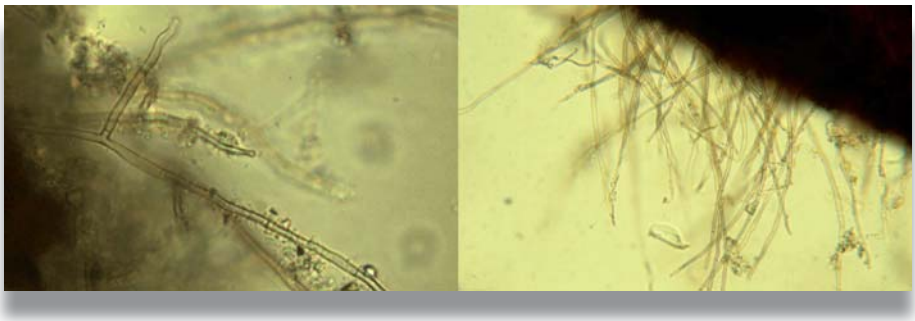

Fig. 2 Details of the emanating hyphae from mycorrhizae of *T. melanosporum* (compound microscope). Note the right-angle branching of hyphae.

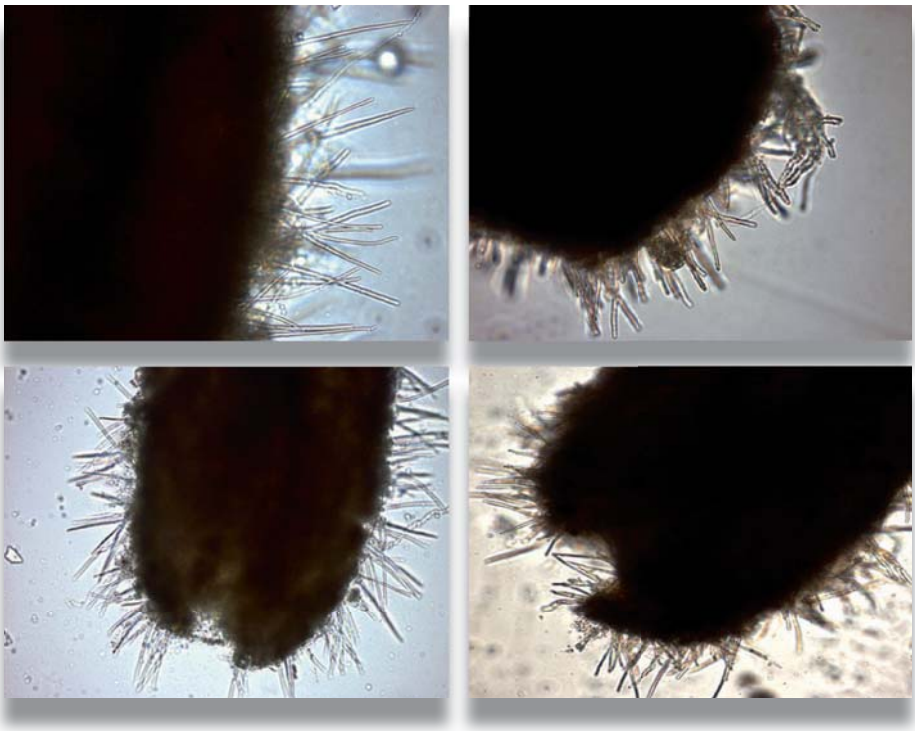

Fig. 3 Details of the root 'hairs' of truffle mycorrhizae with spiky cystidia (compound microscope). *T. maculatum* (top + bottom left); *Tuber brumale* (top + bottom right). Note that both species have spikes that never branch (unlike the 'hairs' of *T. melanosporum*). Although some morphological differences may exist between the two mycorrhizal types (*T. maculatum* spikes are see-through while *T. brumale* spikes are yellow) these two species can look very similar.

Tuber maculatum mycorrhizae were likely to be present on at least 17 trees but this was only confirmed by DNA for 10 trees due to time and cost constraints.

Tuber melanosporum (ie: Périgord Black truffle) mycorrhizae were identified in samples from 23 of the 30 truffières in this survey, on 21 hazels (ie: 66% of the hazels) and 19 oaks (49% of the oaks) (Table 1). Since we were not specifically looking for these mycorrhizae, this result is very likely to underestimate the actual number of trees with *T. melanosporum* mycorrhizae. Some samples showed a very intense mycorrhization by *T. melanosporum* (Fig. 4).

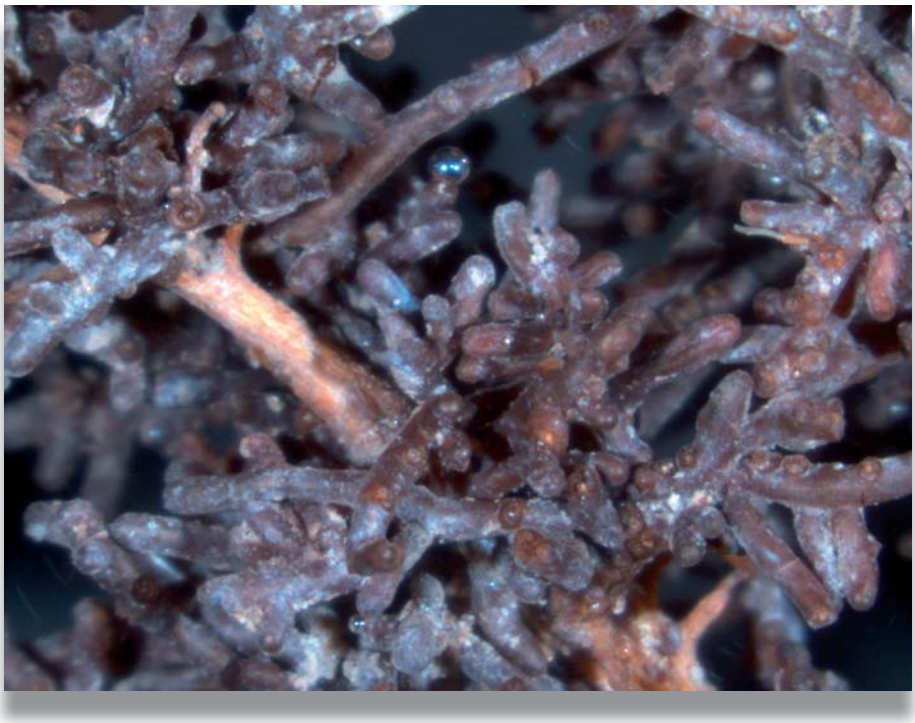

Fig. 4 A cluster of well-developed Périgord black truffle (*Tuber melanosporum*) mycorrhizae on a hazel tree (dissecting microscope). The first *T. melanosporum* truffles were harvested from this site a few weeks following this survey.

Although the presence of *T. melanosporum* mycorrhizae was not systematically confirmed by DNA, when this analysis was carried out it always confirmed our initial identification made using morphological characteristics only.

Compiling data from this study with the preliminary work done by C&FR and MAF, in total *T. brumale* mycorrhizae have been detected in New Zealand in 12 out of 32 truffières, ie: 10 sites in the North Island, 2 sites in the South Island.

Discussion and conclusion

So far *T. brumale* has only been found in its mycorrhizal form. The first New Zealand-grown *T. brumale* truffle has yet to be discovered. It is important to monitor, in the near future, the potential fruiting of *T. brumale* truffles in sites where *T. brumale* mycorrhizae have been detected.

The next logical step for this testing would probably be to estimate the abundance of *T. brumale*, and its abundance relative to *T. melanosporum*, in the truffières which were found positive for this species. In addition, it would be interesting to carry out *T. brumale* surveys in the wild (ie: outside truffières).

Although *T. brumale* and *T. melanosporum* truffles are morphologically distinct, the natural variation within each species makes their distinction based solely on morphology sometimes hazardous (Fig. 5).

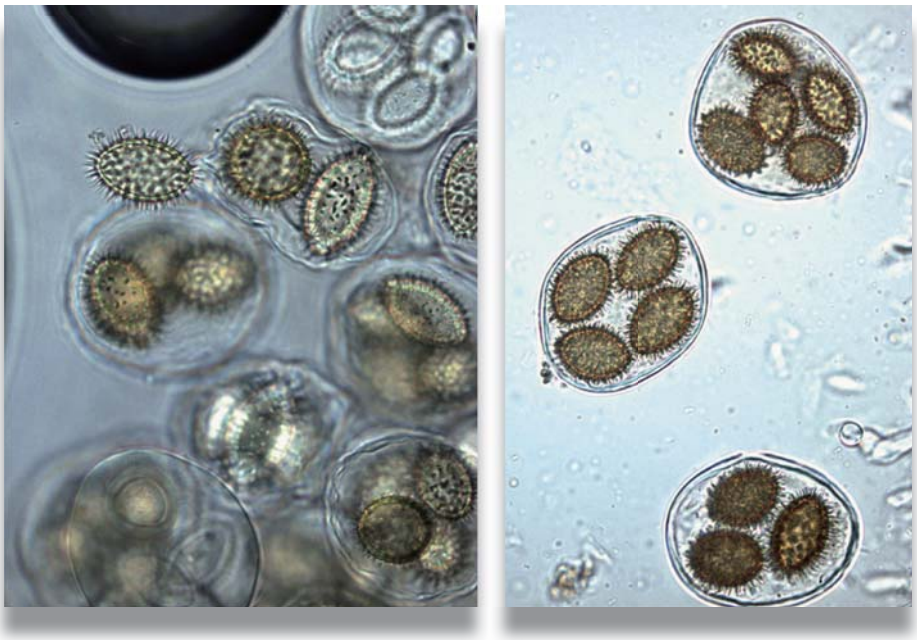

Fig. 5 Although spores of *T. melanosporum* (left) and of *T. brumale* (right) are usually distinguishable, they can also look very similar. The spores pictured here are from specimens whose identity has been confirmed by DNA analysis. Therefore, the DNA testing of truffle inoculum is crucial.

The results of this survey suggest that *T. brumale* is likely to have entered New Zealand truffières as a contaminant on trees inoculated with *T. melanosporum*. Further dissemination of *T. brumale* in New Zealand through the establishment of new truffières can largely be eliminated through the introduction of robust industry standards.

Acknowledgements
The C&FR edible fungi team would like to thank the growers participating in this survey. We also thank Nikki Johnson of the NZTA for helping us organizing this nationwide testing. This work has been fully supported by the New Zealand Institute for Crop and Food Research Limited.

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Notes

[1]Please note that in December 2008 The New Zealand Institute for Crop & Food Research (C&FR) merged with HortResearch Ltd to form The New Zealand Institute for Plant & Food Research (P&FR).

ESTABLISHMENT OF *TUBER AESTIVUM* VITTAD. ECTOMYCORRHIZAE ON *QUERCUS ROBUR* AND *CORYLUS AVELLANA* SEEDLINGS IN POLAND

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Abstract
1-year old seedlings of *Quercus robur* and *Corylus avellana* taken from bare root nurseries were inoculated with spores of *Tuber aestivum,* according to method of IPLA (Torino), which had been collected from fruiting bodies found in Poland in 2007. Ectomycorrhizal development was monitored for 6 months in the greenhouse and compared to the performance of the seedlings. Seedling survival in both host species was at similar level, only 2% of oak and 7% of hazel seedlings died, but mycorrhization showed major differences. The colonization rate was very high in case of *Q. robur* seedlings; 100% of investigated seedlings possessed ectomycorrhizae of *T. aestivum* whereas only 20% of *C. avellana* seedlings were colonized by the fungus. In both species, ectomycorrhiza of adventives fungi could be observed, probably *Scleroderma*. The seedlings have been outplanted in October 2008 to the site with natural soil of pH 7.8.
Preliminary results showed that successful colonization by *T. aestivum* under greenhouse conditions opens up the possibility of cultivating this truffle in Poland.
Key words: *Tuber aestivum, Quercus robur, Corylus avellana*, silviculture, Poland.

Introduction

Over the last a few decades, considerable progress has been made in the controlled infection of forest trees with selected ectomycorrhizal fungi (Perez *et al.,* 2007).
Of particular interest are those fungi that form edible fruiting bodies. The most important in terms of demand and high culinary value are some species of truffles, including *Tuber aestivum.*
T. aestivum grows in an ectomycorrhizal symbiosis with many different tree species e.g., *Quercus robur* and *Corylus avellana* (Chevalier and Frochot, 1997).
Recently, new data on the distribution of *T. aestivum* and other species of truffles have been reported from Poland (Hilszczanska *et al.,* 2008). The aim of our study is to study the feasibility of cultivating the Summer truffle in Poland. In first part of experiment was to investigate the survival of truffle ectomycorrhiza in a pot experiment in a greenhouse 6 months after inoculation. Next part is to check the survival of the ectomycorrhiza and the seedlings, as well, in the experimental plantation one year after establishment.

Materials and methods

In April 2008, 1-year old seedlings of *Quercus robur* and *Corylus avellana* obtained from Polish bare root nursery were inoculated with spores of *Tuber aestivum* fruit bodies found in Poland in 2007 (Hilszczanska *et al.,* 2008).
The seedlings were potted in a previously sterilized mixture of peat moss and vermiculite (4:4, v:v; approx. pH 7.2). After inoculation seedlings were kept for period of 6 months in a greenhouse and watered every 2 days, according to the temperature and substrate condition.
Six months after inoculation, 10 plants of each species were monitored for mycorrhiza formation. Some mycorrhizal tips from all treatments were taken to DNA analysis Amplification by PCR and sequencing was done with the pair of primers ITS5 and ITS7 (Bertini *et al.,* 1999).
Analysis of soil pH and contents of the basic nutrients were done in the site where the seedlings have been outplanted (ISO: 10390 1994; 11261 1995a; 10694 1995c; 11466 1995b).

In October 2008, the seedlings were outplanted to the site that represents Rendzic type of soil (pH ranges from 6.6 to 8.15). Seedlings were planted with 3 m between seedlings within the row and rows were planted 4.5 m apart.

Results

Tab. 1 Identification of the fungal symbiont on root tips of *Q. robur* (1.) and *C. avellana* (2.) seedlings

Fungal taxa	Length of sequenced ITS (bp) or brief description	Accession No. of most similar ITS sequence in Gene Bank	Identities
1. *Tuber aestivum*	690	EU326689	100%
2. *Tuber aestivum*	687	EU32669	99%

ITS, internal transcribed spacer

Tab. 2 Survival and mycorrhization of *Quercus robur* and *Corylus avellana* after inoculation with *Tuber aestivum*

	Q. robur	*C. avellana*
Total seedlings Surviving seedlings after 6 months Total sampled	134 131 (98%) 10	150 149 (92%) 10
seedlings after 6 months Sampled seedlings colonized by *T aestivum*	10 (100%)	2 (20%)

Tab. 3 Soil composition at the site (numbers 1-5 represent different parts of the site) where seedlings inoculated with *T. aestivum* are outplanted

SITE IDENTIFICATION	1	2	3	4	5
pH_{H20}	6.66	7.87	6.95	8.15	8.05
pH_{KCL}	5.83	6.29	6.63	7.28	7.54
$CaCO_3$ (total)%	0.02	0.87	0.03	38.2	0.83
P (g x kg^{-1})	0.12	0.16	0.20	0.43	0.20
Fe(g x kg^{-1})	2.65	3.29	3.44	16.5	5.59
Ca (g x kg^{-1})	0.80	4.89	1.60	177.9	5.31

Mg (g x kg^{-1})	0.53	0.75	0.69	4.46	1.17
K (g x kg^{-1})	0.88	1.17	1.22	6.70	1.73
Ca/Mg	1.5	6.5	2.3	39.7	4.5
K/Mg	1.6	1.6	1.8	1.5	1.5
Fe	2.65	3.29	3.44	16.5	1.73
Carbon (total) %	0.40	1.33	1.14	4.87	1.13
Nitrogen (total) %	0.04	0.10	0.09	0.10	0.09
C/N	10.0	13.3	12.6	48.7	12.5

Fig. 1 Ectomycorrhizae formed by *Tuber aestivum* and *Q. robur* seedlings

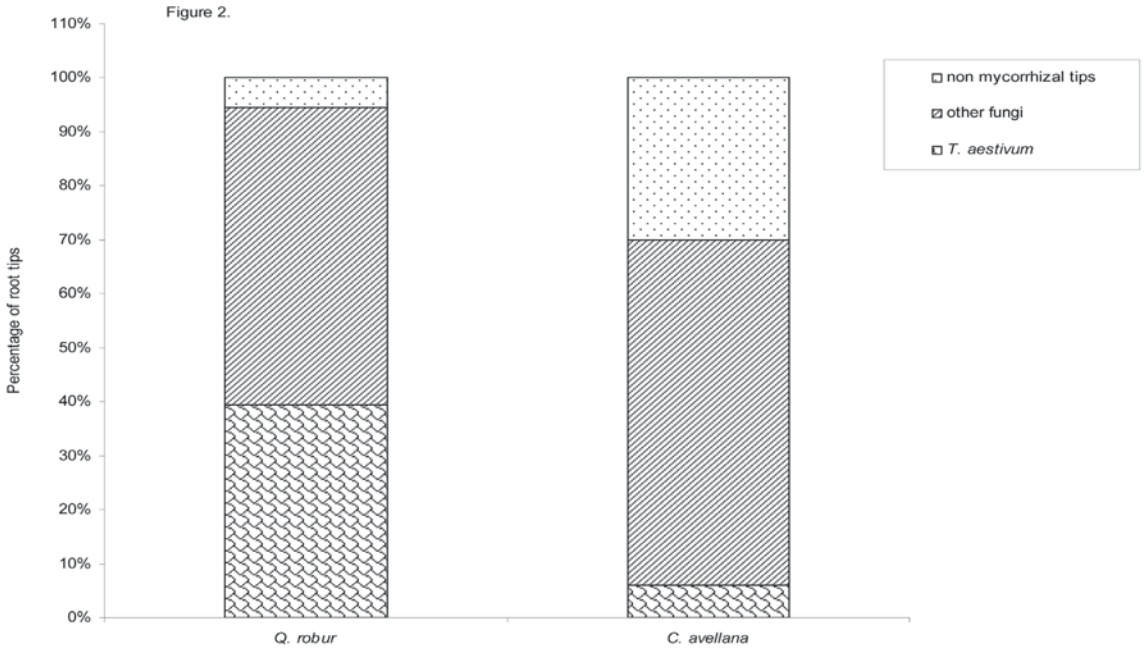

Figure 2 Colonization rates of *Tuber aestivum* and adventives fungi on *Quercus robur* and *Corylus avellana* 6 months after inoculation

Conclusions

1. Seedling survival in both host species was at similar level, only 2% of oak and 7% of hazel seedlings died, but mycorrhization showed major differences.
2. The colonization rate was very high in case of *Q. robur* seedlings; 100% of investigated seedlings possessed ectomycorrhizae of *T. aestivum* whereas only 20% of *C. avellana* seedlings were colonized by the fungus.
3. Preliminary results showed that successful colonization by *T. aestivum* is promising for future truffle cultivation in Poland. Although, our results reflects the situation in a greenhouse conditions, work currently undertaken will examine the survival of *T. aestivum* and the growth of *Q. robur* and *C. avellana* under field conditions.

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TRUFFICULTURE'S GREEN REVOLUTION STARTS IN TERUEL (SPAIN)

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Abstract

The climate and the soils of the highlands of Teruel have never been capable of producing sufficient amounts of agricultural products to support a large population. Nowadays this is changing with truffle cultivation. There are more than 3,000 hectares of truffle orchards in this area and the yields are starting to support more people living in better conditions. The bases of this change were laid in the late 80's with the first successful plantations, and continued in the 90's with a strong program of subsidies to farmers. These efforts have created a strong truffle based sector of the economy that now is demanding technological support. The initial "plant and wait" approach was adequate to obtain the first truffle crops but a completely new body of knowledge is needed to bring trufficulture into the realm of modern agriculture, where yields are consistently high and predictable, and product quality meets global standards. With this objective in mind we have established a network of experimental plots in this area to provide answers to the questions that the growers identify as the most critical to their activity: watering strategies to achieve precocity and maximum sustained production, impact of cultivation decisions, and the influence of different soil textures in mycelia behaviour and fruitbody production. We are accompanying these experiments with monitoring of *Tuber melanosporum* mycelia development in a yearly cycle and during the early years of a plantation, as well as the evolution of the populations of other soil microorganisms as plantations age.

Key words: black truffle, cultivation.

ESTIMATION OF CROP COEFFICIENT FOR IRRIGATION IN BLACK TRUFFLE ORCHARDS DURING ITS FIRST STAGES

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Abstract

The symbiosis between *Tuber melanosporum* and *Quercus ilex* was analyzed from the point of view of the atmospheric evaporative demand on *T. melanosporum*-inoculated seedlings in young truffle orchards. Reference evapotranspiration (ETo), based on data from the climatic stations nearest to five truffle orchards studied, was determined using the Penman-Monteith method. The ETo was used to establish five doses of irrigation: 0, 25, 50, 75 and 100% of the ETo. Three treatment application periods were tested: from April through July, August and September, and from April through September. The experimental design consisted of 5 irrigation treatments and 3 periods in 5 blocks yielding 75 experimental units. Every unit consisted of a tree inoculated with *T. melanosporum*. The irrigation doses were chosen to cover a wide range of the crop coefficient (Kc). The highest numbers of *T. melanosporum* ectomycorhiza were observed on seedlings irrigated with the dosis of 50% of ETo during the April through July period. From the data on irrigation and rainfall we suggest that one of the characteristics of the Kc for young truffle orchards would be a decreasing function from April to August to increase again in September. Based on these observations, when the air temperature reaches the highest values of the year, the still non-productive young truffle orchards should be maintained in moderate water stress.

Key words: holm oak, irrigation, evapotranspiration, ectomycorrhiza, *Tuber melanosporum*.

OBTENCIÓN DE TRUFA NEGRA (*TUBER MELANOSPORUM* VITTAD.) A PARTIR DE PLANTACIÓN CULTIVADA, EN TERRENO TRADICIONAL DE REGADÍO

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Abstract: Black truffle (*Tuber melanosporum* Vittad.) obtention in a mycorrhized oak orchard of a traditional irrigated land.
The background, features and farming methodology to set up a *Tuber melanosporum* Vittad. mycorrhized oak orchard in order to harvest black truffle harvest in Zaragoza (Ebro Valley, Spain), are described in this paper.
Although truffle cropping is a common activity of diversification in mountain depressed areas the establishment on irrigated and calcareous soils could be considered in the same way as a fruit orchard.
A first black truffle was obtained in an experimental plot of Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), located in Zaragoza. This new event in this field supports this assertion in contrast with the orthodoxy of the current trends in truffle farming.
Key words: Truffle cultivation, agricultural techniques, alternative crops, mycorrhization.

Introducción y antecedentes

El desarrollo de la truficultura en España ha experimentado un impulso extraordinario en esta última década, fundamentado en la aptitud edafoclimática de muchas partes de nuestro territorio, que lo hace particularmente propicio para la instalación de plantaciones de árboles micorrizados con el objetivo de producir trufa negra (*Tuber melanosporum* Vittad.).
En los últimos 10 años se ha duplicado prácticamente la superficie dedicada a este cultivo, alcanzando las 5.000 hectáreas y creciendo anualmente a un ritmo medio de 400 hectáreas/año. Las razones de este desarrollo espectacular se basan no sólo en la aptitud de los suelos y climas antes aludida sino también en la mejora de los conocimientos y de toda la tecnología e investigación dedicada a los sistemas de producción. El dinamismo creciente del sector privado ha tenido también fuertes apoyos por las Administraciones Públicas, concretados en la apertura de líneas de ayuda, subvenciones, organización de Jornadas y eventos, que han influido de forma notable en la necesaria información técnica y económica que debe promover la decisión de la puesta en marcha de una plantación trufera.
Coincidiendo con Reyna (2007), la truficultura se puede considerar como una actividad de transición entre lo forestal y lo agrícola. De lo forestal tiene parte de las especies con las que se asocia para el cultivo (encina, roble, quejigo, etc.), la baja intensidad de las operaciones culturales pero, ante todo, los plazos largos en los que se mueve la inversión. Con la actividad agrícola coincide en multitud de técnicas culturales: preparación previa del terreno, laboreo continuo, poda, riego, etc., y muy especialmente en la utilización de terrenos dedicados tradicionalmente a cultivos clásicos, como cereal, viña o almendro, por poner un ejemplo.
La visita realizada en el año 1992, por parte de algunos de los autores, a las instalaciones francesas del Institute National de la Recherche Agronomique (INRA) de Burdeos, Clermont-Ferrand y Dijon nos permitió comprobar dos hechos importantes referentes a la truficultura. El primero de ellos la gran potencialidad y las enormes expectativas que el cultivo de la trufa podía representar en nuestro país, mientras que el segundo afectaba a la filosofía

propia de nuestra actividad investigadora, investigación aplicada, para la que la truficultura había perdido el carácter de universalidad y donde había fuertes intereses económicos que impedían el transvase de conocimientos entre Institutos Públicos de Investigación, habituales en otras muchas áreas, al estar sometidos a virtuales patentes industriales. Se mostraban los fundamentos de los trabajos y el producto final o los resultados, pero nunca se desvelaba con el detalle necesario el apartado de "material y métodos".
Estos hechos motivaron que los Proyectos de Investigación que se iniciaron al abrigo de las convocatorias del Plan Sectorial del INIA fueran abordados con una gran escasez de información científica precedente, siendo necesario preparar el cobijo logístico donde se plasmaría toda la actividad técnica y científica: un invernadero para el ensayo de técnicas de micorrización y una parcela de 11.000 m^2 para el estudio y optimización de los factores biológicos y agronómicos que supuestamente afectaban al cultivo de los plantones micorrizados con trufa negra. Tanto el terreno como la climatología de su ubicación, no eran recomendables para la instalación de una plantación, rompiendo la ortodoxia de la truficultura moderna que aconsejaba suelos de estructura granulosa, con máxima aireación, textura equilibrada y buen drenaje así como climas mediterráneos templado húmedos o fríos subhúmedos. A pesar de ello se elaboró un programa de actuaciones cuyos diseños y resultados constituyen la base del presente trabajo.

Material y métodos

Se partió de una finca de 11.000 m^2 ubicada en las instalaciones del actual Centro de Investigación y Tecnología Agroalimentaria (CITA) del Gobierno de Aragón, en el Campus de Aula Dei, municipio de Zaragoza, con unas coordenadas UTM: X=682284, Y=4621599, Huso 30 y una altitud geográfica de 210m. (Figura 1)

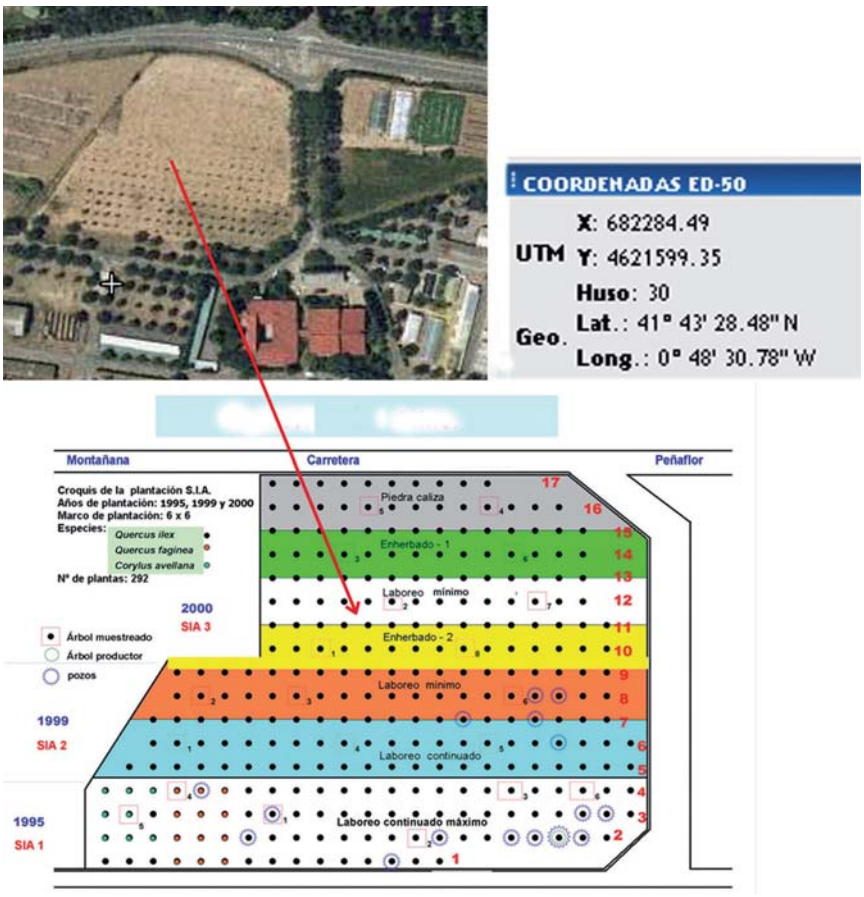

Fig. 1 Localización y croquis de la parcela. Los distintos colores corresponden a los diversos tratamientos.

Fig. 1 Location and sketch of the plot. The different colours represent the different treatments

El suelo pertenecía a un antiguo terreno de aluvión, pedregoso, con textura franco-arcillosa de tipo fino, con una alta capacidad de retención de humedad y de nutrientes.
La reacción del suelo (pH) era moderadamente básica, con un contenido medio de materia orgánica y sin concentraciones altas de sales solubles. La densidad aparente del suelo en superficie alcanzaba valores cercanos a 1,6.
Todos los parámetros referentes a los análisis del suelo están contenidos en el cuadro 1.

Laboreo
Previamente a la plantación se realizó una labor de desfonde, 45-50 cm, seguida de varios pases de cultivador para eliminar las malas hierbas. El criterio seguido a lo largo de los 12 años de la plantación fue el de evitar la competencia por el agua de la flora adventicia presente en la zona de influencia o proyección de la copa de los árboles plantados, mediante pases muy superficiales de cultivador (10-15cm). La frecuencia de estas labores varió de acuerdo con las subparcelas consideradas, eligiendo algunas con tratamientos o pases de cultivador sin ninguna restricción (labores continuas), siempre que la infestación de malas hierbas así lo exigiera, en contraposición con otras subparcelas en las que los pases de cultivador eran mínimos y siempre coincidiendo con los meses de marzo y abril, en los que el daño teórico al micelio y las micorrizas de los árboles se considera mínimo.

Riego
Se realizó una instalación fija de riego por aspersión, para poder asegurar las necesidades hídricas en los meses secos, especialmente importantes durante los periodos

Cuadro 1 Resultados del análisis de suelo de la parcela SIA 1.
Tab. 1 Results of the soil analysis in the plot SIA 1.

Parámetros	Resultado	Valores extremos admitidos
Granulometría	Franco-arcilloso	
Arena total (0,05 - 2 mm), %	27.50	1-79
Limo grueso (0,02 - 0,05 mm), %	14.04	8-79
Limo fino (0,002 - 0,02 mm), %	27.47	
Arcilla (< 0,002 mm), %	30.99	6-69
Fertilidad		
pH al agua 1:2,5	8.02	7,1-8,6
Prueba de salinidad (C.E. 1:5) dS/m a 25ºC	0.23	0,03-0,19
Materia orgánica, %	2.04	0,8-17,4
Relación C/N	8.73	4,8-26
Carbonatos totales. %	29.25	
Caliza activa, %	7.3	1,9-15,6

estivales. En la figura 2 quedan reflejados los aportes anuales realizados y las precipitaciones totales anuales. La mayoría de estos aportes se realizaron durante los meses de julio y agosto, aunque hubo que actuar en ocasiones en algunos años con primaveras y otoños particularmente secos.

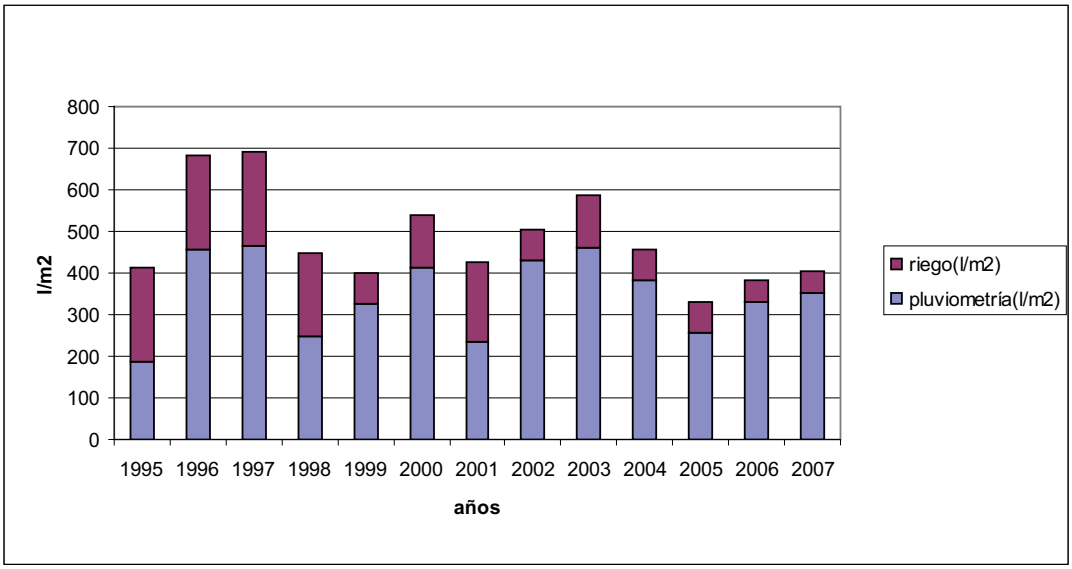

Fig. 2 Precipitación total anual y aportes hídricos realizados en la parcela.
Fig. 2 Total annual rainfall and water provided to the plot.

Plantación y marco

Se eligió un marco de plantación de 6 x 6 que es el más habitualmente utilizado en la truficultura, aunque hoy en día intentan ampliarse hasta 7 x 7. Estos marcos facilitan la labor entre calles y favorecen la aireación e insolación de la zona de proyección de las copas de los árboles, fundamental para el desarrollo de los carpóforos de la trufa.

La plantación se inició el 1 de marzo de 1995, con planta micorrizada de 1 savia proveniente de un vivero de Teruel (Micotec), aunque a partir de los años 1999 y 2000 ya se utilizó planta micorrizada por nuestro propio Centro de Investigación (CITA).

La apertura de los hoyos se realizó con 1 semana de anterioridad al transplante de los plantones. Éstos fueron protegidos mediante tubos de polipropileno de 60 cm con doble pared (TUBEX ©) y tutor, para evitar los daños por viento, heladas y/o roedores (Fig. 3). Los Tubex se mantuvieron hasta el 14 de enero del año1998.

Fig. 3 Secuencia de la plantación y la aplicación del Tubex©
Fig. 3 Sequence of the plantation and application of the Tubex©

Poda

La utilización y mantenimiento de los Tubex durante casi 3 años produjo una autopoda de las ramas laterales en el interior del cilindro que facilitó la formación del árbol, completándose al cabo de dicho tiempo con una poda de formación dirigida hacia la consecución de 3 ramas principales, con una estructura de cono invertido. Con el paso de los años se realizaron podas de mantenimiento durante los meses de febrero y marzo, procurando facilitar un equilibrio

entre la parte aérea y radical a la vez que se eliminaban los chupones y rebrotes en la base del tronco de los árboles (Fig. 4).

Fig. 4 Poda de formación y mantenimiento realizada en la plantación.
Fig. 4 Shape and maintenance pruning made in the plantation.

Distribución del experimento

La plantación se inició en 1995 (83 plantas: 6 avellanos, 9 robles y 68 encinas), ejerciendo sobre ella un laboreo continuo, siempre que la infestación de malas hierbas así lo aconsejase, sin restricción de fechas. Esta subparcela se denominó SIA 1.

En el año 1998 se amplió la parcela con la plantación de 100 encinas, que se separaron en 2 bloques, el primero de ellos contemplaba un laboreo continuo análogo al de la subparcela anterior y en el segundo se aplicaron labores mínimas (2-3 máximo), siempre en los meses primaverales. Estos 2 bloques constituían la subparcela denominada SIA 2.

En el año 2000, se volvió a incrementar la plantación con 110 encinas separadas en 4 bloques. En el 1º de ellos se aportaron 20t de piedra caliza de 50-60 mm de granulometría, repartida en superficie, sobre la que se realizó un laboreo mínimo. En el 2º y 3º bloque se realizaron sendas siembras y empradizamiento con 2 tipos de festuca, *Festuca ovina* y *Festuca arundinacea*. El 4º bloque sólo contemplaba la variante del laboreo mínimo establecido en las subparcelas anteriores, 2 ó 3 como máximo, en los meses de abril, mayo o junio. Los 4 bloques constituyeron la subparcela SIA 3.

No se intentó hacer una valoración estadística de las parcelas y bloques sino que toda la actividad se centró en el análisis de raíces de 20 árboles escogidos al azar, en número y distribución que se resume en el cuadro 2.

Año y subparcela	Laboreo Continuo	Laboreo mínimo	2 Enherbados	Piedra caliza
1995 – SIA 1	6	-	-	-
1999 – SIA 2	3	3	-	-
2000 – SIA 3	-	2	4	2

Cuadro 2 Nº de árboles muestreados en las diferentes subparcelas.
Tab. 2 Number of sampled trees in the different sub-plots.

Análisis de raíces

La toma de muestras de raíces se realizó a partir del año 2000 hasta el 2007. Para el estudio de los factores que afectan al desarrollo de los simbiontes, solamente cabía el análisis del estatus micorrícico de las plantas en función de los tratamientos aplicados. Este análisis, siempre cualitativo, nos permitiría conocer la evolución en sentido positivo o negativo del desarrollo y proliferación de las micorrizas de *T. melanosporum* frente a otras posibles micorrizas competidoras, que podía conducir al inicio de la producción trufera o a su desaparición, simplemente cuantificando la proporción de árboles con presencia de *T. melanosporum* en los diversos tratamientos.

Para conocer el estado de la micorrización fue necesario la toma periódica de muestras estacionales, con especial atención a la primavera y el otoño, por ser estas épocas en las que el micelio se desarrolla más rápidamente y con mayor actividad. Se trataba básicamente de tomar muestras de las raíces en la zona superficial (10-20 cm de profundidad) en los límites del quemado y, en el caso de árboles jóvenes, en proximidad del cuello ó en la proyección de la copa sobre el suelo (Fig. 5). Para ello se utilizó la metodología propuesta por Giraud (1988) descrita como “método global”, porque permite obtener una idea general de la micorrización de la parcela, aplicándola a todos los árboles muestreados, cualquiera que sea su edad.

Fig. 5 Toma de muestras para el análisis de las micorrizas de las plantas.
Fig. 5 Samples taking for the analysis of the plants mycorrhizae.

El siguiente paso fue el de lavar “in situ” las raíces extraídas y depositarlas en frascos o bolsas convenientemente etiquetados. Para la observación macroscópica de las ectomicorrizas se siguieron las directrices marcadas por Agerer (1987-2007) teniendo en cuenta aspectos como el tipo de ramificación, longitud del sistema micorrícico, longitud de las puntas sin ramificar, diámetro de estas puntas y de los ejes, el color, si había o no rizomorfos y características de la superficie del manto de la micorriza.

Para la determinación de las especies, mediante observación microscópica, utilizamos numerosos trabajos que describen las características de las principales especies competidoras que aparecen en las truferas. (Pacioni, 1990; Bencivenga *et al*., 1992; Granetti, 1995; Zambonelli *et al*., 1993; De Miguel y Sáez 2005; González Armada *et al*., 2007) así como el ya citado de Agerer (1987-2007).

Resultados y discusión

Una de las grandes incógnitas de la truficultura moderna es la relación entre determinadas

prácticas culturales y la consolidación de la simbiosis entre el hongo y el árbol a lo largo de los años, culminando con la aparición de las primeras trufas. Los conocimientos adquiridos a partir de trabajos anteriores (Palazon *et al*., 2000) nos habían permitido realizar el análisis del estatus micorrícico de las plantaciones y hacer una estimación de los niveles de la micorrización en lo que afectaba a la presencia de *T. melanosporum* y sus competidores, observando su posible variación en función de las técnicas culturales aplicadas, que suelen afectar a la frecuencia del riego y al tipo y número de labores. Este análisis es fundamental a la hora de establecer previsiones de cosecha, siendo el único valor objetivo que puede aportar información a este respecto.
La figura 6 resume el análisis de la micorrización en el total de los árboles muestreados de las diferentes subparcelas, estableciendo el porcentaje de plantas en el que *T. melanosporum* fue detectada en el análisis de todas las micorrizas presentes en su

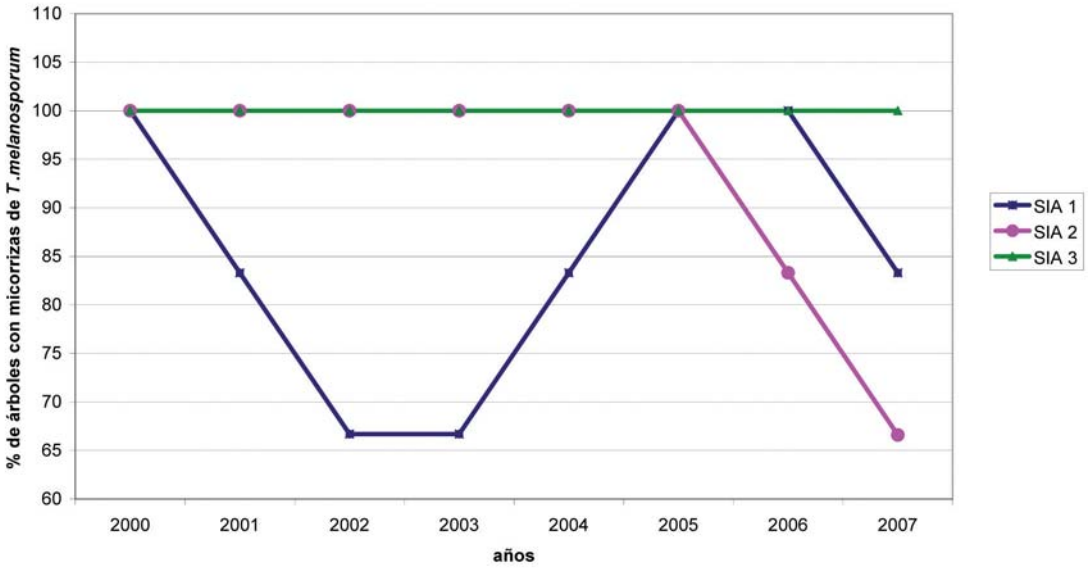

Fig. 6 Evolución temporal del porcentaje de árboles con micorrizas de *T. melanosporum*.
Fig. 6 Time evolution of the percentage of trees with T. melanosporum *mycorrhizae*

sistema radical. En él puede observarse que todas las parcelas consideradas tienen unos valores porcentuales muy altos de árboles con micorrizas de *T. melanosporum*, con alguna fluctuación en el caso de la subparcela SIA 1 y con una disminución, todavía no preocupante, en el caso de la subparcela SIA 2 que deberá ser vigilada en los análisis de 2008.
El momento más importante en la historia de una plantación trufera es aquel en el que se inicia la producción. Los plazos son dilatados, de 6 a 10 años por término medio, y van directamente relacionados con la elección del terreno y su enclave, con la climatología, con la calidad de los plantones micorrizados y finalmente con el uso de prácticas culturales adecuadas. En nuestro caso, tanto el terreno como la climatología de su ubicación, no se caracterizaban por su idoneidad dentro de la ortodoxia de la truficultura moderna, sin embargo sí que se cumplían los otros condicionantes de la ecuación de una truficultura racional y que afectan a la calidad de la planta micorrizada y a las labores adecuadas de cultivo tanto en la plantación como a lo largo de su vida.
El hecho concreto es que el 24 de enero de 2008, en la subparcela SIA 1, se “cazó” con ayuda de perro la primera trufa negra de la plantación (Fig. 7), justamente en la zona de mayor pedregosidad de la parcela. Se encontraba a una distancia de 80 cm del tronco del árbol y a unos 10 cm de profundidad. Tanto el aroma como la textura y color del peridio y la gleba indicaban que sus cualidades organolépticas estaban algo afectadas a causa de haber sobrepasado su periodo de madurez y frescura. Su peso en fresco fue de 7,25g. Las

características del peridio y la observación microscópica de las ascas y ascosporas, permitieron determinar que se trataba de la especie *T. melanosporum*.
La obtención de esta 1ª trufa negra (*T. melanosporum*) en las parcelas experimentales del Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) es para los autores un hecho sin precedentes y que rompe muchos moldes y teorías de todos los tratados en truficultura, abandonando muchos tópicos existentes. Es un logro de la Agronomía con mayúsculas. Si consideramos que el hecho se ha producido en un terreno agrícola enclavado en una zona urbana, fuera de toda norma, compacto y poco propicio, con una flora y fauna propia de un cultivo de regadío y con una climatología fuera de rango, propia de un clima continental extremado, a 210 metros de altitud, con una parada de autobús a pocos metros de los árboles, el hablar de recolectar trufa negra parece casi una frivolidad.

Fig. 7 Carpóforo, gleba, asca y ascosporas de la trufa encontrada.
Fig. 7 Sporocarp, gleba, asca and ascospores of the found truffle

Otro aspecto importante de este trabajo radica en la apertura de expectativas hacia este cultivo, poniendo en valor a la parcela del CITA, que pasa a simbolizar las grandes posibilidades de muchos terrenos y territorios de nuestro país, hasta ahora desaconsejados por las guías y tratados en truficultura.
No es una panacea. Hacen falta cuidados, dinero, bastantes análisis y mucha fe. Pero el hecho es posible. Los beneficios en la aplicación de las modernas técnicas de cultivo, que son la base de la truficultura moderna, se han puesto de manifiesto en el marco de Proyectos de Investigación desarrollados con anterioridad y se han concretado en la obtención de trufa negra, *T. melanosporum,* de la manera y formas precedentemente descritas.
El trabajo que aquí se ha presentado incide de un modo importante en las consideraciones previas y en la ortodoxia de una actividad como la truficultura, al ampliar el rango del terreno agrícola utilizable hasta las zonas de regadío tradicionales, en donde ésta nunca ha sido posible, ni siquiera una alternativa a considerar.

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PLANTATION DENSITY OF HAZEL TREES INFLUENCES THE PRECOCITY AND KINETICS OF BURGUNDY TRUFFLE PRODUCTION

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Abstract
Since the first Burgundy truffle mycorrhized plants available in the market in 1975, orchard owners are waiting for agronomically reasoned practices in order to optimize the truffle production.
In this context, a study was launched 23 years ago, with the objective to test the influence of tree density on the production of burgundy truffle and its evolution up-to-date.
A Burgundy truffle hazel orchard was planted in 1985, with four plantation densities in Commercy located in the North East part of France (48°45'42 N, 5°35'31 E), 250 km East of Paris. The densities tested were 2000, 1000, 666 and 333 trees per ha.
The first harvest was obtained at the highest densities (2000 and 1000 trees. ha^{-1}), 11 years after plantation. Then, high densities produced during four years and then ceased almost to produce 9 years after the first harvest (from the 20^{th} year after plantation). The lowest plantation density (333 trees. ha^{-1}) starts to produce significantly 15 years after the establishment of the orchard. Then, the production increased every year and its peak is not reached yet.
Recommendations to the growers of Burgundy truffle orchard need to include advices (plantation density and truffle orchard management), not only at the establishment, but also during the life span of the truffle bed. This is crucial in order to optimize the economic yield of this crop.
Key words: Burgundy truffle (*Tuber aestivum* Vittad. usage name *Tuber uncinatum* Chatin); hazel orchard (*Corylus avellana* L.), plantation density, production.

Introduction

The Burgundy truffle is an ectomycorhizal hypogeous fungus naturally present in North Europe. Like the black truffle, the Burgundy truffle grows in carbonated soils, in association with trees. Nevertheless its ecological needs are different; it grows more likely in the shadow, in humid but not waterlogged soils (Chevalier and Jalade, in Chevalier & Frochot, 2002). Moreover, it is harvested in autumn.
Unlike the black truffle, for which orchards have been settled for several decades (Pradel, 1914), the first Burgundy truffle-producing trees were planted thirty five years ago (Frochot *et al*, 2001). Like for each truffle species, the expected decline of production for burgundy truffle mycorhized orchard was not quoted in the literature. Information from the oldest burgundy truffle bed, being now 30 to 35 years old, is not available. This is a reason why no recommendation adapted to the management of an old Burgundy truffle orchard has been formulated yet. High density plantations produce earlier than densities advocated for black truffle (Frochot *et al*, 2001) but the effect of tree density on production of Burgundy truffle has not been studied. Thus, the objective of this work was to assess the influence of tree density on the production of burgundy truffle and the evolution of production along the years following the first harvest.

Materials and methods

Description of the truffle orchard

Experiments were carried out at Commercy located in the North East part of France (48°45'42 N, 5°35'31 E), 250km east of Paris.

The climate of the region is semi continental with an oceanic influence; it is characterised by an 18-year mean annual temperature of 9.6° C and annual precipitation of 1050 mm. Most of the precipitations fall during the winter. The high contrasts of climate may induce long dry periods during summer, detrimental to production. Air temperature can be cold in winter (mean of - 2° C in February 1991) and hot in summer (mean of 22.1° C in July 2006).
Climatic data were obtained from the nearest official weather station. The plot has a northern orientation, with a 5% slope. Soil is classified as a calcaric Leptosol. The texture at a depth of 5cm is silty clay (Jamagne *et al*., 1967) with 330, 520 and 150 $g.kg^{-1}$ of clay, silt and sand respectively.
The truffle bed was planted in 1985, with four plantation densities of mycorhized hazel trees following the experimental protocol established by Frochot (Frochot, 1995). Four replicates per density treatment were carried out. The four replicates are in a row on the same contour line (Fig. 1).
It was decided to plant hazel in regular spacing lines (5m) in order to facilitate the management of the truffle bed. So, to vary density, space between trees was made different. There is one meter between trees in the highest density (2000 trees/ha), two (1000 trees/ha), three (666 trees/ha) and six meters in the lowest density (333 tree/ha).

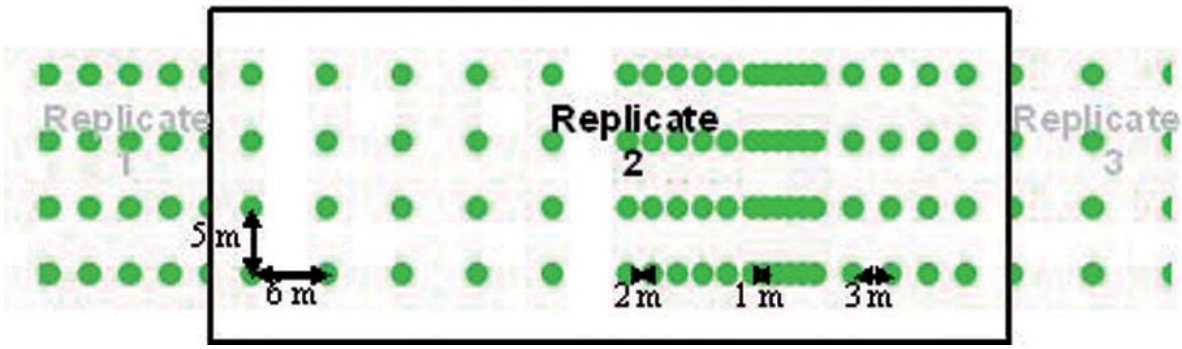

Fig. 1 Scheme of an experimental plot including the four plantation densities. Each green point represents a tree. For each density within a block, trees are planted in micro-plots of twenty trees.

Harvest method
Harvests were made by people members of the truffle growers' association (AMPPTL), owner of the site. A dog was systematically used for harvest.
Each harvested truffle was registered on an index card, indicating distance to the nearest tree and azimuth.

Data collection and processing
We created a database thanks to all of index cards, allowing to map each truffle harvested, and to analyse data. During the three years 2002-2003-2004, no harvests were made due to unavailability of volunteers, and to dry summers affecting the production.

Statistical methods
The amount of truffle recorded each year was highly variable. Consequently, a non parametric test based on ranked data was used to test differences in production, due to the plantation density and harvest year.

Fig. 2 Photography of the experimental orchard at 1000 trees ha-1 density. Shot 22 years after orchard establishment.

Results

The first production occurred eleven years after plantation (Fig. 2). The first remarkable result is figured on an evolving map representing the position of the truffles found each year. The harvested truffles correspond to the colourful points on the graphs. The four replicates were merged in only one graph.

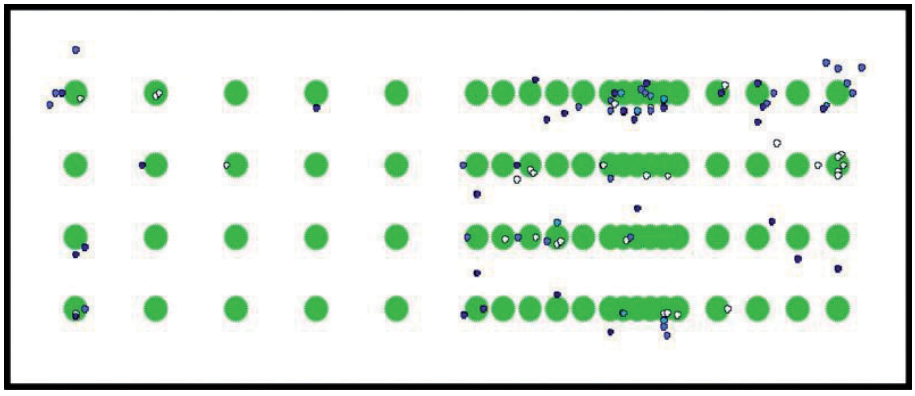

Fig. 3 Truffles harvested between 1996 (white) and 1999 (dark blue)

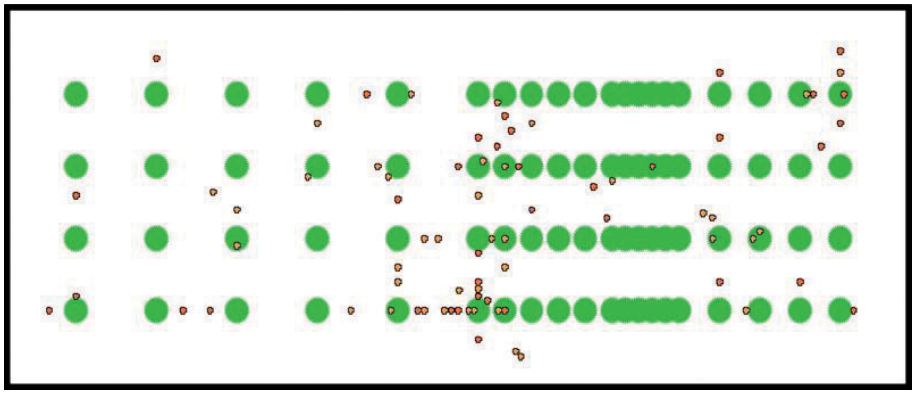

Fig. 4 Truffles harvested in 2005 (orange) and 2006 (dark orange) twenty years after plantation

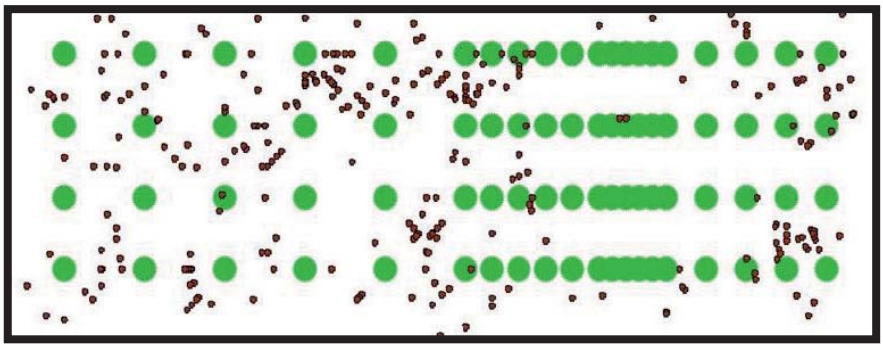

Fig. 5 Truffles harvested in 2007 twenty three years after plantation

Due to large variations of harvested truffle between years attributed to water deficits during summer (Beaucamp, 2001), we show results of each density in percentage of the total truffle bed production.

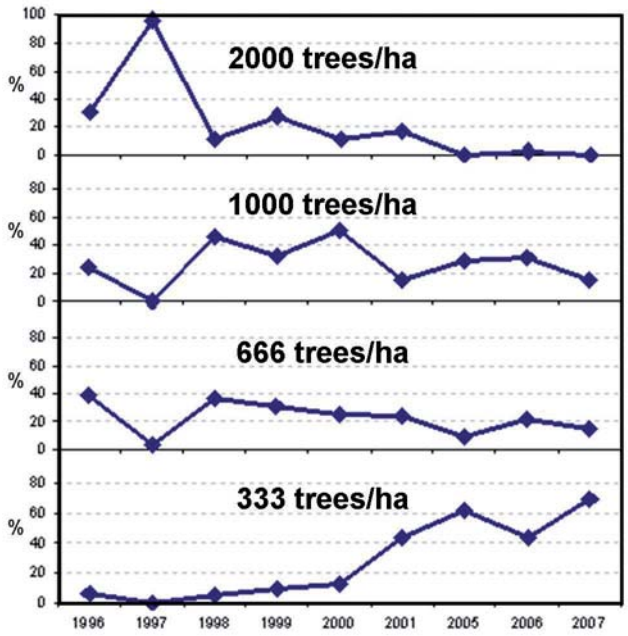

Fig. 6 Kinetics of the production for the four plantation densities, expressed for each density in percentage of total orchard (whole densities) production.

Highest plantation density (2000 trees/ha) produced early and during the first four years after the beginning of production. This density ceased almost to produce after the 20th year after plantation.

Medium plantations densities (1000 and 666 trees/ha) showed the same history; the optimum was reached between 13 and 16 years after plantation. Afterwards the harvest decreased every year, but still produced 23 years after plantation. The production peaked 15 years after plantation for these two densities.

The lowest plantation density started to produce truffles 16 years after plantation, and therefore was the latest to initiate production. This density is the most productive at the present time.

Due to the restricted number of harvested truffles before 2007, we are able to make statistics on results in 2007 only.

Production under the 333 tree.ha^{-1} density was highly significantly higher (P=0.01) than production under the other densities. Production at 2000 trees.ha^{-1} was significantly lower (P=0.05) than production at 1000 and 666 trees.ha^{-1}.

Discussion and perspectives

This work is the first showing the influence of plantation density on the kinetics of Burgundy truffle production. With regard to range of densities investigated (333 to 2000 trees.ha^{-1}), we showed that the precocity of the first production and the end of production are correlated to the increase of tree density. Production began after 11 years, in accordance with other Burgundy orchards (Frochot *et al*., 2001) that produced their first truffles between 8 and 12 years for 400 and 700 trees.ha^{-1} densities.

The highest tree density (2000 trees.ha^{-1}), which has no practical relevance, showed the biggest decline. The evolution was observed between 11th and 23rd year after plantation.

For black truffle orchards, for which more data are available, the effect of density on production precocity and its decline were established empirically by Pradel (1914). According to this author, the appearance and the decline of truffle bed are all the more fast as trees are closer. His interpretation was the excessive shade and root density. Indeed, shade is now known as detrimental for the black truffle production (Ricard *et al.,* 2003), while it is recognized as a positive factor for Burgundy truffle (Chevalier, 2002).

In the case of Commercy orchard, different hypotheses can be formulated in order to explain the end of production in high density zones:

- Higher soil compaction because of higher root density. The truffle orchard soil has a high silt percentage. Consequently, this soil is vulnerable to compaction. This could explain the end of production as degraded soil physical properties entail a poor aeration and permeability and strong soil strength (Horn *et al*., 1995).
- Very low light intensity under the canopy. The trees and their leaves intercept the light, creating a dark microclimate with consequences on soil temperature. Strong shading may alter the decomposition of organic matter and may modify water retention potential, nutrient availability and soil microfauna and –flora composition.
- Water deficit. The rain interception by leaves and the consumption of water by trees are higher in dense than in spaced out trees.
- Slower root turn-over. The regeneration of root could be limited by a high root density. Mycorhiza stay alive on young tree roots only (Chevalier, personal communication). A deficit of root generation could explain the truffle production decline.

Since 1990, it has been advocated to establish hazel orchard mycorhized with Burgundy truffle at a density of 800 trees per hectare in order to produce earlier. Up-to-date, there were any experimental answers about the evolution of old truffle bed. With this experimental truffle bed, it is possible to indicate that 800 trees.ha^{-1} is too high for hazel trees after fifteen years in these growth conditions (climate and soil).

Recommendation for truffle orchard regeneration needs to be formulated if the owner wants to maintain or restart truffle production. In this case, an adapted management of the top soil layers around the trees with specific tools, aiming to cut some roots and re-aerate the soil is promising and needs to be tested (Wehrlen, in Dessolas *et al.*, 2007). A second suggestion would be to chop down some trees to rejuvenate the orchard.

Acknowledgements

We thank the "*Association Meusienne des Planteurs et Promoteurs de la Truffe en Lorraine*" (AMPPTL) for the free access to its hazel orchard and to Lorraine Regional Council for the financial support and especially the year grant given to JS POUSSE. Thanks are due to the members of the AMPPTL who contributed to the harvests and truffle recording.

The authors dedicate the paper to G. Lorsin and L. Ballureau, pioneers in this experimental truffle orchard.

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EFFECTS OF SOIL MANAGEMENT ON THE EVOLUTION OF YOUNG PLANTS MYCORRHIZED WITH *TUBER MELANOSPORUM*

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Abstract
In this trial, we study the effects of different crop management techniques on the evolution of young plants mycorrhization and on soil quality. After 6 years, plastic mulches give the greatest growth and black polypropylene weaving, especially, accelerate the mineralization of the free organic matter in the soil. This technique modifies also the ectomycorrhizal complex of roots.
Key words: truffle cultivation, mycorrhization, organic matters.

Soil management of young truffle plantations is a recurrent problem. In this trial, we study the effects of different crop management techniques on the evolution of *T. melanosporum* mycorrhization and on soil quality (organic matter and microbial biomass). The experimental plot was designed in 2002 by the Ctifl, in collaboration with the Creysse experimental station and the Martel truffle association.

Materials and methods

- Experimental site

The plot is located on the 'Causse de Martel' (Lot-Aquitaine region). The sub-soil is a hard cracked limestone (Jurassic period), with red clay pockets and a shallow stony surface (5 to 15 cm) (photo 1). The texture is silt loam-sandy-clayey with a C/N ratio of 13.5 and 7.7% organic matter. The plant material consists of controlled one-year-old oaks (*Quercus ilex* and *Quercus pubescens*) inoculated with *T. melanosporum.* The trial studied 4 treatments without repetitions (comparison test): mechanical tillage (2.5m on both sides of the rows), chemical weed control (glyphosate), plastic mulch (black polypropylene weaving, width 1.65m) and waterproof ensilage plastic mulch (width 1.5m). Every unit contained 30 trees (plant density 1.5x10m) (photo 2). The plantation was carried out in March 2002.

Photo1 and 2 The soil and the plot in 2008.

- Mesurements

Tree growth was measured each year (trunk diameter and size). In 2007, organic and biological analyses of one soil sample per treatment were performed. A mycorrhization study was carried

out, in 2008, on 15 trees (3 samples /tree) to compare the effect of soil management on the mycorrhizal development and to control the presence of *T. melanosporum* and others ectomycorrhizes (*T. brumale*, *Coenococcum graniforme*, *Scleroderma* sp.) on the basis of morphological identification.
For statistical interpretation, mean comparison (Test t Student, α=0.05) and Khi2 tests were used.

Results

After 6 years, the results (Table 1) show that the growth of *Quercus ilex* trees is greater than the growth of *Quercus pubescens,* whatever the soil management method used. For both oak species, the two treatments of plastic mulch give a greater trunk diameter than the mechanical tillage. No significant difference was observed between the two plastic mulch treatments. Intermediate growth was observed with *Quercus pubescens* trees under chemical weed control but the difference is not significant compared to plastic mulch treatment. The least growth was observed under mechanical tillage.

	Waterproof ensilage plastic mulch	**Plastic mulch (black polypropylene weaving)**	**Chemical weed control**	**Mechanical tillage**
Q.pubescens mean trunk diameter (mm)	28.7 (a)	27.3 (a)	25.4 (a)	14.9 (b)
Q. ilex mean trunk diameter (mm)	46.2 (c)	44.1 (c,d)		37.9 (d)

Tab. 1 Tree trunk diameters (mean value) in 2008 (6 years of growth)

We compared the results of soil analysis for each treatment to the grassed inter-row. A variation of at least 10% was considered to be significant (Tab. 2). The results show that total organic matter decreases under plastic mulches, especially with black polypropylene. This decrease comes from a decrease in free organic matter (>50µm), which is also significant with mechanical tillage. The ratio of fine organic matter/total organic matter is the lowest with the polypropylene material (19%). The quantities of mineralised carbon (mgC/kg/28days) and the ratio Cmin28/%Corg are lower under plastic mulches. The microbial biomass content is also the lowest levels with plastic mulches, especially with the waterproof ensilage plastic, where the ratio microbial biomass/% Carbone organic (the output of this biomass) is particularly very low.

	Organic Matters (%)				Total Nitroge	C/N ratio			Microbial biomass		IAM	mineralised Carbon	
Sites	total OM	free OM >50 um	fine OM <50 um	free OM/total OM	(g/kg)	2 mm	free OM	fine OM	mgC/kg	%Corg.	A490/h	mgC/kg/28j	%Corg.
Chimical weed control (glyphosate)	6,53	1,47	5,06	22%	3,38	11,2	13,4	10,7	603	1,6	0,47	538	1,4
Waterproof ensilage plastic mulch	6,12	1,59	4,53	26%	3,90	9,1	12,2	8,4	371	1,0	0,16	396	1,1
Plastic mulch (black polypropylene weaving)	5,41	1,04	4,37	19%	2,88	10,9	14,1	10,4	509	1,6	0,23	448	1,4
Mechanical tillage	6,27	1,31	4,96	21%	3,34	10,9	14,7	10,2	581	1,6	0,56	823	2,3
Grassed interrow	6,47	1,60	4,87	25%	3,41	11,0	13,8	10,3	747	2,0	0,57	853	2,3
	value <10% compared to the grassed interrow												
	value >10% compared to the grassed interrow												

Tab. 2 Organic and biological analysis of the soil for the different treatments

The mycorrhization analysis (Figg. 1 and 2) show that the *Q. pubescens* are well associated with *T. melanosporum,* while *T. brumale* is more frequent on *Q. ilex* root samples. Other mycorrhizal species developed on the root system: *Coenococcum* sp. and *Scleroderma sp*. on pubescence oak, and *Basidiomycetes* and "unidentified species" on holm oak. For *Quercus pubescens*, the mechanical tillage treatment provided the highest *T. melanosporum* frequence and the lower contamination level while the polypropylene mulch show a good *T. melanosporum* conservation but an important colonization with others ectomycorrhizal.

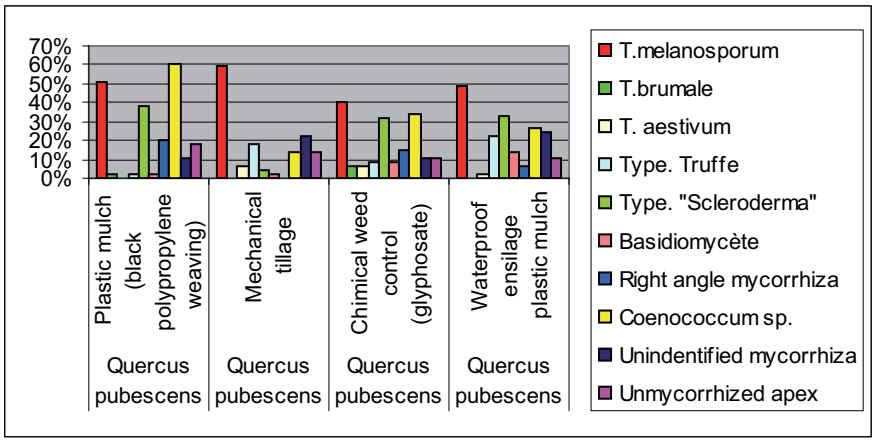

Fig. 1 Mycorrhization analysis: percentage of the presence of ectomycorrhizes according to the number of roots samples analyzed.

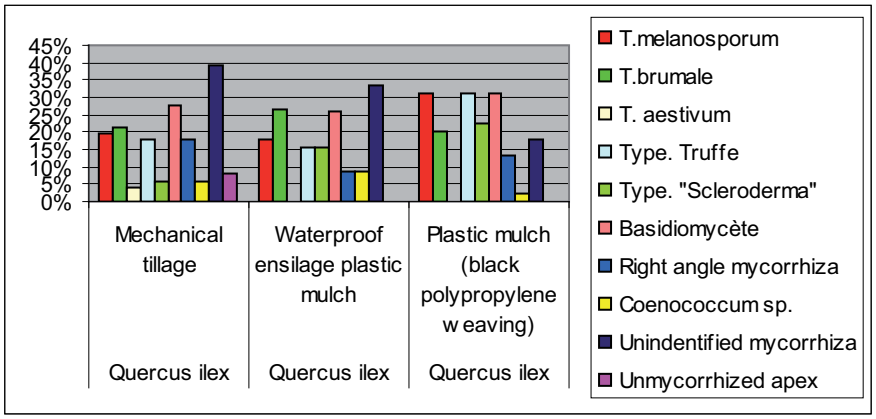

Fig. 2 Mycorrhization analysis: percentage of the presence of ectomycorrhizes according to the number of roots samples analyzed.

Discussion

The quantity of mineralised carbon (mgC/kg/28days) is an indicator of the microbial organic degradation activity and therefore also of the energy available. The ratio Cmin28/%Corg indicates the capacity of organic matter to be mineralised (mineralisation index). The quantities of energy and the mineralisation indexes are lower under plastic mulches showing that mineralisation has occurred under these treatments. However, for the waterproof ensilage plastic, these indicators, and particularly the output of microbial biomass, are very low suggesting that the environmental conditions under this mulch are not favourable for a good mineralisation. Under this mulch, the soil is frequently dry and the level of aeration is lower than under polypropylene mulch. So, mineralisation is greater under polypropylene material. Mechanical tillage also has an effect on organic matter mineralisation but to a lesser extent. This increase of mineralisation, which produces a higher lever of nitrogen supply in the soil,

can explain, in part, the increase of growth of the trees under mulch. Changes in climatic conditions around the roots can also explain these results, because the temperatures are more buffered.
The mycorrhization analysis shows surprising results because *Q. ilex* seems to be more contaminated with *T. brumale* than *Q. pubescens* oaks, which does not coincide with common knowledge. *Q. ilex* are less mycorrhized with *Coenococcum* sp. and *Scleroderma sp*. than *Q. pubescens.* The plastic mulches seem to modify the composition of ectomycorrhizes of pubescence oak and to increase the frequency of *Basidiomycotina* but this result doesn't occur with *Q. ilex,* which forces to be careful when interpreting the results. Other sampling will be necessary to confirm this data.
During the winter 2007/2008, the first truffles were harvested 5 years after plantation under evergreen oak with plastic mulch (4 trees produced *T. brumale* and *T. melanosporum*), confirming the idea that this technique could help to increase organic matter transformation if necessary and be useful for the management of young truffle plants.

Conclusion

These preliminary results show the positive effect of plastic mulching on the growth of young oak trees. This technique accelerates the mineralization of the free organic matter in the soil leading to a ratio which seems to be beneficial for *T. melanosporum* fructification. However mycorrhization analyses 6 years after planting show a high colonization with other ectomycorrhizal fungi of which the influence will be measure in the future.

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CULTIVATION OF TUNISIAN *TERFEZIA BOUDIERI* CHATIN IN FIELDS CONDITION

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Abstract
The transplantation of inoculated host plant of *Terfezia boudieri* Chatin (*Helianthemum sessiliflorum* Desf.) by ascosporous on an experimental field (containing two types of soils originated from the south of Tunisia, the first one is constituted by sand and the second by gypsum) and the inoculation of the same plants directly in the field permitted to produce a fruit body after two years of transplantation. For the sowed and inoculated plants, fruition takes place after the third year of cultivation.
Key words: *Terfezia boudieri*, inoculation *Helianthemum sessiliflorum*, cultivation, Tunisia,

THE VIRULENCE OF *TUBER MELANOSPORUM*

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Abstract
The production of *Tuber melanosporum* is generally that much better when the spread of the burnt area exceeds that of the host tree. This phenomenon, marked by a balance between the size of the tree and that of the burnt area has been called the 'virulence' of the truffle. It shows up on the root system of the tree which is deteriorated in the place where burning is the most active.
Studies of this phenomenon have allowed the definition of different characteristics relating to the state of the habitat and its cultivation. When the virulence is correct, the radius of the burnt aera (Rb) is usually greater than or equal to one and a half times the radius of the foliage (Rf); that is Rb ≥ 1.5 Rf. In function of the fertility of the soil or its water reserves, this equilibrium can be naturally encouraged (shallow soil) or restrained (deep soil) if the growth of the host trees is too rapid. The progression of the burnt area (Pb) is of the order of 10 to 15 cm per year under a good productive tree. This advance (Pb) can be faster when there are positive trophic points in the development zone. The plants beneficial to truffle grounds (dog roses, blackthorns or sloes and grapevines) are natural elements favouring the progress of the burnt area and the presence of fruiting bodies close to or in contact with their roots. The presence of animals on a truffle ground or plantation can be a factor which stimulates the virulence, with increased truffle production.
One experiment demonstrated that heavy pruning, unnecessary for the desired equilibrium, led to a zero progression of the burnt area (even a regression), with no yield at all in the harvesting season. Observing the virulence - or more precisely the Rb ≥ 1,5 Rf balance - has practical consequences for the management of the plantation. Tree-pruning is carried out taking care to limit the spread of the foliage. The potential development zone is reduced by pruning the trees, followed by thinning to regain space.
In some situations we have observed trees where the burnt area remains confined under the foliage and advances very slowly; with production at greater depths than usual. On the contrary, in other circumstances very marked virulence may be accompanied by lack of production despite a mycorrhizal profile dominated by *Tuber melanosporum*. The concept of virulence has been extended to include the productivity of truffle plantations, with characteristics common to truffle cultivation in France, Spain and Italy.
Key words: *Tuber melanosporum* Vittad., burnt area, foliage tree, equilibrium, tree pruning.

1. Problématique et objectif de l'étude

Le concept de virulence a été développé à la suite de l'observation dans la vallée du Tarn en aval de Millau (France) d'une gradation dans l'étendue ou la puissance des brûlés sur une plantation truffière en fonction de la position des arbres sur un coteau calcaire correspondant à d'anciennes terrasses viticoles.
Cette observation, à la faveur d'une caténa des sols, a montré que plus les sols étaient superficiels, moins les arbres étaient vigoureux, plus les brûlés étaient proportionnellement étendus par rapport au volume des arbres, et meilleure était la production truffière.
A partir de ce constat, le concept de virulence a été exploré dans différentes situations et à différents niveaux du système truffier, en particulier du brûlé provoqué par le *Tuber melanosporum*. Nous avons émis l'hypothèse que le brûlé est la traduction de l'agressivité du *Tuber melanosporum* dont on ne peut dire si elle est directement ou indirectement provoquée par le champignon. Cette hypothèse suppose que si l'arbre est dans des conditions qui pénalisent sa vigueur, cette agressivité se traduit par un brûlé particulièrement étendu avec fructification du champignon.

Les illustrations ci-dessous montrent des chênes verts mycorhizés de la terrasse inférieure du coteau par opposition avec un chêne vert d'une terrasse supérieure.

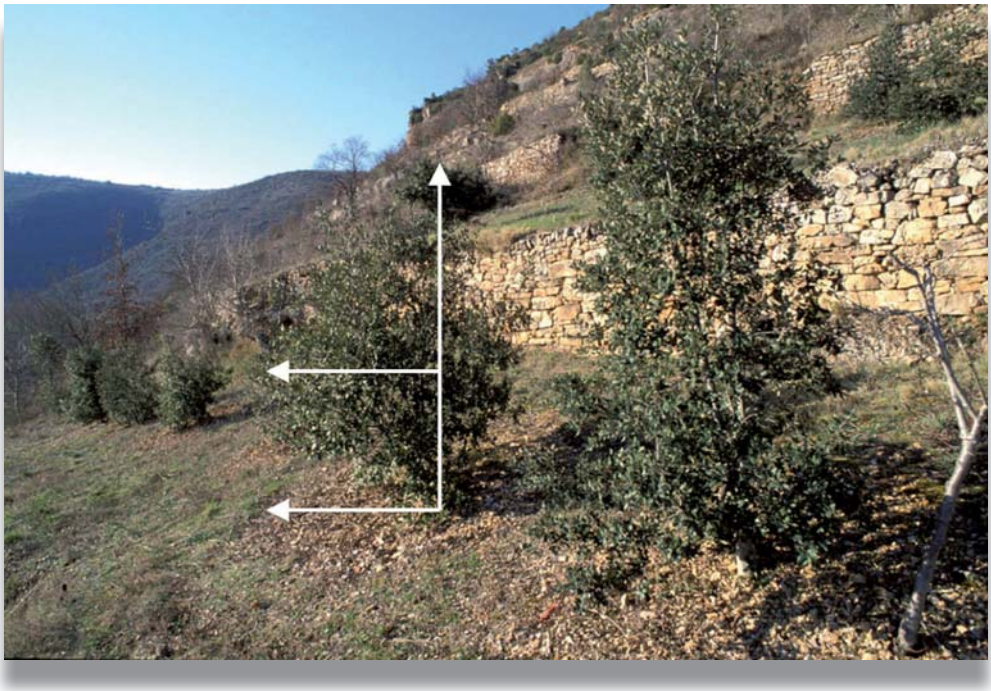


Photo 1 chênes verts sur une terrasse inférieure avec début de production tardif et peu abondant. Le brûlé est peu visible car il dépasse à peine l'aplomb de la frondaison des arbres.

Photo 2 chêne vert sur une terrasse supérieure ayant débuté la production à 5 ans (520 grammes de truffes), âgé de 8 ans au moment de la prise de vue, et montrant un brûlé de dimension exceptionnelle par rapport à la taille de l'arbre.

L'observation du phénomène a été illustrée avec le schéma ci-dessous qui positionne les arbres truffiers en fonction du type de sol situé sur un sous-sol rocheux jurassique. De très bons arbres producteurs sont en bordure de terrasse, pouvant produire jusque dans le mur

de soutènement, ce qui laisse penser que le drainage du milieu peut aussi avoir un effet très bénéfique.

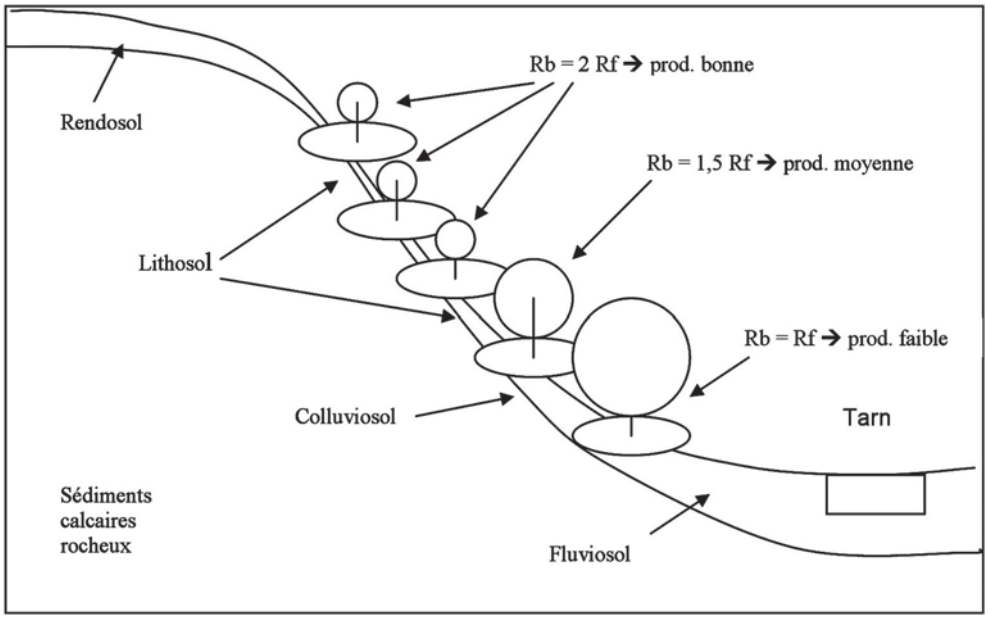


Schéma 1 montrant la vigueur des arbres truffiers et l'étendue de leur brûlé en fonction de leur position dans l'enchaînement des sols plus ou moins profonds sur un coteau de la vallée du Tarn.

Afin de rendre plus clair le phénomène de virulence de la truffe noire, celui-ci a été résumé en mettant en évidence l'équilibre entre la vigueur de la truffe et celle de l'arbre selon les éléments du schéma ci-dessous.

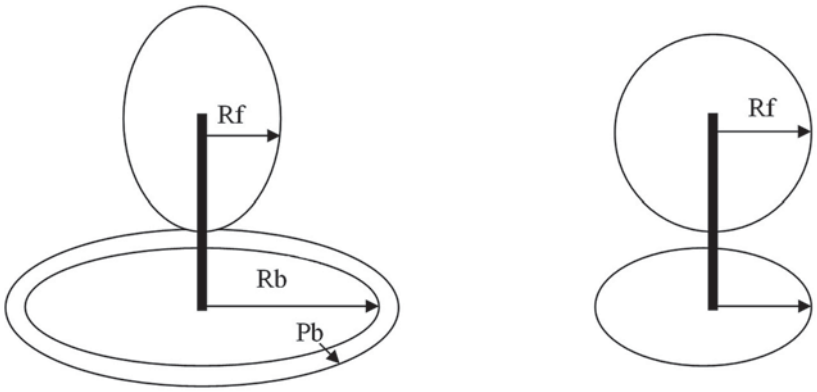


Schéma 2

Arbre truffier producteur
avec truffe virulente
(sol superficiel, pas de taille)

Arbre truffier non producteur
avec truffe non virulente
(sol moyen à profond, pas de taille effectuée)

Les références abrégées sont expliquées ci-dessous:

- Rb = Rayon du brûlé (mesuré sur le pourtour extérieur car celui-ci devient en couronne sur les vieux arbres, délaissant une partie au plus près du tronc)
- Rf = Rayon de la frondaison de l'arbre (en faisant une moyenne sur l'extrémité des branches latérales)
- Pb = Progression annuelle du brûlé (repérable avant le travail du sol)

On considère que si Rb est égal ou supérieur à 1,5 Rf, la truffe *Tuber melanosporum* est en mesure de fructifier, si bien évidemment l'arbre est porteur des mycorhizes de cette espèce. Pour que la vigueur soit à l'optimum, il faut pouvoir observer la progression du brûlé Pb, phénomène possible à noter en avril dans les truffières naturelles ou celles cultivées manuellement avant le travail du sol. Ceci est le cas de la plantation aveyronnaise en raison de l'impossibilité d'introduire une mécanisation sur les terrasses.

On peut ainsi formuler un indice de virulence:

$$Ivt = \frac{Rb \times (1 + Pb)}{Rf}$$

Plus cet indice est élevé, plus la production a des chances d'être importante. Un bon indice (avec des valeurs en m) se situe entre 1,5 et 2. On verra qu'il peut être plus élevé avec le chêne vert et plus faible dans des sols sablo-calcaires profonds.

2. Matériels et méthodes

Avant de présenter l'expérience qui donnera lieu à des mesures précises, il convient de souligner des observations et analyses conduites sur des plantations truffières en France au niveau de la dynamique du brûlé à la base du phénomène de la virulence. A ceci, on ajoutera des observations sur les transformations opérées sur le système racinaire de l'arbre truffier dans la zone de production. Pour plus d'informations sur le sujet, on se référera au document «Questions d'écologie appliquées à la trufficulture».

2.1. Rappel sur des observations et analyses effectuées à partir de 2004 sur des plantations truffières

Les observations entreprises dès 2004 dans de nombreuses situations en France et en Italie, pour vérifier si le phénomène se reproduisait, ont permis de faire les constats suivants:

- lorsque la progression du brûlé est nulle ou négative (plantation de Miers, Lot), la production de truffes régresse ou disparaît malgré un statut mycorhizien qui peut être exclusivement à base du *Tuber melanosporum*,
- quand un arbre est faiblement mycorhizé par *Tuber melanosporum* et davantage par d'autres espèces fongiques, si la progression du brûlé est nette, la fructification du *Tuber melanosporum* est tout de même observée,
- quand les sols sont fertiles, la production est d'autant meilleure que les arbres sont taillés ou élagués vigoureusement (Touraine, Tricastin),
- sur des plantations aux sols hétérogènes, la production du *Tuber melanosporum* débute généralement sous les arbres mycorhizés situés dans des zones où le sol est plutôt superficiel et drainant (Lot, Aveyron, Charente maritime, en Côte d'Or, etc.),
- les «bons arbres truffiers» sont généralement moins vigoureux (ou rabougris), soit parce que la présence du *Tuber melanosporum* pénalise leur croissance (nécrose le système racinaire), soit parce que le caractère drainant ou séchant d'un sol ne leur permet pas une croissance rapide, soit parce que les deux éléments se conjuguent.

Les illustrations suivantes montrent comment se comportent les brûlés dans différentes plantations. Les deux premières plantations n'ont été cultivées que pour la reprise des arbres, la première et la deuxième année. La troisième plantation est située sur un sol profond de Touraine, ce qui appellera une observation dans le chapitre sur la formation du cortège fongique.

Photo 3 le meilleur chêne vert de la plantation S11 (Rb > 2 Rf) à Miers (Lot, France)

Photo 4 jeune plantation (5 ans) dans le Tarn en pelouse calcicole réalisée avec la butte de terre ayant commencé à produire avec en général Rb = 1,5 Rf. (ici Rb = 2 Rf).

Photo 5 brûlé autour d'un arbre coupé mais ayant rejeté au premier plan (Rb > 2 Rf). A l'arrière plan, on observe des arbres taillés jusqu'à plus de la moitié de la hauteur du tronc. Cette plantation performante est en Touraine sur sol profond.

2.2. Observations sur la virulence du *Tuber melanosporum* en liaison avec la structure des racines

Dans les brûlés producteurs du *Tuber melanosporum,* on remarque la présence de nombreuses cicatrices d'abscission sur les racines longues à l'instar de la trace d'implantation des feuilles visibles après leur chute en automne (voir photo ci-dessous). On note la présence d'un bourrelet de cellules autour de la racine (qui va se détacher) semblable à celui qui se forme à une autre échelle autour des branches mortes sur les chênes. Ce phénomène ne se remarque pas sur les racines dans les brûlés du *Tuber brumale* ou au-delà ou en-deçà de la zone de fructification du *Tuber melanosporum* (voir cortège fongique dans Questions d'écologie appliquées à la truffiiculture).

En fait, tout se passe comme si le phénomène du brûlé était également efficace à l'encontre des racines de l'arbre hôte. L'impact du brûlé s'observe en surface avec la disparition de la flore et dans le sol sur les racines, mais jusqu'à un certain niveau. Dans les sols superficiels (15 à 20 cm de terre) sur sous-sol calcaire jurassique, son effet ne s'exerce généralement pas dans l'horizon rocheux où l'on trouve entre les lits de roche des mycorhizes aussi vivantes que sur les jeunes plants truffiers.

Photo 6 racines avec nombreuses cicatrices provenant de truffières naturelles performantes (la grosse à gauche de Sainte-Beauzile (Tarn), les deux à droite de Miers (Lot))

2.3. Travaux relatifs à l'impact de la taille sur le phénomène de la virulence

Le suivi d'une plantation truffière depuis plusieurs années, sur laquelle a été réalisée une forte intervention en termes de taille et d'élagage des arbres en août 2006, a permis d'élaborer des éléments de réponse à la problématique de la virulence. Cette plantation a fait l'objet depuis 2005 de mesures de la progression annuelle des brûlés sur une douzaine d'arbres. A partir du printemps 2006, tous les arbres ont été mesurés. Pour des raisons de commodité d'analyse, on a privilégié les mesures effectuées sur la douzaine d'arbres fortement taillés en introduisant une population témoin non taillée après l'épisode de taille d'août 2006.

Description et performances de la plantation

- 190 arbres mycorhizés Agri-Truffe en novembre 1996, principalement des chênes verts, soit 1 ligne de noisetiers mycorhizés, 1 chêne pubescent à chaque tête de ligne de chênes verts.
- Sol argilo-calcaire sur sous-sol marneux tertiaire (calcaire lacustre).
- Précédent cultural: friche après ancienne vigne.
- Culture: travail du sol au motoculteur limité au brûlé; girobroyeur entre les rangs.
- 1ère récolte: 11 kg l'hiver 2002-2003 (6 ans), pas de truffes en 2003-2004 (sécheresse et canicule de l'été 2003), 6 kg en 2004-2005 (sécheresse en juin et jusqu'au 20 juillet 2004), 4 kg en 2005-2006 (mauvaises conditions climatiques), pas de truffe en 2006-2007 (incidence vraisemblable de la taille du mois d'août), 10 kg en 2007-2008 (retour de la production).

La taille ou l'élagage d'août 2006

Cette taille ne répondait à aucun besoin cultural précis. Elle a été réalisée pour satisfaire à la curiosité d'une personne férue de radiesthésie et convaincue de son impact très positif. Son niveau d'intervention a été tel que la plupart des arbres ont vu leur volume de frondaison diminué de moitié, voire des deux tiers. Pour certains arbres, leur hauteur a été divisée par deux. Si la valeur de cette intervention n'a pas été appréciée du point de vue de «l'art» du tailleur, elle a tout de même opportunément servi à évaluer l'impact d'une taille ou d'un élagage sur la progression des brûlés et la production truffière en situation performante.

Mesures de la progression des brûlés

Les mesures ont débuté sur les meilleurs arbres producteurs en 2005 (21 avril), avant le travail du sol réalisé au motoculteur la semaine suivante (25 avril). La mesure de la progression du brûlé a été faite entre la trace du travail du sol de l'année précédente et la limite actuelle du brûlé. Le 13 juin 2005, de nouvelles mesures ont été réalisées sur tous les arbres des lignes des arbres producteurs pour ajouter à celles-ci, notamment celle du diamètre au collet. Ces mesures au collet ont montré leur faible intérêt, notamment parce que certains arbres étaient formés sur plusieurs troncs.

En 2005-2006, la progression du brûlé a été plus faible en moyenne que celle de 2004-2005, probablement en raison d'un manque de repérage sur l'année 2004 (le suivi physique n'avait pas encore commencé). Les brûlés ayant le plus progressé en 2006 sont ceux des arbres qui avaient produit normalement l'hiver 2005-2006, confirmant la bonne corrélation entre la progression du brûlé et la production truffière. La progression du brûlé a été faible sous les autres arbres de même que la production truffière, à la suite notamment d'un manque de pluie estivale au cours de l'été 2005. Les mesures des années suivantes ont été effectuées le 18 juin 2007 et le 16 mai 2008.

Tableau de la progression du brûlé exprimée en mètre sur 13 chênes verts producteurs avec une taille vigoureuse à l'été 2006

Arbre	Pb 2005	Pb 2006	Pb 2007	Pb 2008
Ch. vert K8	0,46	0,10	0,00	0,20
Ch. vert J6	0,37	0,20	-0,20	0,20
Ch. vert J7	0,35	0,14	-0,04	0,40
Ch. vert J12	0,45	0,10	0,10	0,10
Ch. vert I13	0,35	0,10	-0,10	0,20
Ch vert I11	0,41	0,10	-0,10	0,80
Ch. vert I9	0,34	0,40	-0,30	0,80
Ch. vert I7	0,35	0,10	0,00	0,45
Ch. vert H9	0,38	0,30	0,00	0,50
Ch. vert H12	0,39	0,45	-0,10	0,40
Ch. vert G10	0,38	0,20	0,00	0,20
Ch. vert G9	0,38	0,20	0,10	0,40
Ch. vert G7	0,39	0,20	-0,10	0,50

Tableau de la progression du brûlé exprimée en mètre sur 6 chênes verts peu ou pas producteurs sans taille au cours de l'été 2006

Arbre	Pb 2007	Pb2008
Ch. vert A5	0,25	0,45
Ch. vert A6	0,55	0,15
Ch. vert A7	0,25	0,95
Ch. vert A8	0	-0,3
Ch. vert A9	0,3	0,5
Ch vert A10	0,3	0,6

Photo 7 vue sur la plantation au printemps 2006 (11 mai 2006)

Photo 8 vue sur les mêmes arbres de la plantation le 13 décembre 2006

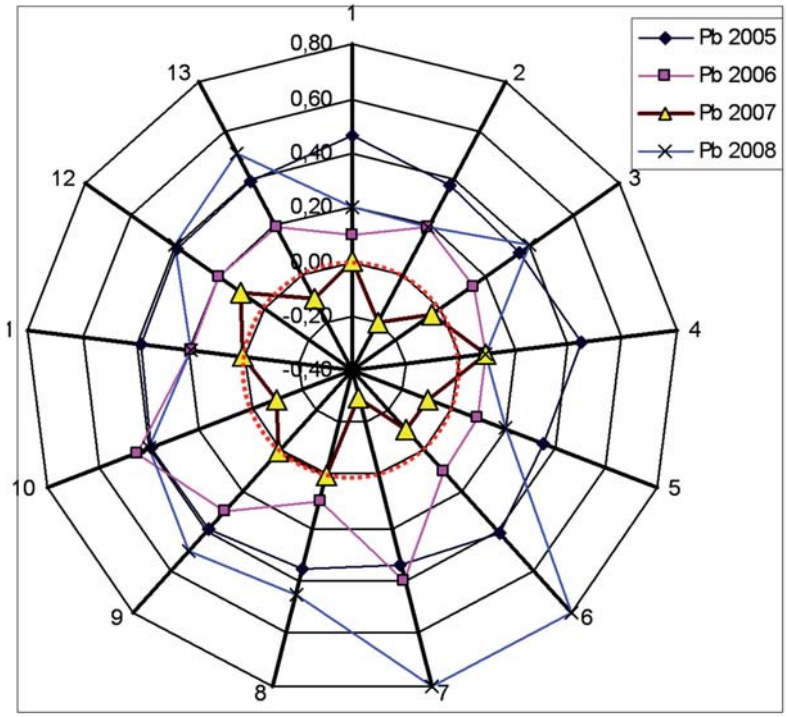
Une taille excessive sur des arbres producteurs a stoppé la progression du brûlé et la production truffière.

Photo 9 vue sur les mêmes arbres de la plantation le 18 juin 2007 avec un retour de la virulence

3. Résultats

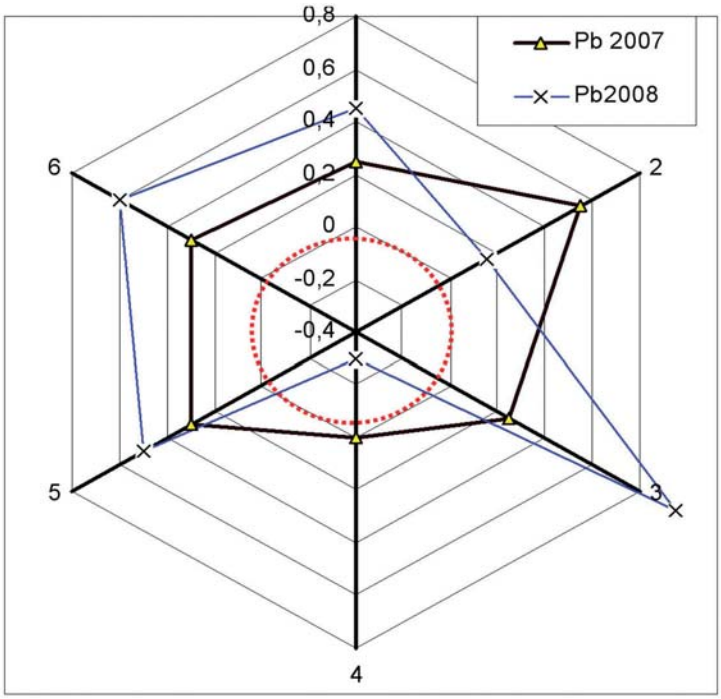
En juin 2007, les mesures ci-dessus montrent que la taille d'août 2006 a bloqué la progression des brûlés, voire même entraîné leur régression d'une dizaine de centimètres pour la plupart. Les mesures effectuées sur les témoins montrent que ceux-ci n'ont pas été affectés dans leur progression. Toutefois, ces arbres, plus développés que ceux de la partie taillée, n'ont commencé à produire que l'hiver 2006-2007, c'est-à-dire à 10 ans au lieu de 6 ans.

Il est curieux de noter que la taille du mois d'août 2006 a, non seulement affecté la progression du brûlé de la saison 2006-2007, mais également eu pour conséquence l'absence de production truffière au cours de l'hiver 2006-2007. Etant donné qu'il existe une deuxième période de progression du brûlé à l'automne, c'est probablement sur celle-ci que la taille a eu des conséquences. Par contre, on ne comprend pas pourquoi, l'hiver 2006-2007, aucune truffe n'a pu être récoltée sur cette plantation: la climatologie de l'été 2006 a connu une répartition correcte des pluies; de plus, les truffes naissant généralement en mai et juin (2006), comment se peut-il qu'une taille en août ait pu avoir des conséquences sur des truffettes déjà formées et dont le mode de nutrition est connu pour être indépendant de l'arbre?



Graph. 1 de la progression du brûlé sur 13 chênes verts producteurs avec une taille vigoureuse à l'été 2006. L'échelle des axes présentant la progression des brûlés est en mètre.

L'analyse du graphique ci-dessus montre comment, en juin 2007, la progression du brûlé était négative sous certains arbres. Ensuite, en mai 2008, on peut observer des progressions exceptionnelles donnant l'impression d'un rattrapage du brûlé sur l'année antérieure. Sur certains arbres qui avaient régressé de 10 à 30 cm, la progression du brûlé a pu atteindre 80 cm l'année suivante. A noter que la progression du brûlé peut ne pas être homogène dans tous les sens. A Escayrac, on n'observe pas d'orientation particulière de la progression par rapport à l'ensoleillement naturel.



Graph. 2 variation de la progression du brûlé sur 6 chênes verts peu ou pas producteurs sans taille au cours de l'été 2006. L'échelle des axes présentant la progression des brûlés est en mètre.

Le graphique des arbres non touchés par la taille montre que la progression a été forte lors de la saison 2007-2008, probablement à la suite d'un printemps humide en 2007 (mai et juin très pluvieux). L'exception du 4ème arbre (A8) est intéressante à noter. Cet arbre, qui n'avait pas progressé en 2007 a carrément régressé en 2008, indiquant la perte de son potentiel truffier.

4. Discussion

Bien que les mesures de rayon du brûlé (Rb) soient d'une précision relative (étant donné les irrégularités du cercle de brûlure), on constate que, sur de jeunes arbres performants, si une forte taille a un impact négatif sur la progression du brûlé (Pb) et la production truffière, le système se rétablit l'année suivante: Pb redevient normal, voire tend à rattraper l'année perdue; la production reprend son niveau antérieur.

En revanche, si l'on compare les résultats de cette situation avec celle d'un système truffier en voie de déclin, on constate que celui-ci n'a pas la capacité de se rétablir, probablement parce que les processus ou les éléments liés à la virulence du *Tuber melanosporum* sont altérés de façon plus ou moins irrémédiables. Dans les caractéristiques du système en voie de déclin, le statut mycorhizien dominé par *Tuber melanosporum* n'est pourtant pas immédiatement affecté, tout au moins la ou les premières années. Le phénomène de déclin semble présenter une inertie à toute intervention, semblable à l'inertie au déclenchement de la fructification dans certaines jeunes plantations qui tardent à produire.

En règle générale, les observations et mesures conduites depuis l'année 2004, année de la perception du phénomène de la virulence, montrent que la progression moyenne du brûlé (Pb) sous un bon arbre producteur est de 10 à 15 cm par an. On a vu plus haut que dans le cas d'un rattrapage de la virulence (à la suite d'une forte taille), Pb pouvait largement dépasser cette valeur, la doubler, voire la tripler. Dans des truffières naturelles, on a pu observer que Pb pouvait prendre des valeurs exceptionnelles si un point trophique existait dans la zone de conquête du brûlé. Ces points trophiques ou qui «nourrissent» le brûlé sont généralement représentés par des plantes telles que *Rosa canina* L., *Prunus spinosa* L., *Vitis vinifera, Juniperus communis* L., *Lavandula angustifolia* L., *Thymus vulgaris* L. C'est généralement au pied de ces plantes que seront récoltées de magnifiques truffes dès lors que le brûlé les aura atteintes depuis 2 à 3 années.

L'observation de l'évolution de ces plantes montre que celles-ci meurent progressivement après avoir passé plusieurs années à l'intérieur du brûlé et avoir servi de points trophiques au brûlé et à la fructification du *Tuber melanosporum*. La mort de ces plantes peut être plus ou moins progressive en fonction de leur vigueur. C'est le cas du genévrier commun *Juniperis communis* L. entre les racines duquel sont récoltées des truffes tant que celui-ci est en vie et même parfois une ou deux années après sa mort. Le cas du ciste cotonneux (*Cistus albidus* L.) est intéressant à analyser en particulier lorsqu'il est producteur. Dans les Corbières, on observe nettement sous celui-ci l'installation du brûlé pendant un ou deux ans, suivi de la production du *Tuber melanosporum* pendant 1 ou 2 années, puis de sa mort. Or, contrairement au genévrier commun, le ciste cotonneux est à lui seul en capacité de produire des truffes.

Photo 10 cistes cotonneux en train de développer un brûlé du *Tuber melanosporum* au-dessus de la muraille de pierres sèches (Pézilla de Conflent, Pyrénées orientales, France)

Ce qui se passe sous le ciste cotonneux semble indiquer que, au-delà d'un certain stade d'infection racinaire par le *Tuber melanosporum*, la plante trophique de la virulence va mourir. Si celle-ci est capable de produire des mycorhizes, cette mort peut se traduire par les phénomènes d'abscissions racinaires présentés plus haut et observés sur les racines des chênes. En revanche, sur le ciste cotonneux, dont le système racinaire est relativement peu différencié (avec différents types de racines) et moins puissant que celui du chêne, la mycorhization ne suffira pas à protéger la plante de l'agression ou de la virulence du *Tuber melanosporum*.

Il convient également de relever que la virulence peut être fortement stimulée par la présence d'animaux. Le pastoralisme a maintes fois été souligné comme une pratique ayant accompagné la truffiiculture à une époque où les résultats étaient exceptionnels dans des paysages certes très ouverts et où était pratiquée une agriculture vivrière. On a vu des brûlés avec des progressions exceptionnelles et une capacité à détruire les plantes herbacées, semi-ligneuses et ligneuses, à des vitesses inhabituelles après la fin d'un parcage de chevaux pendant quelques années dans une lande avec chênes pubescents et genévriers communs. Dès lors que les chevaux ont été enlevés, les brûlés sont devenus très actifs, producteurs de truffes, et particulièrement agressifs, notamment à l'égard de la population de genévriers communs. L'existence de témoins voisins, aussi bien en matière de genévriers que de types de sols ou de végétations, a permis de mettre en évidence que le parcage des chevaux avait été à l'origine d'un retour de virulence du *Tuber melanosporum*.

Cette virulence est surtout active au printemps, de la fin mai à début juillet, puis à l'automne, de la mi-septembre à la mi-octobre. Le suivi des plantes thérophytes permet de situer la période pendant laquelle l'agressivité du *Tuber melanosporum* n'a pas lieu. Parmi ces thérophytes, on citera *Myosotis ramosissima* Rochel*, Alyssum alyssoïdes* L., *Valerianella olitoria* Poll*., Cerastium semidecandrum L..* Ces plantes annuelles apparaissent dès la fin de l'automne sur le brûlé, qui s'estompe voire disparaît, et fleurissent au printemps avant sa reprise d'activité intense. En été, elles sont sous forme de graines.

La persistance sur le brûlé en été d'une crassulaceae, *Sedum sediforme* (Jacq) Pau, peut donner une indication sur une des stratégies de l'agressivité du *Tuber melanosporum* à l'égard des plantes vivant dans l'espace de conquête du brûlé. Il est très probable que la truffe exerce

son agression en captant notamment la ressource en eau du milieu. Mais il ne s'agit là que d'une stratégie puisque ce type d'agression ne suffit pas à expliquer la mort des plantes ligneuses déjà citées, notamment le genévrier commun. Les tissus racinaires des plantes ligneuses sont très probablement investis par le mycélium du *Tuber melanosporum* qui s'y comporte comme un parasite à défaut de former des mycorhizes avec ces végétaux. Ces plantes ligneuses et d'autres semi-ligneuses font l'objet de recherches préliminaires sur la présence de l'ADN du *Tuber melanosporum* dans leur système racinaire. Celui-ci a été observé, notamment dans les racines de *Sanguisorba minor* Scop., plante semi-ligneuse pouvant persister un certain temps à l'intérieur du brûlé.

5. Conclusion

L'observation de l'équilibre entre l'étendue du brûlé et celle de la frondaison de l'arbre, à l'origine de la formule indiquant une bonne virulence (Rb ≥ 1.5 Rf), a des conséquences pratiques, notamment en termes de taille des arbres. On n'a pas fini d'explorer ce qui se passe entre l'arbre et le champignon à partir de cet équilibre que l'on peut observer avec régularité en France, Espagne, Italie et même en Australie ou Nouvelle-Zélande.

Dans certaines situations très productives, le brûlé peut rester confiné sous la frondaison de l'arbre. Les truffes sont alors récoltées à des profondeurs inhabituelles, entre 10 et 20 cm en moyenne. En revanche, on peut aussi observer des brûlés très étendus sous certains arbres avec une production nulle en dépit de la présence de mycorhize du *Tuber melanosporum*. La première situation peut impliquer une biomasse mycélienne du *Tuber melanosporum* très abondante et en profondeur (voir travaux de Laura M. Suz) tandis que la seconde peut indiquer une sexualité inopérante du *Tuber melanosporum (*hétérothallisme nécessaire du *Tuber melanosporum)*. Dans le premier cas, il convient de rechercher les éléments qui vont favoriser la biomasse mycélienne (biodiversité faunistique et floristique) tandis que dans le deuxième, on pourra envisager l'introduction de souches mycéliennes diverses du *Tuber melanosporum*. C'est actuellement sur ces voies que sont orientés les travaux de la Station trufficole du Montat (Lot, France).

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RESEARCH ON HYPOGEOUS FUNGI AND FIRST HARVEST OF A CULTIVATED TRUFFLE IN AUSTRIA

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Abstract

1. In the past decade, a program for the inoculation of tree seedlings with Burgondy truffle (*Tuber aestivum* f. *uncinatum*) and Périgord truffle (*Tuber melanosporum*) was initiated. The first Burgondy truffle was harvested in 2008, on a 5 years old plantation established in an alluvial plain with naturally calcareous soils, about 50 km south of Vienna. Thus far, no harvest of Périgord truffles was recorded.

2. Accompanying inventory research on truffles revealed the presence of about 13 naturally occurring taxa of the genus *Tuber*, depending on species concepts. *Tuber aestivum* f. *uncinatum*, *T. mesentericum*, *T. brumale* f. *brumale*, *T. brumale* f. *moschatum*, *T. rufum, T. excavatum* and *T. fulgens* were most frequently found. There are no Austrian records of the most precious edible truffle species, *T. magnatum*, *T. melanosporum* and *T. macrosporum*.

3. Molecular analyses of anamorphs present in the vicinity of *Tuber* ectomycorrhizae revealed the first known conidial states in the genus *Tuber*, the anamorphs were linked to the *T. borchii* species group by phylogenetic placement. Published species affiliations of the *Tuber* anamorphs need to be revised, according to recent DNA database information.

4. An ongoing research project is dedicated to the investigation of the role of small mammal mycophagy in vectoring fungal spores, particularly those of hypogeous ectomycorrhizal fungi, in Austrian mountain forests. First results indicate that mycophagy is widespread but variable among different species of forest small mammals. Species of many different genera of hypogeous fungi, including *Tuber,* were recorded. Only few of the species vectored by small mammals could be detected in the mountain forest ECM communities, including *Tuber* cf. *puberulum* and *Elaphomyces* sp.

Key words: truffle cultivation, *Tuber* diversity, mycophagy, spore dispersal.

1. Production of inoculated trees and establishment of truffle plantations – first results

In the past decade, a program for the inoculation of tree seedlings with one autochthonous truffle species (*Tuber aestivum* f. *uncinatum,* in the following referred to as *T. aestivum*) and with one introduced truffle species (*Tuber melanosporum*) was initiated, motivated by the success of truffle cultivation in France, Italy, Spain and overseas, and by increasing awareness of the silent decline of autochthonous truffle populations.

Continuous research and experimentation resulted in the rediscovery of the local history of truffling, in the production of mycorrhized plantlets, in the establishment of experimental plantations and in the generation of publicity for this kind of agriculture new for Austria. In 2003, a specialised nursery was founded by the first and second author of this article. Ever since, a few thousand controlled mycorrhized saplings of various host tree species (*Carpinus betulus, Corylus avellana, C. colurna, Quercus* spp., *Pinus nigra* susp. *austriaca* and *Tilia platyphyllos*) were sold to gardeners and landowners or planted in cooperative projects. The majority of planted trees were mycorrhized with *T. aestivum*, *T. melanosporum* was introduced on an experimental scale only. Costumers and cooperation partners were supplied with information concerning site choice, site preparation and recommended cultivation practices. For certain plantations, we had the opportunity to follow-up the development of the host trees and of their mycorrhizal

status, and we start to assist in truffle search. We already checked mycorrhizal samples from a variety of locations which are fairly representative of the diversity of potentially suitable soil types occurring in eastern Austria, mostly soils formed on sedimentary deposits of various origins (marine, alluvial, glacial, eolian). In most samples the mycorrhization with *T. aestivum* was very good at first controls, typically 2-3 years after outplanting. For *T. melanosporum*, results were generally less uniform. In a few sites mycorrhization was satisfactory, while in other cases mycorrhizae of *T. melanosporum* were no more detectable in the root samples. It seems that in these cases *T. melanosporum* had been planted in unsuitable edaphic or climatic conditions, sometimes against our advice. Thus far, no harvests of *Tuber melanosporum* were recorded in Austria.

The first Burgondy truffle (*Tuber aestivum)* was harvested under *Corylus colurna* on October 26th, 2008, on a five years old plantation established in cooperation with Gerald Gruber. The plantation (about 150 *C. colurna* inoculated with *T. aestivum* planted at roughly 3 m x 3 m) was managed according to the model “calcareous grassland” (pelouse calcicole, terrain enherbé; Chevalier & Frochot, 1997). The grass and herbal cover was cut regularly, at least 2-3 times annually, to reduce competition with the tree seedlings and to limit the plantation´s attractivity for rodents. No fertilizers were used. Irrigation was applied in periods of drought. Tree growth was rather slow, probably due to the calcareous, stony soil and due to competition by the grass cover. After five growth seasons, the height of most of the trees was comprised between 1.5 m and 2 m. No mechanical or chemical treatment was used to assist the formation of *brulés*, which started to appear in the fourth year after planting, characteristically below the best developed trees.

The site of the plantation is an alluvial plain with naturally calcareous soils, about 50 km south of Vienna. The plain is situated at the north-eastern margin of the Alps (longitude 16° 16’, latitude 47° 48’, altitude 270 m a. s.). Mean annual temperature is about 9.4 °C, mean annual precipitation about 600 mm. The appellation “Steinfeld” (stone field) of the central and southern part of this plain refers to the composition of its sediments, which consist of huge deposits of calcareous gravel covered by a thin horizon of clay and gravel. These deposits gave rise to the formation of Rendzinas, which have a pH (H_20) of about 8 and which are covered by calcareous grasslands and Pine plantations or used for agriculture. The region was regarded as the most productive area of wild truffles (*T. aestivum*) in Austria, but in the late 20th century, a severe decline of truffle harvests was recorded (Urban & Mader, 2003, Urban & Pla, 2008). While the annual variation of truffle harvests correlates well with climatic fluctuations, long-term changes in land use and collateral effects of industrialisation might be causal for the overall decline of truffle growth (Urban & Mader, 2003). The further development of truffle orchards will inform to which degree the regions past fertility for truffles can be restored.

2. Research on local truffle diversity

Inventory research on truffles revealed the presence of about 13 naturally occurring taxa of the genus *Tuber*, depending on species concepts. *Tuber aestivum* f. *uncinatum*, *T. mesentericum*, *T. brumale* f. *brumale*, *T. brumale* f. *moschatum*, *T. rufum*, *T. excavatum* and *T. fulgens* were most frequently found. The group of small white truffles was less represented in our collections, but this bias may be due to our targeted search for *T. aestivum* f. *uncinatum.* There are no Austrian records of the precious edible truffle species, *T. magnatum*, *T. melanosporum* and *T. macrosporum*.

Currently a revision of *Tuber* species found in Austria, based on recent collections and herbarium specimens, combining nuclear ribosomal DNA markers and morphological studies is under way. Preliminary results hint at an unexpected molecular diversity among the minor, non-commercial truffle species. Species concepts and delimitations of the minor truffles are still a matter of debate, and the full assessment of the molecular and morphological diversity of the genus *Tuber* in Europe will require further work (Mello *et al.,* 2000; Montecchi & Sarasini, 2000; Riousset *et al.,* 2001; Ceruti *et al.,* 2003; Halász *et al.,* 2005; Jeandroz *et al.,* 2008).

3. Molecular studies on terricolous microfungi reveal novel anamorphs of two *Tuber* species

Molecular analyses of anamorphs found on forest soil in the vicinity of *Tuber* ectomycorrhizae revealed the first known conidial states in the genus *Tuber* (Urban *et al.,* 2004). The anamorphs (Fig. 1) were genetically linked to two species of the taxonomically difficult group of small white truffles based on DNA database data available at that time, particularly on an earlier version of sequences contributed by Halász *et al.,* (2005). The reference sequences originally deposited as *T. borchii* were later relabelled as *T. puberulum*, and those deposited as *T. oligospermum* were relabelled as *T. borchii* in the public DNA database records. This revision is consistent with the final species labels used for publication (Halász *et al*., 2005) but unfortunately this update could not be considered by Urban *et al*., (2004), who used the old, erroneous labels for the phylogenetic placement of *Tuber* anamorphs. According to Halász *et al.,* (2005), the correct species designations would be *T. puberulum* and *T. borchii*. In any case, a widely accepted consensus on the taxonomy of European minor white truffles seems to be missing thus far, judging from a recent comparative study including DNA database data generated by various researchers (Jeandroz *et al.,* 2008). A revision of white truffles found in Austria including nrDNA sequence data is under way, to contribute to the resolution of this species complex.

Apart from the taxonomic problems, many questions concerning the function of *Tuber* conidia and their relevance for the dispersal and reproduction of truffle species are still open. Are these conidial structures restricted to certain species of small white truffles or do they occur throughout the genus? What is their role in the truffle life cycle? Recent progress concerning the life cycle of *T. magnatum* (Paolocci *et al*., 2006) might pave the way for further research on the function of *Tuber* conidia.

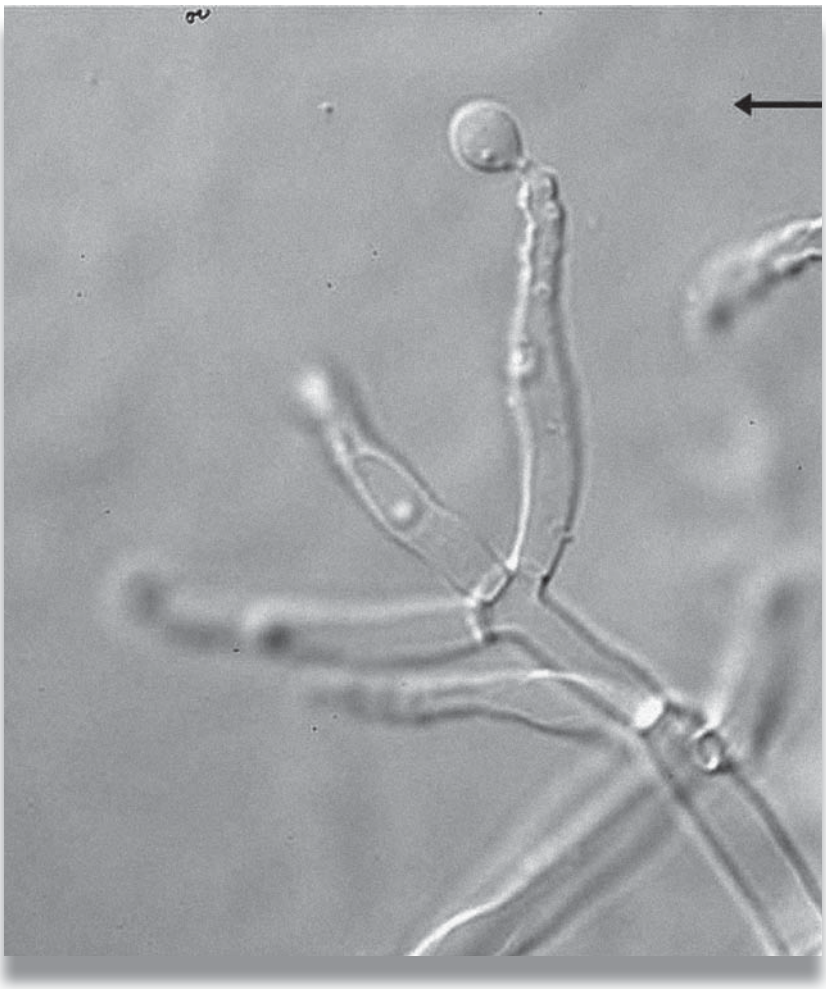

Fig. 1 Conidial state of *Tuber borchii.* Length of arrow = 5 µm.

4. Small mammal mycophagy in mountainous forests in Austria

An ongoing research project is dedicated to the investigation of the role of small mammal mycophagy in vectoring fungal spores, particularly those of hypogeous ectomycorrhizal fungi, in Austrian mountain forests dominated by *Picea abies*, *Fagus sylvatica* and *Abies alba* (Schickmann *et al.,* 2007).

Hypogeous fruiting is evolutionarily linked with the loss of active spore discharge and aerial spore dispersal. Alternatively, spores are dispersed by animals, particularly by endozoochory. The evolutionary trend to hypogeous fruiting is found repeatedly in all major groups of ECM-fungi suggesting that zoochory is a successful way of spore dispersal (Trappe & Claridge, 2005).

Only little information on mycophagy of small mammals in European mountain forests is available, even though these animals may play a major role in the dispersal of fungal spores. We started to investigate the following questions in two study sites in Lower Austria, the Wilderness Area Dürrenstein and the Demonstration Forest Heuberg/Rosalia:

- Which of the small mammal species occurring in the area are mycophagous?
- Which fungal species are consumed?
- Are there differences between mammal species concerning the degree of mycophagy and the fungal species consumed?
- Does the type of habitat (managed forest, primeval forest, disturbed habitat - windthrow) have an impact on small mammal species composition, fungal species composition and mycophagy?

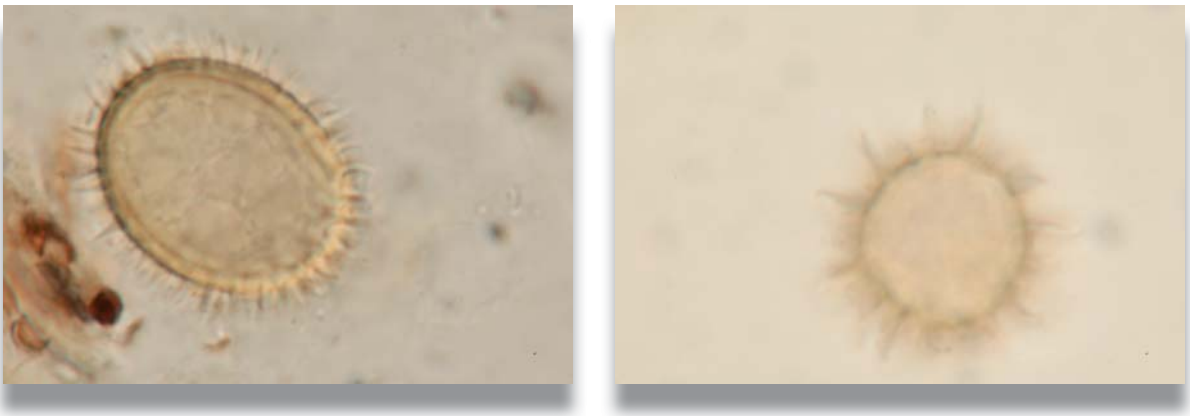

Fig. 2 Spores from small mammal faeces: *Tuber rufum* (left), *Tuber* cf. *puberulum* (right)

A multidisciplinary approach, including life trapping of small mammals, the microscopic and DNA based analysis of fungal spores in faecal pellets, the inoculation of tree seedlings and the inventory of the ECM fungal communities is being employed. Microscopical identification of spores of hypogeous fungi was based on personal experience and on the descriptions and figures provided by Joseph Astier (1998), Montecchi & Sarasini (2000) and Riousset *et al*, (2001).

First results indicate that mycophagy is widespread but variable among different species of forest small mammals. The red backed vole (*Myodes glareolus*) is preferentially mycophagous, while the yellow-necked mouse (*Apodemus flavicollis*) and shrews (*Sorex* spp.) were found to be opportunistic mycophagists. Spores of many different genera and species of hypogeous fungi were detected, including *Elaphomyces* spp.*, Tuber rufum* (Fig. 2 left)*, Tuber* cf. *puberulum* (Fig. 2 right)*, Choiromyces meandriformis,* cf. *Genea* spp.*, Balsamia* cf. *polysperma, Picoa carthusiana, Melanogaster* spp.*, Chamonixia caespitosa, Octavianina polysperma, Hymenogaster* spp.*, Hysterangium* spp.*,* as well as a variety of conidiospores (*Haplotrichum* sp., *Cladosporium*-like, and others) and some spores of putative epigeous fungi (Boletacea sp., Psathyrellaceae spp., Pyronemataceae sp.). Some of the species which are frequently consumed by small mammals are usually regarded as rare, since there are only few herbarium

records of most hypogeous species. Only few of the species vectored by small mammals could be detected in the mountain forest ectomycorrhizal communities, including *Tuber* cf. *puberulum* and *Elaphomyces* sp. The nrITS (nuclear ribosomal internal transcribed spacer) sequence obtained from *Tuber* cf. *puberulum* ectomycorrhizae is a novel genotype not yet represented in public databases. The study of this species will add another piece to the mosaic of truffle diversity.

Acknowledgements
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LA COLTIVAZIONE DEL TARTUFO IN SICILIA: RISULTATI PRELIMINARI

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Astract: **Truffle cultivation in Sicily: preliminary results**
In recent times truffles were found in several natural forest ecosystems and reaforestation of Sicily. On the basis of these findings a number of research projects were activated. In the framework of an agreement between the Laboratory of Micology of the Department of Botany (University of Palermo) and the Farm "Buontempo-Le Due Sicilie", located in the territory of the Madonie Natural Park, a first attempt to cultivate truffles of economic importance were carried out. In the spring of 2006 the experimental truffle-bed was developed on the basis of 100 seedlings of *Corylus avellana* L. inoculated with *Tuber aestivum* Vittad. (80%) and *T*. *melanosporum* Vittad. (20%). Besides 110 seedlings of *Quercus pubescens* Willd. s.l., inoculated with *T*. *melanosporum* (20%) and *T*. *aestivum* (80%) were planted. The techniques of cultivation and the scheme of planting of the truffle-bed together with the preliminary results of two years of observations are here pointed out.
Key words: truffles, cultivation, Sicily.

Introduzione

In questo contributo vengono riportati i risultati preliminari di uno studio avviato nel 2006 a seguito dell'impianto di una tartufaia nel territorio di Polizzi Generosa, in provincia di Palermo. La sperimentazione è frutto di una sinergia attivata da alcuni anni con la società cooperativa Buontempo-Le Due Sicilie, interessata alla coltivazione di varietà locali pregiate di prodotti orticoli e frutticoli. L'impianto della tartufaia costituisce un primo tentativo di produzione di tartufi pregiati nell'isola basato sulla certezza che gli stessi sono ampiamente diffusi in natura e sull'esistenza di aree vocate nel territorio siciliano (Bencivenga & Venturella, 2001; Pecorella & Venturella, 2006; Venturella, 1995; Venturella *et al*., 2004, 2006).

Caratteri fisiografici dell'azienda

L'azienda Buontempo-Le Due Sicilie, la cui superficie complessiva è di ettari 10.000, è ubicata nel versante sud orientale del territorio del Comune di Polizzi Generosa, Contrada Santa Nicola, ad un'altitudine media di 600 m sul livello del mare.
Le principali colture dell'azienda sono rappresentate da noccioleti, ortaggi e leguminose da foraggio.
L'azienda è stata caratterizzata dal punto di vista pedologico per mezzo di analisi fisico chimiche effettuate sulla base di 5 campioni di suolo prelevati in cinque punti differenti del territorio aziendale. Tutti i campioni di suolo analizzati presentano una reazione sub alcalina mentre il contenuto in $CaCO_3$, sia totale che attivo è variabile. In particolare nei campioni 1, 2 e 4 non si riscontra presenza di calcare sebbene il contenuto di calcio sul complesso di scambio sia elevato. Il $CaCO_3$ risulta più abbondante nei campioni 4 e 5 mentre tutti i campioni di suolo esaminati risultano ricchi di sostanza organica, di azoto e fosforo, sufficientemente dotati di potassio e con alta capacità di scambio cationico.
L'analisi fisico meccanica evidenzia una buona struttura del suolo e caratteristiche di medio impasto idonee all'impianto di una tartufaia.

Ricerca di tartufi spontanei

All'interno dell'azienda sono state individuate produzioni spontanee di *Tuber excavatum*, *T*. *brumale*, *T*. *borchii* e di altri quattro ipogei quali *Genea fragans*, *G*. *verrucosa*, *Hymenogatser*

olivaceus e *H*. *vulgaris*. In particolare *T*. *excavatum* è stato ritrovato nei mesi di gennaio e febbraio in un bosco misto a *Quercus pubescens* e *Corylus avellana*. Nello stesso tipo di vegetazione, a partire da dicembre e fino al mese di febbraio è stato raccolto *T*. *borchii* mentre *T*. *brumale* è stato riscontrato all'interno del noccioleto.

Impianto sperimentale

Nella fase preliminare di progettazione dell'impianto colturale sono stati presi in considerazione i fattori climatici, l'altitudine, l'esposizione, la topografia, le caratteristiche del suolo, le colture antecedenti, la disponibilità di acqua.

Successivamente si è provveduto alla scelta delle piante simbionti privilegiando quelle già diffuse allo stato spontaneo nel territorio oggetto di studio ed in particolare il nocciolo e la roverella. Per la preparazione del terreno è stato effettuato un intervento di taglio delle piante di nocciolo vetuste ed una ripulitura della vegetazione spontanea presente. È stato anche risistemato un canale di sgrondo per lo smaltimento delle acque meteoriche e dell'acqua stagnante. Il terreno è stato lavorato in estate mediante una rippatura ed una successiva erpicatura. Non sono stati utilizzati diserbanti sistemici per l'eliminazione di piccole ceppaie o radici profonde.

Nei terreni destinati all'impianto di piante micorrizate con roverella le avverse condizioni atmosferiche non hanno consentito di effettuare le lavorazioni e quindi si è provveduto a realizzare un impianto a buche.

Le piantine utilizzate per la realizzazione dell'impianto sono state fornite da un vivaio qualificato del Nord Italia che ha rilasciato una idonea certificazione.

Sono state acquistate 100 piante, dell'età di un anno di *C*. *avellana* di cui 80 micorrizate con *T*. *aestivum* e circa 20 con *T*. *melanosporum* e 110 piante di *Q*. *pubescens* di cui 22 micorrizate con *T*. *melanosporum* e 78 con *T*. *aestivum*.

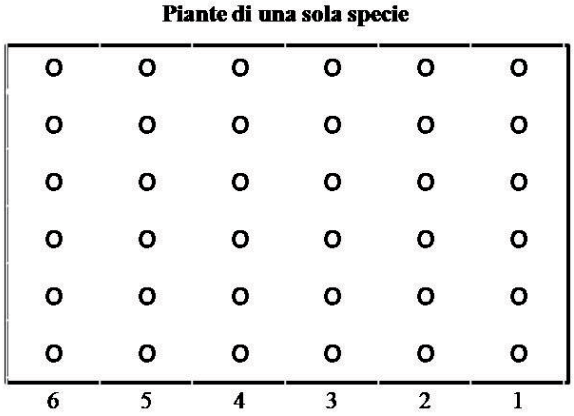
L'impianto è stato realizzato tra la fine di marzo e l'inizio di aprile 2006.

Prima di procedere alla messa a dimora delle piante si è provveduto ad una estirpatura superficiale per rimuovere eventuali erbe infestanti.

Successivamente sono stati sistemati dei picchetti in corrispondenza del punto di collocazione delle piante realizzando delle buche di cm 20 × 20 × 20. Le piantine sono state irrigate prima della sistemazione nelle buche già predisposte e liberate dal vasetto che le conteneva provvedendo a riportare le radici in posizione radiale altrimenti accartocciate con conseguente danno alla crescita dell'apparato radicale. A questo punto le buche sono state riempite con terra fine interrando la parte alta della zolla per 4-5 cm. Ad operazione ultimata il terreno intorno alla pianta è stato sistemato a conca per meglio raccogliere l'acqua piovana o con irrigazione. Sono stati somministrati 5-6 l di acqua per consentire l'assestamento del terreno intorno alle radici. La tartufaia è stata recintata con rete metallica alta 1.80 m sostenuta da paletti di legno.

Schema d'impianto

L'area di impianto si compone di due spazi separati: nel primo, sito in una zona pianeggiante, sono state sistemate le piantine di nocciolo all'interno di un'unica parcella 5 × 3 m con disposizione a rettangolo.

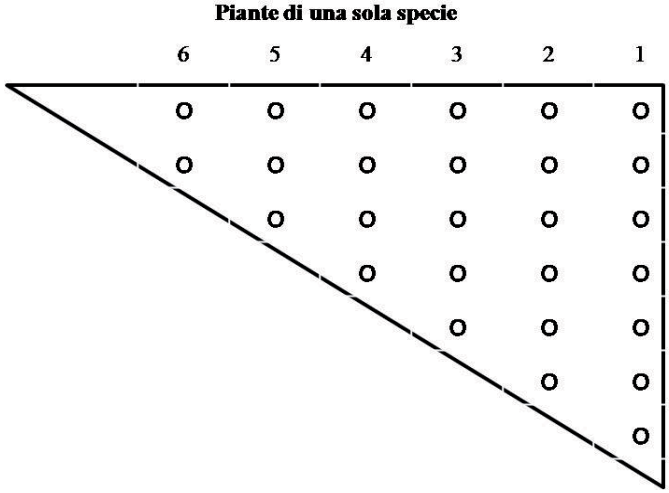


Lungo il filare 1 sono state collocate piantine di nocciolo micorrizate con *Tuber melanosporum*.
Filare 2: nocciolo-*Tuber aestivum*. N° piante 17
Filare 3: nocciolo-*Tuber aestivum*. N° piante 16
Filare 4: nocciolo-*Tuber aestivum*. N° piante 16
Filare 5: nocciolo-*Tuber aestivum*. N° piante 6
Filare 6: nocciolo-*Tuber aestivum*. N° piante 6

Sono state rilevate altezza e diametro per ciascuna pianta:

Filare 1	H max 80 cm	H med -	H min 40 cm
	D max 70 cm	D med -	D min 45 cm
Filare 2	H max 90 cm	H med - 60 cm	H min 15 cm
	D max 90 cm	D med - 45 cm	D min 20 cm
Filare 3	H max 70 cm	H med - 45 cm	H min 15 cm
	D max 75 cm	D med - 35 cm	D min 20 cm
Filare 4	H max 80 cm	H med - 35 cm	H min 30 cm
	D max 60 cm	D med - 45 cm	D min 25 cm
Filare 5	H max 45 cm	H med -	H min 35 cm
	D max 40 cm	D med -	D min 25 cm
Filare 6	H max 70 cm	H med -	H min 55 cm
	D max 60 cm	D med - 45 cm	D min 35 cm

I filari 1 e 2 sono quelli che rilevano un buon sviluppo della pianta.
Nel secondo spazio, che presenta una pendenza maggiore, sono state collocate le piantine di roverella; le parcelle 2, situate a poca distanza l'una dall'altra, misurano 5 × 3 m e la disposizione è a filari irregolari.

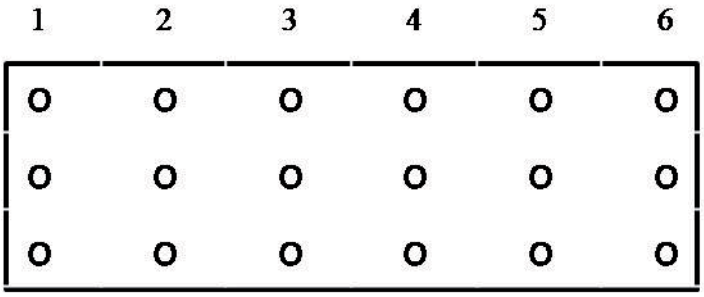


Filare 1: roverella-*Tuber aestivum*. N° piante 20
Filare 2: roverella-*Tuber aestivum*. N° piante 20
Filare 3: roverella-*Tuber aestivum*. N° piante 5
Filare 4: roverella-*Tuber aestivum*. N° piante 6
Filare 5: roverella-*Tuber aestivum*. N° piante 3
Filare 6: roverella-*Tuber aestivum*. N° piante 0

Sono state rilevate altezza e diametro per ciascuna pianta:

Filare 1	H max 80 cm	H med - 45 cm	H min 30 cm
	D max 75 cm	D med - 35 cm	D min 35 cm
Filare 2	H max 15 cm	H med -	H min 10 cm
	D max 20 cm	D med -	D min 10 cm
Filare 3	H max 15 cm	H med -	H min 12 cm
	D max 30 cm	D med -	D min 20 cm
Filare 4	H max 58 cm	H med - 25 cm	H min 20 cm
	D max 40 cm	D med - 40 cm	D min 25 cm
Filare 5	H max 25 cm	H med -	H min -
	D max 20 cm	D med -	D min -
Filare 6	H max -	H med -	H min -
	D max -	D med -	D min -

Piante di una sola specie



Filare 1: roverella-*Tuber melanosporum*. N° piante 1
Filare 2: roverella-*Tuber melanosporum*. N° piante 3
Filare 3: roverella-*Tuber melanosporum*. N° piante 3
Filare 4: roverella-*Tuber melanosporum*. N° piante 3
Filare 5: roverella-*Tuber melanosporum*. N° piante 3
Filare 6: roverella-*Tuber melanosporum*. N° piante 1

Sono state rilevate altezza e diametro per ciascuna pianta:

Filare 1	H max 45 cm	H med -	H min -
	D max 20 cm	D med -	D min -
Filare 2	H max 45 cm	H med -	H min 25 cm
	D max 20 cm	D med -	D min 16 cm
Filare 3	H max 45 cm	H med -	H min 30 cm
	D max 36 cm	D med -	D min 15 cm
Filare 4	H max 30 cm	H med -	H min 20 cm
	D max 15 cm	D med -	D min 11 cm
Filare 5	H max 6 cm	H med -	H min - 4 cm
	D max 3 cm	D med -	D min - 1 cm
Filare 6	H max -	H med -	H min -
	D max -	D med -	D min -

Su tutti i sesti di impianto non è stata effettuata la pacciamatura al fine di ridurre l'evaporazione dell'acqua dal suolo, di contenere lo sviluppo delle erbe infestanti e di consentire un migliore attecchimento delle piante.

Cure colturali

Le cure colturali sono necessarie per favorire lo sviluppo della parte aerea delle piante e del loro apparato radicale e per realizzare le migliori condizioni pedologiche per la crescita delle micorrize, la formazione e la maturazione degli ascomi. Nell'impianto oggetto di studio si è provveduto a mantenere un manto vegetale erbaceo per problemi legati all'utilizzo di mezzi meccanici e per rendere meno impegnativa e costosa la conduzione della tartufaia. In particolare è stata effettuata solo una leggera erpicatura superficiale eliminando le erbe infestanti a mano. Successivamente è stata eseguita una zappettatura superficiale del terreno facendo attenzione a non tagliare o danneggiare le radici delle piante tartufigene che in prossimità del tronco sono superficiali. Questo intervento di aerazione del terreno è stato fatto verso la fine dell'inverno per ridurre il rischio di costipare il terreno a causa della forte umidità. Al momento non sono state effettuate potature in attesa che le piante siano meglio sviluppate ed attecchite.
La somministrazione dell'acqua è stata effettuata in vicinanza del fusto utilizzando un impianto di irrigazione a goccia con un gocciolatoio per pianta. Nel corso dell'estate l'irrigazione è stata effettuata con cicli giornalieri di 40 min (circa 2 litri di acqua per pianta). La periodicità delle irrigazioni è stata variata a seconda delle esigenze delle piante tartufigene e in rapporto alle situazioni pedologiche e climatiche del luogo.

Controllo delle piante micorrizate

È stato utilizzato il metodo morfologico, sicuramente il più utilizzato in tartuficoltura.
Sono stati prelevati n. 2 campioni di piante di cui:
8 roverelle micorrizate con *T. aestivum*;
8 noccioli micorrizati con *T. aestivum*;
2 roverelle micorrizate con *T. melanosporum*
2 noccioli micorrizati con *T. melanosporum*
Le roverelle micorrizate con *T. aestivum* si presentano in buono stato fitosanitario e con apparato radicale ben sviluppato. La percentuale di micorrizazione è elevata. È stata rilevata la presenza di inquinanti quali *Pulvinula costellatio*.
Sia le roverelle che i noccioli micorrizati con *T. melanosporum* presentano un buono stato fitosanitario con apparato radicale abbastanza sviluppato. La percentuale di micorrizazione è inferiore a quella di *T. aestivum*, ma più che sufficiente. I noccioli micorrizati con *T. aestivum* sono in buono stato fitosanitario con apparato radicale ben sviluppato. Gli apici radicali non presentano un grado di micorrizazione elevato ed è stata rilevata la presenza di *P. costellatio*.

Conclusioni

Lo studio condotto ha evidenziato una buona rispondenza delle caratteristiche climatiche del sito di Polizzi Generosa all'impianto di una tartufaia con piante di nocciolo e roverella micorrizate con *Tuber melanosporum* e *T. aestivum*.
Si tratta di una prima esperienza sperimentale che dovrà essere valutata nel tempo in quanto, come è noto, le tartufaie di nuovo impianto trascorrono un periodo improduttivo variabile da 4 a 6 anni.
I risultati della ricerca possono essere così sintetizzati:

- L'area ricadente all'interno dell'azienda Le Due Sicilie è vocata per la produzione spontanea di tartufi;
- L'area oggetto di studio è anche vocata all'impianto di tartufaie sperimentali;
- L'impianto di piante micorrizate con scorzone appare al momento il più affidabile considerata anche la abbondante presenza in natura di questo tartufo;

- L'impianto di piante micorrizate con tartufo nero pregiato rappresenta un tentativo logico di sperimentazione di un tartufo, al momento non riscontrato in natura, ma che per caratteristiche ecologiche potrebbe proporsi come valida e più pregiata alternativa allo scorzone;
- Il grado di micorrizazione delle piante a distanza di circa 2 anni dall'impianto è buono ma necessita di costante valutazione nel tempo;
- Lo stato vegetazionale delle piante è complessivamente buono tranne alcune fallanze cui si è provveduto al reintegro con l'acquisto di nuove piante.

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THE MAGNATUM PROJECT (MONITORAGGIO DELLE ATTIVITÀ DI GESTIONE DELLE TARTUFAIE NATURALI DI *TUBER MAGNATUM*)

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Abstract

MAGNATUM is the acronym for a four year inter-regional cooperative project co-funded by the Abruzzo, Emilia – Romagna, Molise and Tuscany Regions established through a public initiative and coordinated by ARSIA (Agenzia Regionale per lo Sviluppo e l'Innovazione nel settore Agricolo-forestale) of the Tuscany region. The aim of this project is to obtain scientific information that will be of use in safeguarding natural *T. magnatum* truffières. It will integrate well established methodology and molecular technology.

Four natural *T. magnatum* truffières, one in each of the collaborating regions will be chosen which will be closed to the public so that the collected scientific data will be more meaningful. The chosen truffières will also have pedological, vegetational and climatic characteristics considered ideal for each region.

In each truffière the effects of selective thinning and different methods of soil tillage will be observed for their effect on truffle production, the ectomycorrhizal fungal communities, and soil physical and chemical characteristics. Their effects on the presence and dynamics of *T. magnatum* mycelium in the soil will be also evaluated by real time PCR techniques.

The integrated use of different methods of analyses should provide insights into truffle biology giving information on the relationship between truffle production and the vegetative development of *T. magnatum* in the soil. The molecular quantification of *T. magnatum* mycelium in the soil of natural truffières, were the variability of fruiting body production often gives results difficult to evaluate, may also allow us to establish the effects of different management treatments.

Key words: *Tuber magnatum*, conservation, ecology, management, soil mycelium, real time PCR.

Tavola Rotonda

Esperienze e problematiche di coltivazione dei tartufi

Modera il Prof. Mattia Bencivenga che tiene la relazione introduttiva

LA COLTIVAZIONE DEI TARTUFI: GIOIE E DOLORI

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Abstract: Truffles cultivation: joy and pain
Truffle cultivation is an agricultural activity sought after by farmers because it allows them:

to produce a valuable non-deficit good, easily placed on the market;
to generate income even on marginal terrains;
to pursue other activities as truffle cultivation does not require continuous maintenance;
to increase the value of other farm products;
to arm with full respect for the environment and the landscape.

In recent decades, truffle cultivation has markedly improved thanks to several achievements of scientific research:

the quality of mycorrhizal plants put on the market has improved;
the ecological and productive characteristics of truffle-producing plants are clearer;
knowledge of different truffle species' environmental needs has deepened;
this allows us to choose the truffle species suited to the cultivation environment.
The initial period of non-productivity in a plantation has been reduced from 8-9 years to 4-5 years.
We have begun to get the first research results on techniques of cultivation for mycorrhizal plants after they have been planted.

Along with these scientific successes, new problems have arisen, some of which are still unresolved:

the first problem consists in the production of truffles belonging to a different species from that being cultivated;
a serious problem is that of the early exhaustion of some truffle orchards' productive activity: plantations begin to produce, have satisfactory production for a few years and then exhaust production;
changes in the environment and, above all, the increase of droughts and summer temperatures damage plantations;
Techniques for the cultivation of truffle-producing plants in the field are only partially acquired;
In Italy, truffle orchard production is not reported, giving rise to legal problems and to a lack of interest in truffle cultivation on the part of public bodies.

One hopes that scientific research can solve the problems I have just referred to, as well as others which will no doubt arise, so that truffle cultivation as an agricultural activity, can become both more and more reliable and profitable. The goal of the round table which we are about to begin is precisely that of understanding how farmers approach, and, we hope, have resolved, some of the problems listed above, and whether there are other problems we should add to the list.
Key words: truffle farming, truffle production, problems of truffle cultivation.

Riassunto
La tartuficoltura è un'attività agricola richiesta dagli agricoltori perché consente loro di:

produrre un bene pregiato, non eccedentario e facilmente collocabile nel mercato;
produrre reddito anche nei terreni marginali;
rendere possibili altre attività in quanto la tartuficoltura non richiede la presenza continua di manodopera;
valorizzare altri prodotti aziendali;
operare nel rispetto dell'ambiente e del paesaggio.

Negli ultimi decenni la tartuficoltura è sensibilmente migliorata per alcuni traguardi raggiunti dalla ricerca scientifica:

è migliorata la qualità delle piante micorrizate che vengono messe in commercio;
sono più chiari i caratteri ecologici e di produttività delle piante tartufigene;
sono state approfondite le conoscenze sulle esigenze ambientali richieste dalle diverse specie di tartufo; ciò consente di scegliere la specie di tartufo idonea per l'ambiente di coltivazione; è stato ridotto il periodo improduttivo iniziale di una piantagione tartufigena da 8-9 a 4-5 anni;
si iniziano ad avere i primi risultati delle ricerche sulle tecniche di coltivazione delle piante micorrizate dopo la loro collocazione a dimora.

Insieme a questi successi scientifici sono emersi nuovi problemi, alcuni dei quali ancora irrisolti:

un primo problema consiste nella produzione di tartufi appartenenti a specie diverse da quella coltivata;
un problema grave è rappresentato dall'esaurimento precoce dell'attività produttiva di alcune tartufaie: le piantagioni entrano in produzione, forniscono produzioni soddisfacenti per qualche anno e poi si esauriscono;
i cambiamenti ambientali e soprattutto l'aumento della siccità e delle temperature estive provocano danni alle piantagioni;
sono parzialmente acquisite le tecniche di coltivazione delle piante tartufigene in campo;
in Italia non vengono rese note le produzioni delle tartufaie causando problemi legislativi e la mancanza di interesse da parte degli Enti pubblici verso la tartuficoltura.

Si auspica che la ricerca scientifica possa risolvere i problemi appena accennati, e altri che probabilmente sorgeranno, al fine di rendere la tartuficoltura un'attività agricola sempre più certa e redditizia. Lo scopo della tavola rotonda che stiamo iniziando è proprio quello di conoscere come gli agricoltori affrontano, e speriamo abbiano risolto, alcuni dei problemi elencati e se ne esistono altri da aggiungere all'elenco.

Parole chiave: Coltivazione tartufi, Produzione tartufi, Problemi tartuficoltura.

Premessa

Gli agricoltori italiani, a causa della scarsa remunerazione fornita dalle colture agrarie che vedono l'aumento dei costi di produzione e la costanza dei prezzi di vendita, nonché la necessità di fornire reddito nel rispetto dell'ambiente, si stanno orientando verso la coltivazione dei tartufi che consente loro di:

produrre un bene pregiato, non eccedentario e facilmente collocabile nel mercato;
produrre reddito anche nei terreni marginali;
rendere possibili altre attività in quanto la tartuficoltura non richiede la presenza continua di manodopera;
valorizzare altri prodotti aziendali che vengono venduti insieme ai tartufi;
operare nel rispetto dell'ambiente e del paesaggio.

La specie più largamente coltivata è *Tuber melanosporum* Vittad. seguita da *Tuber aestivum* Vittad. nella forma estiva ed autunnale, *Tuber borchii* Vittad., *Tuber brumale* Vittad. e *Tuber macrosporum* Vittad.. In passato era stata avviata la coltivazione di *Tuber magnatum* Pico, pressoché interrotta a causa di problemi biologici (Bencivenga, 2001).

La coltivazione dei tartufi è migliorata sensibilmente negli ultimi anni, ma purtroppo esistono ancora margini di rischio in quanto non è possibile garantire, con assoluta certezza, l'entità della produzione e neanche il successo produttivo della piantagione.

Le produzioni medie registrate nelle piantagioni realizzate negli ultimi 10-15 anni sono:

- *Tuber melanosporum* (tartufo nero pregiato): attualmente il 95% delle tartufaie fornisce produzioni variabili tra 5-6 kg/ha e oltre i 100 kg/ha con punte che hanno raggiunto, in particolari stagioni, i 300 kg/ha. Le produzioni medie annuali sono di 30-40 kg/ha (Bencivenga e Di Massimo, 2000).

- *Tuber aestivum* (scorzone): la maggior parte delle tartufaie è recente e pertanto i risultati non sono ancora consolidati. Sembra che la produzione sia più tardiva rispetto a *Tuber melanosporum*, ma molto soddisfacente.
- *Tuber borchii* (bianchetto): ha fornito produzioni soddisfacenti (30-80 Kg/ha) e durature nei terreni soffici, tendenzialmente sabbiosi, e con pH da lievemente sub-acido a sub-alcalino.
- *Tuber brumale*: anche in questo caso si tratta di piantagioni recenti realizzate negli ambienti dove *Tuber brumale* si sostituisce naturalmente a *Tuber melanosporum.* Le produzioni ancora non possono essere valutate.
- *Tuber macrosporum* (tartufo nero liscio): alcune prove di coltivazione stanno fornendo risultati interessanti (Vezzola, 2010).

Tuber magnatum (tartufo bianco): le piantagioni sono state realizzate fino agli anni 90' quando l'analisi molecolare delle micorrize, fino ad allora ritenute di tartufo bianco, ha evidenziato che sono prodotte da tartufi del gruppo del bianchetto (*Tuber borchii* Vittad., Paolocci *et al*., 1999, 2001). Alcune delle tartufaie realizzate in passato, comunque, stanno fornendo produzioni di tartufo bianco comprese tra 1 e 15 Kg/ha (Gregori *et al*., 2010). Le produzioni sono tardive (15-20 anni dopo l'impianto) e limitate ai siti dove si sono create le condizioni ambientali idonee alla fruttificazione del tartufo: condizioni ambientali particolari e difficilmente realizzabili in una tartufaia coltivata.

Negli ultimi anni sono emersi nuovi problemi, come ad esempio l'esaurimento produttivo precoce di alcune piantagioni, che richiedono nuove indagini per rendere sempre più remunerativa la coltivazione dei tartufi.

Successi della tartuficoltura: GIOIE

Negli ultimi due decenni la tartuficoltura è sensibilmente migliorata grazie ai vari traguardi scientifici.

Il primo traguardo riguarda il miglioramento della qualità delle piante tartufigene utilizzate nell'impianto delle tartufaie. In Italia quasi tutte le piante che vengono messe a dimora hanno una micorrizazione certificata sotto il profilo morfologico e molecolare (Bencivenga *et al*., 1987). Va rilevato che molti vivai, oltre al controllo delle piante, richiedono il controllo e la certificazione dei tartufi che utilizzano per produrre le piante micorrizate. Contemporaneamente sono migliorate le conoscenze sulle caratteristiche produttive delle diverse specie simbionti; nelle piantagioni realizzate in passato si preferivano *Corylus avellana* L. e *Ostrya carpinifolia* Scop., specie oggi pressoché abbandonate a vantaggio di *Quercus pubescens* Willd. e *Quercus ilex* L. che hanno la capacità di conservare a lungo le micorrize del tartufo (Baciarelli Falini *et. al*., 2000).

Un altro successo è rappresentato dal miglioramento delle conoscenze ambientali richieste dalle diverse specie di tartufo per accrescersi e fruttificare (Bencivenga *et al*., 1990; Bencivenga e Granetti, 1990; Bencivenga *et al*., 1992; Bencivenga, 1994; Bencivenga *et al*., 1996; Bragato *et al*. 2001; Lulli e Primavera 2001; Raglione *et al*., 2001). Queste conoscenze consentono di valutare, con buona approssimazione, l'idoneità tartufigena di un ambiente. In Italia, dove le condizioni ambientali sono molto variabili e dove si coltivano più specie di tartufo, è importante individuare, per ogni sito di coltivazione, la combinazione idonea di pianta-tartufo. Va precisato, comunque, che le ricerche sugli ambienti idonei allo sviluppo di alcune specie di tartufo (es. *Tuber melanosporum*) sono state condotte negli ambienti dove cresce attualmente allo stato naturale: si tratta spesso di zone di rifugio, essendo le aree migliori sottoposte a coltura. Per questo motivo e per conoscere la microflora e la microfauna implicate nello sviluppo dei tartufi, si ritiene importante un approfondimento di tali ricerche.

Dal punto di vista scientifico sono migliorate le conoscenze sulle micorrize prodotte dai tartufi, le quali consentono una ottima valutazione delle piante micorrizate (Granetti, 1994; Granetti, 1995; Granetti *et al.,* 1995a, 1995b; Mello *et al*., 1998, 2001; Paolocci *et al*., 1995).

Un altro successo è rappresentato dalla riduzione del periodo improduttivo di una tartufaia. In passato una piantagione iniziava a produrre tartufi dopo 10-15 anni dall'impianto, oggi tale periodo si è dimezzato. Anche le produzioni unitarie sono sensibilmente migliorate.

Un ultimo successo, ancora parziale, consiste nella individuazione delle tecniche colturali

da adottare nei confronti delle piante simbionti dopo la loro collocazione a dimora (Baciarelli Falini e Bencivenga, 2002). E' ancora radicata, in molti tartuficoltori, l'idea che la tartufaia non debba essere sottoposta ad interventi agronomici: pur mancando ancora una adeguata sperimentazione, si osserva che le tartufaie non sottoposte ad interventi colturali come l'irrigazione, la sarchiatura la potatura delle piante simbionti, l'inoculo sporale, ecc. sono improduttive o forniscono produzioni irrisorie.

Attuali problemi della tartuficoltura: DOLORI

Insieme ai miglioramenti della tartuficoltura sono sorti nuovi problemi alcuni dei quali ancora irrisolti.

Un problema che riteniamo sia risolto è rappresentato dalla sostituzione delle micorrize di un tartufo con quelle di altri funghi o altri tartufi che si verifica in alcune tartufaie coltivate. Ciò accade quando l'ambiente di coltivazione non è perfettamente idoneo al tartufo che si intende coltivare. Va chiarito, comunque, che questo fenomeno si verifica più frequentemente nei terreni dove in precedenza erano presenti piante provviste di ectomicorrize e, di conseguenza, dove sono presenti propaguli di funghi che possono diventare competitori del tartufo coltivato. Il problema si risolve valutando correttamente l'ambiente di coltivazione ed utilizzando solo terreni seminativi.

Un problema preoccupante è rappresentato dall'esaurimento precoce dell'attività produttiva di alcune tartufaie: le piantagioni entrano in produzione, forniscono produzioni soddisfacenti per qualche anno e poi si esauriscono. Il problema dovrà essere affrontato in maniera razionale valutando la diminuzione o l'accumulo di alcuni elementi nel suolo indispensabili alla fruttificazione del tartufo.

Altri problemi derivano dai cambiamenti ambientali: la forte siccità e le alte temperature registrate nelle ultime stagioni hanno causato la perdita parziale o totale della produzione, soprattutto dei tartufi neri. Questi fenomeni atmosferici hanno determinato anche il danneggiamento delle micorrize nelle radici superficiali, probabilmente con grave danno per le produzioni future. In seguito a tali fenomeni e/o all'inquinamento ambientale, nell'Italia centrale, si osserva una costante e progressiva moria delle tartufaie naturali. Si tratta di problemi gravi che possono essere parzialmente risolti con l'adozione di alcune tecniche agronomiche come la pacciamatura e l'irrigazione.

Un altro problema deriva dalla carenza di conoscenze sulle tecniche colturali da adottare alle tartufaie durante il periodo produttivo. Dalle poche esperienze condotte si deduce che le tecniche di coltivazione non possono essere generalizzate, ma devono essere studiate caso per caso: un intervento agronomico favorevole in un determinato ambiente può risultare indifferente o nocivo in un altro. Il tartuficoltore, pertanto, non ha modelli di coltivazione a cui fare riferimento.

Esiste, infine, un problema causato dai tartuficoltori che tengono nascosti i risultati positivi al contrario di quelli negativi. Ciò favorisce la credenza secondo la quale la tartuficoltura è fallimentare: credenza diffusa soprattutto nelle sedi preposte allo sviluppo del territorio; ciò frena:

- l'erogazione di contributi a favore dei tartuficoltori;
- l'erogazione di fondi per la ricerca di settore;
- l'emanazione di leggi che incentivano la tartuficoltura.

Conclusioni

Vista la crescente richiesta di coltivazione dei tartufi da parte degli agricoltori, è necessario il contributo di tutti per rendere più certe e più produttive le piantagioni tartufigene. Un compito importante è affidato ai ricercatori che devono avviare una serie di studi, coordinati a livello internazionale, per affrontare e risolvere i problemi che affliggono la tartuficoltura. Dovranno essere approfondite le ricerche di base e soprattutto quelle che tendono a svelare il ciclo biologico delle specie pregiate di tartufo ed i periodi in cui si completano le diverse fasi; vanno incentivate, inoltre, le ricerche applicate che devono individuare le tecniche colturali capaci di

far conservare e diffondere la micorrizazione iniziale delle piante simbionti e promuovere le condizioni per la formazione degli ascocarpi, così come sosteneva Ceruti (1990) venti anni fa: in questo settore i progressi compiuti nell'ultimo ventennio sono limitati.
E' necessario, infine, che vengano resi noti i risultati produttivi delle tartufaie coltivate per favorire lo sviluppo della tartuficoltura, che si ritiene molto importante soprattutto per lo sviluppo socio-economico delle aree interne.
Si spera che i ricercatori che hanno consentito lo sviluppo della tartuficoltura (Fontana, Chevalier, Palenzona, Granetti) e che oggi ringraziamo ufficialmente, vengano sostituiti da giovani che possano proseguire, con analogo entusiasmo, le ricerche applicate alla tartuficoltura ed essere di supporto ai tartuficoltori e agli Enti preposti allo sviluppo del territorio.

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Discussione

Moderatore - Ritengo di aver introdotto molti temi di discussione e mi aspetto un vivace dibattito dal quale possano emergere esperienze e problemi di tartuficoltura importanti per orientare le ricerche future.

Alfredo Brofferio - Sono un imprenditore agricolo che da 2-3 anni si dedica alla tartuficoltura. Innanzitutto il Congresso, per me che vengo dal settore merceologico, mi ha dato fiducia su quello che come imprenditore volevo intraprendere mettendo la mia azienda a disposizione della ricerca. Sono d'accordo con quanto è stato detto dal moderatore perché in qualsiasi settore, se non esiste la ricerca di base e la ricerca applicata, non esiste la produzione e non esiste il mercato. Noi rischiamo di perdere questo prezioso prodotto se non investiamo nella ricerca. Devo aggiungere che c'è un problema di educazione, in altre parole nella mia attività, al di là degli aspetti climatici, il maggiore nemico è l'uomo nelle sue diverse sfaccettature. Non vorrei parlare da politico, ma le leggi devono essere fatte con il supporto della ricerca: prima di avventurarsi in campi che non conoscono, è bene che i politici e i legislatori abbiano un attimo di umiltà, consultino il settore scientifico e considerino il tartufo non solo un elemento per migliorare la visibilità di una zona, ma come una risorsa economica. Altro aspetto è che i cavatori sono troppi in un territorio limitato. Andrebbe fatto uno studio per valutare la sostenibilità del numero dei cavatori che può sopportare un comprensorio. Nel mio territorio si parla di 2000 cavatori registrati, cioè provvisti di tesserino che autorizza la raccolta, e di oltre un migliaio non registrati; ciò crea solo un danno perché sulla stessa cava passano più cavatori al giorno con il risultato che distruggono tutto quello che c'è. Sono confortato dall'entusiasmo dei giovani ricercatori che hanno parlato e pertanto continuerò testardamente in questa attività di tartuficoltore.

Pierre Sourzat - Nel prendere la parola presenta una slide dove si leggono quattro punti:
- La gestione della pressione di contaminazione dei funghi in ecologia forestale (*Tuber brumale*).
- L'impatto su *Tuber melanosporum* delle precedenti colture con utilizzazione di prodotti chimici (pesticidi e fertilizzanti) e la risposta del suolo.
- Le pratiche attuali per migliorare la biodiversità utile per la nutrizione del tartufo (fauna, flora, virulenza).
- L'aumento dell'inoculo di tartufo con apporto di spore.

La prima domanda per me è quella di limitare la pressione della contaminazione causata dai funghi estranei come *Tuber brumale,* che preferisce un ambiente boschivo. Noi in Francia abbiamo molti problemi e lo stesso accade in altri paesi. Io penso che se noi capiamo questi problemi, possiamo affrontare e risolvere le difficoltà delle piantagioni tartufigene.
Uno dei problemi riguarda l'impatto delle colture precedenti l'impianto delle tartufaie sulla produzione di *Tuber melanosporum*. Si possono notare molti problemi negli impianti realizzati su terreni seminativi in cui erano stati usati fertlizzanti e pesticidi. Il problema principale sono i trattamenti che modificano la biodiversità del suolo, migliorano la flora e la fauna, ma causano una violenza nei confronti del suolo che può aiutare a nutrire il tartufo. Per me è importante migliorare il quantitativo di inoculo nel suolo con le spore riducendo la contaminazione all'inizio e alla fine del ciclo. Credo di aver esposto il mio problema e potete ora discutere di questo perché la pressione della contaminazione è un problema fondamentale.

Federico Paci - Sono un naturalista e la mia estrazione mi permette di considerare la natura nel suo complesso. Da questo congresso non ho sentito parlare di piante comari che in alcuni testi ho letto sono importanti per favorire la produzione dei tartufi, volevo sapere se l'introduzione di altre specie arboree o erbacee è una pratica che viene fatta o è stata abbandonata.

Pierre Sourzat - Farò semplicemente un cenno, se lo permettete, alla biodiversità della flora e

della fauna. Abbiamo queste famose piante comari che partecipano alla biodiversità e citerò un esempio concreto: nel 2003 in Francia abbiamo avuto la siccità e alte temperature e, malgrado questo clima sfavorevole conosciamo delle piantagioni dove la produzione è di 50 chili in confronto ai 70 chili degli anni precedenti.
La produzione si è concentrata particolarmente sull'esperienza delle piante di lavanda messe a dimora in associazione con le piante tartufigene; in questo particolare caso il tartufo ha aumentato la sua resistenza alla siccità.

Virgilio Vezzola - Parlerò di un'esperienza che ho fatto nei pressi del Lago di Garda. Con la locale Comunità montana abbiamo realizzato impianti partendo dagli anni 90 su terreno vocato per il tartufo nero pregiato, utilizzando piante micorrizate da *Tuber melanosporum*: abbiamo avuto buoni risultati, in alcuni casi ottimi con il 100% delle piante in produzione. Essendo il Lago di Garda una zona turistica, ci siamo resi conto che era interessante avere tartufi durante l'estate; abbiamo fatto piantagioni con il tartufo estivo. Ora cominciamo a verificare i risultati delle piantagioni fatte nel '90-'95; in particolare un impianto ha cominciato a produrre l'estivo dopo 5-6 anni, all'ottavo anno ha prodotto 8 Kg, al nono ne ha prodotti 17, quest'anno 30. Si tratta di una piantagione mista di carpino, nocciolo e roverella; in totale sono 120 piante impiantate con sesto abbastanza stretto perché l'estivo vive in ambiente abbastanza ombreggiato. Delle tre specie la roverella è entrata in produzione un po' più tardi. Abbiamo fatto altri impianti a 1000 metri di quota e a 300 metri di altitudine con un tartufo raccolto a 100 metri e poi abbiamo fatto piantagioni alle stesse quote con un tartufo raccolto a 1000 metri di quota. È venuto fuori che quello raccolto a 100 metri di quota ha prodotto in tutte e due le altitudini, mentre quello raccolto a 1000 metri non ha mai prodotto a quota bassa. Porto anche un'altra esperienza: una piantagione di tartufo estivo di circa un ettaro, dopo 8 anni ancora non aveva prodotto, al nono anno ha prodotto solo qualche carpino nero; visitando la tartufaia ci siamo resi conto che l'impianto era stato fatto mettendo a dimora le piante troppo in profondità, l'apparato radicale era molto profondo e questo probabilmente ha ritardato la produzione.
Piantagioni realizzate utilizzando solo il nocciolo micorrizato, sempre con tartufi autoctoni della stessa altitudine, hanno fornito risultati ottimi con il 100% delle piante in produzione. Vorrei considerare una cosa, che quando si parla di piante micorrizate con il tartufo estivo bisogna stare attenti a utilizzare tartufi raccolti in loco e alla stessa altitudine.

Jean Claude Pargney - Credo fermamente nello schema presentato da Sourzat relativo alla biodiversità da realizzare nelle tartufaie, perché favorisce lo sviluppo della flora e della fauna che sono molto importanti per la nutrizione del tartufo. Credo che le piante introdotte nell'ecosistema tartufigeno possano contribuire allo sviluppo del sistema radicale e a fornire zone di rifugio per la fauna. Si creano, inoltre, zone d'ombra più o meno umide, favorevoli alla conservazione dell'acqua nel suolo che contribuiscono a soddisfare il fabbisogno idrico delle piante ed inoltre non va sottovalutato l'apporto di sostanza organica e di conseguenza di humus, che rientra nella modificazione del suolo, localmente dove si sviluppa il tartufo.

Marcello Raglione - Andando a rilevare i terreni nelle tartufaie naturali, presenti nel nostro territorio, ho avuto l'occasione di fare una serie di osservazioni. Innanzitutto, in riferimento al grosso calo delle produzioni che si sono verificate in Francia e in Italia, al contrario di quello che dicono alcuni, il numero dei raccoglitori di tartufi va aumentando continuamente, l'anno scorso per esempio la Provincia di Rieti ha fatto cinque sessioni di esami per la concessione dei tesserini: per ogni sessione c'erano 20 nuovi aspiranti per cui sono stati concessi 100 nuovi tesserini in una provincia di 150000 abitanti. Questo si ripercuote con una eccessiva frequentazione del territorio, tanto è vero che le cave spesso sono lavorate fino in profondità, addirittura sembra che ci siano passati dei trattori con degli aratri che hanno rivoltato il suolo; qualcuno potrà dire che la lavorazione è indispensabile, però assicuro che in questi casi vengono esposte all'aria anche grosse radici. Altro problema è l'attività dei selvatici come i cinghiali.
Altra osservazione è che le tartufaie nuove, in particolare di estivo e nero, le troviamo tutte su

quello che viene chiamato il bosco "secondario", cioè nei pressi delle piante isolate che nascono nei terreni abbandonati e una volta coltivati a uliveto, frutteto o vigneto; successivamente, man mano che il bosco avanza, non più regolato dalla presenza dell'uomo, aumenta l'ombreggiamento e le tartufaie si esauriscono. Ciò è dimostrato dal fatto che entrando nel bosco si ha la percezione che c'erano vecchie tartufaie con pianelli ancora evidenti ma più o meno inerbiti e improduttivi. Perciò lo spopolamento delle campagne, la riduzione del pascolo che conteneva lo sviluppo degli arbusti, la raccolta eccessiva e indiscriminata e irrazionale nonché lo scarso controllo ha provocato la riduzione della produzione. Quindi, l'abbandono dei terreni ha due aspetti contrastanti, uno positivo che comporta l'aumento delle tartufaie naturali e dall'altro, mancando il controllo della vegetazione, si verifica l'infoltimento con la conseguente riduzione delle tartufaie. Pertanto le nostre tartufaie naturali devono diventare tartufaie controllate in modo che ci sia una raccolta equilibrata, cioè affidata a gruppi di persone che mantengono inalterato questo patrimonio.

Moderatore - Grazie Marcello, questa mattina ho ascoltato una comunicazione di un ricercatore spagnolo che faceva vedere il diradamento del bosco per conservare l'habitat naturale del tartufo. Queste cose in Italia diventano difficili perché le norme forestali spesso sono in contrasto con gli interventi da praticare sulle tartufaie.

Marcello Raglione - Ciò che hai detto mi ha fatto ricordare una cosa che prima ho dimenticato. Le tartufaie dovrebbero essere censite, perché un altro modo per difenderle è quello di sapere dove sono, in modo da programmare una gestione corretta. Per esempio noi abbiamo trovato una tartufaia di circa tre ettari di *Tuber magnatum* che ogni anno produceva decine di chilogrammi di tartufi e pertanto decine di migliaia di euro, ebbene l'anno scorso è stata ceduata; vorrei sapere come poche centinaia di euro provenienti dal legname possano compensare la perdita di decine di migliaia di euro fornite dal tartufo. Questo succede anche nei boschi dove sono presenti *Tuber aestivum* e *T. melanosporum*, il censimento dovrebbe garantire un taglio del bosco mirato alla conservazione del tartufo.

Leonardo Baciarelli Falini - Vorrei portare la mia esperienza di tecnico che ha seguito molte tartufaie. Vorrei rispondere a Vezzola che ha riferito come la roverella nelle tartufaie di *Tuber estivum* abbia prodotto più tardi rispetto al carpino nero. La mia esperienza è diversa, si dice che i noccioli e i carpini producono prima delle querce, però sia nelle tartufaie di *Tuber melanosporum* che in quelle di *Tuber aestivum*, i lecci e le roverelle quasi sempre hanno prodotto prima dei carpini e dei noccioli, i quali hanno formato pianelli ben evidenti, ma con produzione limitata e spesso tardiva.

Moderatore - Queste esperienze dimostrano come in ambienti diversi le piante simbionti si comportino in maniera differente e gli interventi colturali non hanno lo stesso effetto.

Ian Hall - Io penso che la Nuova Zelanda e l'Australia hanno qualche cosa da offrire. Quando noi abbiamo introdotto *Tuber melanosporum* e *Tuber borchii* nei nostri paesi noi non abbiamo importato insetti come collemboli e qualsiasi altra cosa che convive con il tartufo nei vostri paesi. Noi abbiamo un'enorme possibilità di fare sperimentazione nei nostri paesi perché non abbiamo quegli insetti (collemboli) o i funghi che invece sono presenti nel vostro suolo, se questi insetti fossero stati importati non avremmo avuto produzione, ma noi abbiamo avuto produzioni di tartufo. Per cui le cose suggerite in questa sede, per noi non sono rilevanti.

Moderatore - Hall ci dovrebbe riferire come ottiene buone produzioni di *Tuber melanosporum* su terreno sub-acido.

Ian Hall - Alcuni dei suoli che noi utilizziamo sono totalmente inadatti, sono troppo umidi, il sottobosco, la porosità, tutto è diverso dai vostri, però noi otteniamo enormi quantitativi di

tartufi. Ciò è incredibile. Io penso che ci sia un grosso potenziale nelle nostre piantagioni in Nuova Zelanda e credo che se avessimo i finanziamenti potremmo andare a vedere cosa sta succedendo comparando con ciò che si ha in Europa. Credo che studiare le piantagioni in questi nuovi paesi sia molto utile, perché si possono controllare meglio vari fattori.

Moreno Moraldi - Sono il responsabile dell'UmbraFlor, un vivaio che produce piante tartufigene: non sono qui per pubblicizzare il vivaio, ma per portare un'esperienza di coltivazione. Nel 1990 una partita di piante di farnia (*Quercus robur*), micorrizate da *Tuber aestivum*, essendo rimasta invenduta è stata utilizzata in azienda per fare un rimboschimento su un terreno di 7000 mq, perfettamente in pianura, molto argilloso (49% di argilla il resto limo) assolutamente privo di scheletro. Le piante furono messe a dimora molto fitte (m 2,5 x 2,0) perché c'era la volontà di utilizzarle successivamente per le alberature stradali. Ora le piante sono alte 8-10 metri, alcune dominanti, altre dominate e si è formato un sottobosco di biancospino, sanguinello e altre specie. Passeggiando nella piantagione vedevamo tutto privo di erba come se fossero pianelli uniti l'uno all'altro: credevamo fosse l'ombra operata dalle piante. L'anno scorso, per la prima volta sono stati trovati qualche decina di chilogrammi di tartufo estivo, quest'anno è stato trovato un centinaio di Kg di tartufo. Vorrei avere una spiegazione di questi risultati perché se un cliente mi viene a chiedere di fare una tartufaia su un terreno analogo e con la stessa fittezza, io lo sconsiglio. Ai miei clienti, seguendo quanto si trova in bibliografia, fornisco indicazioni completamente diverse.

Moderatore - Noi siamo abituati a impiantare le tartufaie nei terreni peggiori, non idonei per le ordinarie colture agrarie. Questo è un errore causato dal fatto che seguiamo quanto osserviamo oggi in natura; le tartufaie naturali le troviamo negli ambienti impervi, dove in passato non è stato possibile disboscare per ottenere terreni da coltivare. Abbiamo studiato l'ecologia del tartufo nelle zone di rifugio e non è detto che i migliori terreni agrari non siano anche i migliori terreni tartufigeni.

Guy Rethore - Sono un giovane tartuficoltore di Lot-et-Garonne Francia; nel 2000 ho effettuato una piantagione su un terreno calcareo con caratteri ritenuti favorevoli al tartufo nero; sono state messe a dimora, in un ettaro, 300 piante micorrizate di roverella e di leccio in parti uguali. Durante i primi tre primi anni sono state fatte sarchiature del suolo e in seguito è stato seminato un miscuglio di graminacea, nella dose di 1,5 kg/ha. Dopo la semina abbiamo constatato che intorno alle piante non c'era germinazione e quindi la zona era priva di erba. Quest'anno per la prima volta ho raccolto i primi tartufi sia sotto le roverelle che sotto i lecci. Alcuni sostengono che il leccio sia più precoce della roverella: non è il caso della mia piantagione.

Gian Luigi Gregori - Sono il responsabile del Centro Sperimentale per la Tartuficoltura di Sant'Angelo in Vado. Prima una considerazione e poi due testimonianze. Mi sembra che la filosofia di approccio al problema dall'ottantotto a oggi sia cambiata; si è messo in evidenza, a diversi livelli e con diverse competenze, che bisogna agire considerando la coltivazione un sistema integrato, un sistema nel quale tutte le componenti giocano un ruolo importante. Come diceva Bragato, dobbiamo scegliere prima il modello sperimentale e le modalità con cui vogliamo rappresentare i dati per la loro elaborazione, così nella coltivazione dobbiamo scegliere prima quale è il sistema con cui vogliamo arrivare alla produzione quindi quale è l'itinerario da seguire e su questo non mi dilungo.
Come testimonianza vorrei portare, a proposito della capacità di resistenza vegetativa del micelio di *Tuber melanosporum,* un'esperienza su una tartufaia impiantata con sesto di 5x5m su terreno idoneo, sottolineo che l'ambiente dal punto di vista climatico e pedologico è fortemente vocato al tartufo nero pregiato. Le piante dopo la loro messa a dimora sono state completamente abbandonate perché il proprietario è dovuto emigrare. Un nuovo proprietario, a distanza di circa 10 anni, sapendo che era una vecchia tartufaia ha ritenuto opportuno fare una ripulitura: è entrato dentro con una macchina decespugliatrice, ha tolto tutti gli arbusti e

con sorpresa, nell'arco di tre anni, sotto le piante che erano rimaste vive (solo 180 su 300) hanno iniziato a manifestarsi i pianelli. Il proprietario ha iniziato a sarchiare i pianelli per favorire la loro formazione; quando il pianello si era ben affermato non lo ha più sarchiato limitando questo intervento alla sola cerchia di espansione. Bene, nell'arco di tre quattro anni da questi interventi, la tartufaia è entrata in produzione a testimoniare il fatto della potenzialità vegetativa del micelio che è rimasto in vita nelle condizioni in cui non poteva fruttificare. Il proprietario non ha fatto altro che creare uno spazio aperto e il tartufo ha cominciato a fruttificare.
La seconda esperienza che vorrei portare è a proposito del tartufo bianco pregiato di cui nel 1988 se ne è parlato molto, mentre a Aix-en-Provence di meno e purtroppo oggi siamo a parlare di ecologia piuttosto che dei meccanismi con cui si forma il corpo fruttifero, di cosa ha bisogno, in altre parole di come si coltiva. Chiaramente i risultati produttivi che riferisco nella mia esperienza sono inferiori rispetto al nero pregiato, ma va sottolineata la significatività del raggiungimento di questi risultati: mi sembra come se una strada sia tracciata, dobbiamo capire come avvengono le singole tappe però la strada è tracciata. Preciso che si tratta di una ventina di piantagioni del Centro Italia, però riferisco solo di 5-6 che sono veramente interessanti. Va precisato che nei luoghi dove queste piante sono state messe a dimora, spontaneamente e a memoria d'uomo, non era presente la produzione spontanea di *Tuber magnatum*. Si tratta di piante ottenute con le tecniche di micorrizazione usate per il nero a partire da corpi fruttiferi selezionati. Dopo la messa a dimora delle piante ci si aspettava, nel giro di 6-7 anni, di avere i tartufi; non avendo ottenuto produzioni le piantagioni sono state abbandonate o è stata tagliata solo l'erba. Tutte queste tartufaie, o almeno quelle che perdurano, sono passate da una fase di produzione di tartufi strani appartenenti alla grande famiglia del *Tuber borchii,* però a partire da una certa data è iniziata la riduzione fino all'esaurimento della produzione di *T. borchii* e hanno cominciato a produrre e tuttora seguitano a produrre, nelle quantità che ha indicato prima il prof. Bencivenga, carpofori di *T. magnatum.* Questo risultato mi sembra molto interessante e auspico che si ritorni a studiare il tartufo bianco e a riprendere le ricerche sulla micorrizazione, perché non è sufficiente una pianta prodotta in laboratorio, ma una serie di piante per eseguire nuove sperimentazioni e capirne un po' di più.

Giovanni Pacioni - Durante questi giorni noi abbiamo visto molte relazioni che parlavano di terreni vocati per il tartufo nero e abbiamo visto tutti terreni ricchi di scheletro, però da una decina di anni a questa parte, a seguito della messa a riposo dei campi, dell'agricoltura biologica, della dismissione di aziende, ecc. sono state realizzate una serie di tartufaie su terreni seminativi di collina dove c'erano vigneti, dove addirittura c'erano impianti di irrigazione perché si coltivavano ortaggi; questi interventi hanno riguardato anche grosse estensioni in Friuli, in Veneto, in Romagna, in Abruzzo, nel Lazio, anche su terreni molto argillosi e producono da anni tartufi di grosse dimensioni.
Probabilmente, come ha accennato poco fa Bencivenga, abbiamo mal interpretata l'ecologia del tartufo; noi abbiamo considerato l'ecologia del tartufo nero quella residuale dei terreni marginali, perché lì sono stati spinti dall'uomo che ha operato per ottenere terreni da coltivare; il tartufo cresce bene anche negli ottimi terreni agricoli, ovviamente bisogna stare molto attenti soprattutto con l'irrigazione, perché se i tartufi vengono riforniti di acqua, anticipano enormemente la maturazione. Perciò i terreni che non abbiamo mai considerato tartufigeni sono in realtà altamente produttivi.

Moderatore - Questa è una considerazione estremamente valida.

Gabriella Di Massimo - Sono un agronomo e come tecnico seguo numerose piantagioni di tartufo nero e conosco abbastanza quello che sta succedendo in natura. *Tuber aestivum* la considero una specie in espansione perché sta colonizzando i terreni abbandonati, non mi risulta la stessa cosa per *Tuber melanosporum*. Ho paura che le tartufaie realizzate in ambienti che probabilmente non sono quelli normali, naturali, siano piantagioni in cui la produzione sia destinata a terminare presto. Mi piacerebbe sapere se qualcuno ha esperienze in merito. Nei

riguardi delle piante micorrizate noi stiamo puntando molto sulla scelta di piante realizzate con tartufi locali e piante autoctone; è una scelta non basata su criteri scientifici però l'esperienza insegna che piante ben micorrizate con tartufo del luogo e seme del luogo permettono di abbassare di molto il periodo improduttivo: da circa 10 anni che era il periodo improduttivo in passato, si è ridotto a 4-5 anni. Anche su questo argomento vorrei sapere se qualcuno ha esperienze in merito. Venendo al tartufo bianco credo che dobbiamo studiare molto prima di arrivare alla sua produzione; vanno ripresi gli studi di ecologia che erano stati abbandonati perché se non salvaguardiamo le tartufaie naturali e aspettiamo che la ricerca ci consenta di coltivare questa specie corriamo il rischio, almeno in Italia centrale che conosco meglio, di rimanere senza prodotto.

Virgilio Vezzola - mi riferisco a quello che ha detto Gabriella adesso. Nella mia zona un agricoltore ha preso un centinaio di piante francesi e le ha piantate nel suo terreno altamente vocato al tartufo nero. Cinque anni dopo un programma della Comunità Montana consentiva di produrre e fornire agli agricoltori piante al 50% del costo. L'agricoltore, visto che aveva ancora del terreno libero ha deciso di piantare ancora e abbiamo fornito un po' di piante prodotte in loco con materiale autoctono. Dopo dodici anni le piante francesi che erano ancora perfettamente micorrizate con *T. melanosporum* non avevano ancora iniziato a produrre mentre le piante che avevamo prodotto noi con seme autoctono hanno cominciato a produrre già dopo 4 anni, altre dopo 5 e al settimo anno producevano quasi tutte. Abbiamo poi raccolto i fioroni (tartufi maturati in anticipo) sotto piante in produzione autoctone e abbiamo fatto inseminazioni sulle piante francesi; dopo due anni dalla inseminazione hanno iniziato a produrre anche quelle francesi.

Alberto Cretoni - Sono uno che sta cercando di fare tartuficoltura. La mia esperienza è questa: nel 90 ho messo a dimora delle piante tartufigene in due appezzamenti abbastanza diversi, uno fortemente vocato al tartufo nero e uno meno; il primo ha subìto, l'anno successivo alla piantagione, forti traumi a seguito di una grandinata, le piante erano state quasi completamente distrutte per cui non è stato coltivato in maniera razionale. Questa piantagione non ha fornito grandi risultati anche se un monitoraggio recente ha dimostrato che ancora sono presenti le micorrize del tartufo per cui c'è ancora da sperare. L'altro impianto è stato realizzato con 300 piante, delle quali 260 di nero e 40 di estivo che sono state messe a dimora, sbagliando, in mezzo alle piante micorrizate con il nero. All'inizio le piante sono state irrigate per favorire la loro sopravvivenza e ho avuto un attecchimento del 99%, è stata fatta la potatura per eliminare i rametti dal livello del terreno fino a un metro d'altezza, sono state fatte alcune sarchiature e da 3-4 anni non abbiamo fatto più nulla se non una pacciamatura con tessuto non tessuto per combattere la forte siccità. Questa tartufaia pur essendo in un ambiente meno vocato ha iniziato a dare buoni risultati anche con pezzi di oltre 300 g e quindi più che soddisfacenti.
In mezzo, dove erano state messe a dimora piante micorrizate dal tartufo estivo, è iniziata la produzione e, quest'anno che è stata una stagione abbastanza piovosa in primavera, abbiamo ottenuto risultati apprezzabili infatti sono stati raccolti 15 kg di tartufi in meno di 40 piante. L'acqua perciò è molto importante. E' opportuno che vengano approfondite le tecniche colturali come l'irrigazione, perché è di questo che abbiamo bisogno; la buona volontà è tanta, i sacrifici sono tanti, però non ci abbandonate perché altrimenti non riusciamo a fare nulla. Se si dispone di una buona pianta, di un terreno vocato e di un tecnico con suggerimenti giusti oltre a buone stagioni, molto probabilmente il tartufo continueremo a mangiarlo.

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Moretti - Vengo da Umbertide, la mia famiglia ha una lunga tradizione di raccoglitori di tartufo bianco. L'esperienza di 130 anni di cercatori di tartufi è questa: stiamo distruggendo le tartufaie con l'abbattimento selvaggio delle piante. Vengono abbattute piante come il salicone il cui legno ha uno scarso valore, così come le piante malate che sono quelle che producono il tartufo. Altro problema è il numero dei raccoglitori: a Città di Castello c'erano 60 cavatori di tartufi, oggi, tra Umbertide e Città di Castello che distano circa 20 km, ci sono la bellezza di 4200 cavatori di tartufi e le zone di raccolta sono sempre più ristrette. Ora siamo repressi in tutto e per tutto, perché ovunque troviamo tabelle di divieto di transito, divieto di raccolta funghi e tartufi; sembra che i proprietari terrieri che non sapevano che nella loro terra c'era il tartufo, siano diventati i più grandi tartufai. Il 70% del territorio, tra bandite, oasi di protezione, fondi chiusi, divieto di caccia, ecc. è chiuso: questo è vergognoso. E' ora che la Regione si decida a liberalizzare la raccolta.

Gilberto Bragato - Io non ho esperienze di tartufaio o di tartuficoltore. Riferendomi a questo Congresso voglio sottolineare la partecipazione di una serie di ricercatori provenienti da paesi europei diversi da quelli tradizionali. Interessanti sono le esperienze di produzione, in questo momento solo naturale, di specie da noi considerate secondarie come del tartufo estivo e di *T. macrosporum*. Altre esperienze interessanti sono quelle relative al tartufo bianco, che si verificano soprattutto verso la sponda danubiana. Tutte queste esperienze esterne potrebbero essere utili per la programmazione e la pianificazione delle tartufaie non solo in quei paesi, ma soprattutto da noi, perché secondo me sono quelle situazioni naturali che noi abbiamo perso e che dovremmo studiare. Mi ha colpito molto l'intervento di Moreno Moraldi e l'ho collegato alle diapositive sulle tartufaie di estivo ungheresi, dove il tartufo vive in simbiosi con la farnia. Lo stesso discorso vale per l'esperienza svedese, dove nelle tartufaie c'è sempre la farnia. E' il caso di andare a indagare questi ambienti dove *T. aestivum* interagisce con la sostanza organica, perché la farnia produce una lettiera spessa e quindi tanta sostanza organica tale da arricchire i primi strati di suolo. Sarebbe quindi importante indagare su questo aspetto e soprattutto sull'attività biologica che ne consegue, che può essere utilizzata per fini produttivi nelle tartufaie che verranno impiantate o che sono in corso di produzione. Mi interessano molto le zone dove lo scorzone forma un pianello poco evidente, perché in questi casi c'è qualche altro fattore che favorisce la creazione di uno strato produttivo adeguato. Forse sarebbe il caso di cominciare anche a fare esperienze di questo tipo.

Moderatore - Porto anche io la mia piccolissima esperienza; vivo in un ambiente dove il terreno è privo di scheletro e la terra fine è costituita dal 45% di argilla, dal 40% di limo e dal 15% di sabbia. Su questo terreno nel 1990 ho messo a dimora piante micorrizate dal tartufo estivo, devo sottolineare che si tratta di un terreno lontano da piante arboree e da sempre coltivato con colture erbacee, pertanto privo di funghi simbionti concorrenti del tartufo. Alcune osservazioni radicali hanno evidenziato la presenza pressoché assoluta delle sole micorrize di tartufo e da qualche anno è iniziata la produzione di *Tuber aestivum*. I carpofori, però, crescono molto in superficie, spesso quasi affiorano, e per tale motivo spesso sono di qualità scadente.

Paul Thomas – Io ho una brevissima osservazione dall'Inghilterra. Noi abbiamo una piantagione di poco più di 3.000 alberi. Ha solo 2 anni, ma abbiamo avuto davvero un buon livello di formazione dei brulé. E' di *Tuber aestivum* e già si erano formati i brulé dopo solo 9-12 mesi.
Ma la cosa molto interessante è che il proprietario delle piantagioni che vive vicino, e conosce molto bene le tartufaie, ha notato che quando gli alberi sono stati piantati durante l'estate, sono apparsi un sacco di scarafaggi. Io non ho dati quantitativi su questo: è solo un'osservazione. Ma il sito era coperto di scarafaggi molto piccoli che andavano in giro per la piantagione e soprattutto sopra i brulé e alla base degli alberi. Ho pensato che forse è una osservazione interessante circa le attività degli insetti nelle piantagioni.

Silvano Fabrizi – La mia storia sul tartufo risale agli anni 90, quando per ragioni di lavoro, mi avvicinai al Centro Sperimentale per la tartuficoltura di Sant'Angelo in Vado e entrai in contatto con le prime nove piante che mi vennero regalate. Misi a dimora queste nove piante e successivamente, nel 1991, acquistai altre piante e iniziai questa coltivazione. Nei primi anni ho sarchiato continuamente le piante, le ho potate rifacendomi a quando c'erano le capre nella mia zona, quindi le ho trattate veramente male e poi le ho abbandonate. Al nono anno, per caso sono arrivato sulla tartufaia e i miei cani sono letteralmente impazziti era il 2 febbraio del 2000, e da quel giorno ho avuto una buona produzione, su trecento piante ho raccolto anche 40 Kg di tartufo. Quest'anno ho iniziato la raccolta molto presto perché già alla fine ottobre i tartufi erano maturi e li ho raccolti per non farli rovinare come l'anno passato. L'anno scorso, infatti, vidi bei tartufi verso la fine di ottobre, non li raccolsi nella speranza di poterli raccogliere quando si potevano commercializzare per legge. Quei tartufi io non li ho più ritrovati e per questo motivo quest'anno ho anticipato la raccolta.

Moderatore - Signor Silvano ha notato differenze tra la parte irrigata e quella asciutta? Anche nei riguardi del periodo di maturazione?

Silvano Fabrizi - Quest'anno ho avuto una pioggia il 27 di luglio, successivamente il 29 per cui non ho notato nulla. L'anno scorso a differenza dell'anno precedente che avevo raccolto 40 Kg di tartufi, ho avuto solo 8 Kg e solo nelle piante irrigate.

Moderatore - La tua esperienza è importante perché negli anni siccitosi se non si interviene con le irrigazioni non si ha produzione. L'irrigazione, però, può essere anche dannosa se eseguita in maniera irrazionale. Se c'è qualcuno in sala che può riferire esperienze sulle irrigazioni sarebbe bene se ci riferisce la sua esperienza: sarebbe utile sapere quale tipo di irrigazione adotta, se sotto o sopra chioma, su che tipo di terreno, ecc.

Tartuficoltore marchigiano - Vengo dalle Marche, nei pressi di Roccafluvione in provincia di Ascoli Piceno, una delle migliori zone italiane per la produzione del tartufo nero. Ho circa 10 ettari di tartufaie, ho iniziato a piantare le tartufaie nel 94 su un terreno agricolo e non su terreni marginali come è stata la regola negli anni passati. Alcuni dicevano che quel terreno non andava bene perché le colture precedenti venivano concimate e diserbate: prodotti che danneggiano micorrize. Ciò non è successo perché la tartufaia è riuscita benissimo. La produzione è stata buona soprattutto negli anni passati per la stagione favorevole: si sono avute piogge sufficienti. Hanno prodotto circa l'80% delle piante e qualcuna ha fornito 5-6 kg di tartufo, il terreno è ricco di limo, ma c'è anche il 30-40% di argilla e un po' di scheletro. L'argilla è necessaria perché riesce a mantenere un po' di acqua nel terreno. Piantagioni successive su terreni agricoli stanno fornendo buoni risultati anche se negli ultimi anni purtroppo il clima non ci assiste.

Moderatore - Questa esperienza, insieme ad altre che ho ascoltato in sede congressuale, ci suggerisce di scagionare il pericolo dei funghi concorrenti. I risultati migliori si ottengono nei terreni dove in precedenza non erano presenti piante legnose provviste di ectomicorrize, perché altrimenti il tartufo, presente nelle radici delle nostre piantine, deve superare la concorrenza dei funghi presenti nel suolo, spesso aggressivi, perché ben adattati alle condizioni ambientali del luogo. Ecco perché i risultati migliori si stanno ottenendo nei terreni agrari lontani da boschi e dove non c'era nessuna pianta con ectomicorrize. Credo che questo sia un punto importante da affermare in questo congresso. Oltre alle piante legnose, occorre fare attenzione ad alcune specie suffruticose come alcune *Cistaceae* (gen. *Helianthemum*, *Cistus*) che vivono in simbiosi con funghi ectomicorrizici e che comunemente troviamo nei terreni incolti. Purtroppo in Italia e soprattutto in Umbria, regione ricca di colline e montagne, le tartufaie vengono realizzate spesso nei pezzetti di terreno abbandonato e dove sicuramente si era sviluppata una vegetazione comprendente anche alcune *Cistaceae.*

Pierre Sourzat - Ho ascoltato il precedente coltivatore che ha impiantato una tartufaia su terreno dove era stato coltivato il mais e l'intervento del signore che ha detto che nell'ambiente forestale ha le contaminazioni da funghi concorrenti. Devo dire che la regola generale è di non piantare in un ambiente boscato, così come in ambienti dove sono stati utilizzati pesticidi che possono bloccare l'attività biologica del suolo: attività biologica favorevole a *T. melanosporum.* Tuttavia si possono trovare situazioni di ambienti boschivi dove la produzione di *T. melanosporum* domina sui funghi concorrenti e situazioni di piante micorrizate messe a dimora in terreni precedentemente coltivati a cereali che tardano ad entrare in produzione. In situazioni simili è possibile assistere ad una modificazione del sistema micorrizico verso il tartufo brumale o il tartufo estivo. Ciò vuol dire che ci sono ancora problemi per capire ciò che blocca l'ecosistema tartufigeno e ciò che non lo blocca. In altri termini è importante interessarsi a ciò che funziona come di ciò che non funziona e credo che studiare quello che non funziona potrà fornire ottimi elementi per migliorare la produzione tartufigena.

Moderatore - Grazie Pierre, sicuramente intervengono altri fattori che condizionano i risultati in ambienti diversi, come il bosco e il campo dove sono stati usati pesticidi. Vorrei far notare che sono state portate testimonianze di buona produzione su terreno con il 40-50% di argilla. Marcello Raglione ci ha spiegato che esistono diversi tipi di argilla che hanno comportamenti diversificati. Se Marcello può precisare questo punto è ben accetto.

Marcello Raglione - Per chi era presente alla relazione ha ascoltato che il 40% di argilla è ammissibile, però bisogna vedere che tipo di argilla è cioè se si tratta di argille espandibili oppure stabili. Poi c'è un'altra cosa da tenere presente: le analisi per rilevare il contenuto in argilla sono di tipo granulometrico per cui vengono comprese tutte le particelle che hanno le dimensioni inferiori a 2 micron. Ciò significa che nella classe tessiturale dell'argilla vengono compresi altri composti come i carbonati, perciò l'argilla reale può essere anche molta di meno. Approfitto dell'occasione per ritornare sul discorso che ha fatto il coltivatore prima di me rispetto ai diserbanti: porto un altro esempio, ad Acqualagna lungo un fosso c'era produzione di *Tuber magnatum* sia lungo la sponda destra che in quella sinistra; nella parte destra è continuato ad essere presente un prato mentre nella parte sinistra si è iniziato a coltivare il girasole, il mais ecc, a destra sono rimaste le tartufaie a sinistra no a causa dei diserbanti. In questo caso tutti gli anni venivano distribuiti i diserbanti che venivano trasportati dall'acqua fino alla riva dove era presente il micelio del tartufo che è stato danneggiato. Questa è una situazione diversa rispetto al campo dove venivano utilizzati i diserbanti perché questi hanno una vitalità di pochi giorni o di qualche settimana per cui quando sono state messe a dimora le piante è passato un tempo sufficiente a farli decomporre. Posso quindi tranquillizzare gli agricoltori che se un terreno è stato sottoposto negli anni precedenti a forti diserbi, quando si mettono a dimora le piante l'effetto è scaduto, anzi hanno liberato il campo da tutti quegli altri funghi che possono essere concorrenti con il tartufo.

Moderatore - È stato utile che hai ripetuto queste cose perché questa sera sono presenti molti tartuficoltori che non hanno ascoltato le relazioni tenute durante il Congresso.

Gérard Chevalier – Non sono un coltivatore però ho una grande esperienza maturata in 40 anni di osservazione. Riguardo le piante tartufigene posso affermare che ormai sono una certezza, non un'ipotesi. Attualmente le piante tartufigene sono prodotte partendo da seme, da ghiande, che sono eterogenee e pertanto si producono piante eterogenee che possono dare buoni risultati con produzioni fino al 100%. Quando si producono le piante micorrizate ci si accorge che non esiste nessuna pianta che resiste alla micorrizazione, tutte le piante si micorrizano però non tutte producono nello stesso tempo. La precocità di produzione in condizioni buone è di 3,5 anni dopo la piantagione. Abbiamo commesso un errore utilizzando il nocciolo in cui abbiamo riposto tanta speranza: si diceva che il nocciolo ha tante micorrize, può produrre in anticipo rispetto alle querce, ma ciò non si è verificato, perché il leccio può

fornire produzioni precoci come il nocciolo. Attualmente il leccio è la pianta tartufigena più largamente utilizzata in Francia, è un buon produttore e produce già dopo tre anni dalla sua messa a dimora. Importante è la selezione delle piante e sono d'accordo che bisogna utilizzare preferibilmente l'ecotipo locale; ritengo comunque che la clonazione consentirà una migliore selezione della pianta micorrizata, la produzione di piante molto più omogenee con risultati produttivi più regolari e un sistema radicale molto sviluppato e più adattato alla micorrizazione, anche perché non hanno il fittone e quindi hanno più radici superficiali.
Prove recenti di clonazione hanno dimostrato che i cloni sono migliori delle piante nate da seme solo in determinati ambienti. Al contrario di quanto sostengono alcuni vivaisti, non si può affermare che un clone è il migliore in assoluto, perché può fornire buone produzioni nelle sue condizioni ambientali ideali e un altro clone sarà migliore in altre condizioni. Si devono produrre cloni (coppie di pianta e tartufo) per ogni regione e questi saranno migliori delle coppie eterogenee ottenute da seme. Questo per quanto riguarda la pianta micorrizata.
Dal punto di vista del suolo adatto al tartufo in Auvergne, dove io lavoro, ci sono tante piantagioni in condizioni di suolo molto variabili e si è notato che le condizioni fisico-chimiche del suolo orientano la produzione dei tartufi: in alcuni casi si ha produzione di *T. melanosporum* e in altri casi di *T. brumale*. Ci si è resi conto che un fattore chiave è la sofficità del suolo, pertanto credo che possiamo ricostituire suoli artificiali quando si dispone di suoli non idonei. Si possono modificare i suoli apportando del calcare o elementi minerali quali la pozzolana per alleggerire i suoli e ricostituirli dal punto di vista fisico e chimico; abbiamo fatto alcune prove ma ancora non disponiamo di risultati.
In terzo luogo vorrei parlare degli impianti tartufigeni che esauriscono precocemente la loro produzione: effettivamente ci sono piantagioni che non durano molto. Si dice che la produzione si esaurisce perché gli elementi minerali del suolo sono consumati; ciò non è assolutamente vero perché la produzione dei tartufi non è a tonnellate come le colture agrarie che consumano molti elementi, quello che preleva il tartufo è veramente poco. Il tartufo nero consuma le radici, si nutre di radici, di tannini di legno, di polifenoli, ecc. per cui per avere dei tartufi bisogna avere in continuazione nuove radichette: quando il tartufo non ha più radichette scompare. Bisogna trovare dei sistemi per favorire in continuazione la formazione di giovani radici: come procurare giovani radici da mangiare? Due soluzioni, la prima è la potatura degli alberi, è una soluzione indiretta perché quando si potano gli alberi si favorisce indirettamente la rigenerazione del sistema radicale; la seconda soluzione è lavorare il suolo con attrezzi adatti, affinché si provochi la rottura di alcune radici favorendo la rigenerazione del sistema radicale.
Ritengo che in futuro le piantagioni tartufigene dovranno essere lavorate con attrezzi adattati alla tartuficoltura; attualmente ci sono macchine idonee allo scopo per cui il futuro non sarà quello della zappa, ma dei trattori leggeri e degli attrezzi leggeri che aerano il suolo e che rompono il sistema radicale rigenerando le radici: a mio parere è su questo che bisogna lavorare per il futuro.

Moderatore – In questo pomeriggio non si è parlato della distribuzione delle spore in campo per mantenere viva la micorrizazione e favorire la sopravvivenza delle tartufaie. Ho qualche esperienza in merito, infatti riportando spore di tartufo su tartufaie in produzione o in via di esaurimento mi ha consentito di ottenere un miglioramento della produzione e in qualche caso di recuperare una tartufaia esaurita. Questo si capisce benissimo, perché in passato i tartufai erano pochi, avevano cani poco bravi, pertanto non venivano raccolti tutti i tartufi e le tartufaie si conservavano a lungo. Oggi i tartufai sono tanti, i cani sono diventati professionisti, nel suolo non si lascia assolutamente nulla e ciò crea problemi per la sopravvivenza delle tartufaie.

Moreno Moraldi - Mi riallaccio a quanto detto da Chevalier sui cloni. Sono d'accordo con Gérard sulla possibilità di utilizzare cloni autoctoni delle singole zone dove si va ad operare, perché solo in quel caso si ha la possibilità di avere ottimi risultati. A questo proposito vorrei ricordare a tutti che i cloni non sono ammessi nell'utilizzo in bosco; c'è una direttiva della Comunità Europea che vieta di utilizzare piante clonate in bosco sicché stiamo attenti quando

parliamo di cloni, perché stiamo parlando di piante che non possono essere utilizzate in tartuficoltura che corrisponde ad una forestazione. Questo divieto si giustifica per il rischio di diffusione delle malattie, perché i cloni sono una serie di soggetti tutti perfettamente identici e qualsiasi patogeno potrebbe svilupparsi a dismisura.

Gabriella Di Massimo – Sto per dire una cosa che probabilmente non mi sarei mai sognata di dire "non sono d'accordo con Chevalier" per gli stessi motivi che ha illustrato Moreno Moraldi e soprattutto perché andiamo incontro a un periodo di variazioni climatiche per cui alle nostre piantagioni dobbiamo assicurare una certa variabilità genetica, che ci garantisce una maggiore resistenza. Credo molto nella validità del materiale autoctono e quindi abbiamo fatto la scelta di queste linee consorelle che stiamo realizzando con la Comunità Montana di Spoleto attraverso il progetto "Matriarca" che vi è stato illustrato ieri. La utilizzazione dei cloni, sia di pianta che di tartufo, la ritengo necessaria per creare campi di conservazione per garantirci una riserva di germoplasma per il futuro. Ciò perché le tartufaie naturali sono in continua rarefazione. Nei riguardi delle piante micorrizate vorrei precisare una cosa, ieri si parlava di certificazione ed è venuto fuori il discorso che non si sa ancora oggi quale è la perfetta pianta micorrizata e quante micorrize deve avere, questa è una affermazione molto pericolosa, le piante micorrizate devono essere abbondantemente micorrizate e fortunatamente i vivai italiani, quelli spagnoli e Francesi che conosco producono piante micorrizate di ottimo livello: è questo punto importante e non dobbiamo tornare indietro. Per quanto riguarda l'inoculo sporale in campo abbiamo effettivamente recuperato alla produzione alcune tartufaie che erano in declino produttivo, ma è un risultato che deve essere valutato nel tempo perché non sappiamo se effettivamente ha funzionato l'apporto delle spore o semplicemente la lavorazione che abbiamo fatto per interrarle. La parte difficile per noi che lavoriamo in campo è proprio la molteplicità di parametri di cui dobbiamo tener conto per valutare i risultati.

Moderatore - Grazie Gabriella, quando ti riferivi al mio discorso di ieri relativo a quante micorrize deve avere una pianta per essere giudicata valida ai fini della tartuficoltura, voglio precisare che effettivamente non lo sappiamo e il metodo utilizzato in Italia, che prevede un minimo di micorrizazione del 30%, è scaturito dalle osservazioni che un gruppo di ricercatori ha fatto sulle produzioni dei migliori vivai e precisamente sulle piante di un anno di età e non da prove sperimentali effettuate mettendo a dimora piante con diverso grado di micorrizazione. E' chiaro che più è alta la percentuale di micorrizazione migliore è la pianta e migliori saranno i risultati che potrà conseguire. Non vorrei che la mia affermazione di ieri sia interpretata in modo errato.

William Saenz - Voglio intervenire su due punti. Per quanto riguarda l'inoculo in campo con spore di tartufo mi viene in mente un esempio: nel Lot c'è una tartufaia naturale che produce da più di trenta anni, è esposta a nord e i raccoglitori cessavano la raccolta a metà gennaio perché al di là di questa data i tartufi erano di cattiva qualità. Dunque rimanevano molti tartufi sul terreno, quindi molto inoculo e forse per questa ragione la tartufaia è durata così a lungo.
Un altro punto di cui vorrei parlare è l'influenza della precedente coltura nella riuscita della tartufaia. Il territorio del Lot è oggi molto boscoso, ci sono tante tartufaie ormai improduttive e pochi terreni ancora coltivati. In questa situazione, negli ultimi anni, molte persone provano a impiantare nuove tartufaie nei terreni sottratti al bosco o nelle tartufaie esaurite e non ci sono cattivi risultati. Voglio precisare che queste piantagioni vengono realizzate nei terreni che, dopo disboscati, sono stati sottoposti ad una buona preparazione con strumenti che permettono di eliminare il massimo delle radici.

---------- Sono un tartuficoltore di base. Voglio intervenire per aggiungere qualche cosa nei confronti dei due interventi precedenti. Il primo riguarda la raccolta precoce; prima di venire qui ho venduto 10 chili di tartufi neri che erano di buona qualità e ho raccolto anche tre chili di tartufi putrefatti che non volevo rimanessero nel suolo. Ora ho capito che avrei dovuto lasciare

i tartufi avariati in loco. Quando rientrerò dal congresso mi aspetto di raccogliere una quantità equivalente a quanto raccolto prima di venire a Spoleto. Ho buoni risultati, ma non capisco come devo intervenire per migliorare o conservare la produzione.
Ho seguito il vostro discorso d'apertura quando dicevi che i ricercatori si dedicavano molto al lavoro di laboratorio e che la tartuficoltura ha bisogno di ricercatori che operano sul terreno. I tartuficoltori non hanno la capacità di fare analisi e di trarre le conclusioni per poter individuare un modello colturale idoneo, sarebbero felici di poter disporre di ricercatori che hanno le capacità di analisi e di sintesi. Spero che la nuova generazione di ricercatori possa andare sul terreno, ascoltare i tartuficoltori, andare a casa loro, vedere ciò che succede e studiare in loco. Ci sono molte tartufaie che funzionano per cui è interessante che si conoscano le produzioni e gli interventi colturali in modo che confrontando i differenti modi di coltivazione si possano sviluppare metodiche che garantiscano la riuscita produttiva della tartufaia.

Moderatore – Non essendoci altre richieste di intervento dichiaro chiusa la tavola rotonda che ha offerto ottimi spunti di cui faranno tesoro gli agricoltori e i ricercatori.

Round Table

Moderator - I think I have introduced many topics of discussion and I expect an intriguing debate from which will come out personal experiences and problems of truffle cultivation. This could be important to guide future research in this field.

Alfredo Brofferio – I am an agricultural entrepreneur, and I have devoted the last 2-3 years to truffle cultivation. I come from the merchandising sector, and the Conference has above all made me feel confident about the goals I, as an entrepreneur, wished to achieve, by putting my farm at the disposal of research. I agree with what was said by the moderator because, in any sector, if you don't have basic research and applied research, than you don't have production and you don't have a market. If we don't invest in research, we run the risk of losing this precious product. I must add that there's a problem here, too, of education; by which I mean, in my line of work, apart from the climatic aspects, the number one enemy is man himself in his various facets. I don't want to speak as a politician, but laws have to be made on the basis of the findings of research, rather than going forwards with no knowledge of the subject; politicians and legislators should have a touch of humility, consult the scientific sector, and think of the truffle not just as element which can increased an area's visibility, but as an economic resource. Another problem is that there are too many truffle-hunters in a territory that is limited. A study should be made to evaluate the number of truffle-hunters that a given area can tolerate. In my area, we know that there are 2000 registered truffle-hunters, i.e, who have a license that authorizes them to gather truffles, and more than a thousand who aren't registered. This does nothing but harm – numerous hunters go through the same truffle bed every day with the result that they destroy everything that's there. I am comforted by the enthusiasm of the young truffle hunters who have spoken today, and as a result, I intend to persist, stubbornly, in my work as a truffle-farmer.

Pierre Sourzat - As he starts his talk he presents a slide on which can be read four points:
- The management of the pressure of contamination on mushrooms in ecological forests *(Tuber brumale)*.
- The impact on *Tuber melanosporum* of preceding cultivations which used chemical products (pesticides and fertilizers) and the reaction of the soil.
- Current practices for the improvement of biodiversity useful for the nutrition of the truffle (fauna, flora, virulence).
- Increase in the inoculation of truffles by the use of spores.

The most important issue is, in my view, that of containing the pressure of contamination caused by extraneous mushrooms like *Tuber brumale*, which has a preference for woody environments. In France, we have a lot of problems with this, and the same holds true for other countries. I think that if we understand these problems, we will be able to deal with and resolve the difficulties faced by truffle plant plantations.
One of the problems involves the impact of cultivations which preceded the establishment of the truffle orchards on the production of *Tuber melanosporum.* We can observe many problems in establishments set up on arable lands in which fertilizers and pesticides have been used. The main problem is that treatments which modify the soil's biodiversity improve flora and fauna, but wreak havoc on soil that could help to nourish truffles. In my view, it's important to improve the quantity of inoculation in the soil with spores, thereby reducing the contamination both at the beginning and at the end of the cycle. I think I have described my problem and now you can discuss it, because the pressure of contamination is a fundamental problem.

Federico Paci: I am a naturalist and my training makes it possible for me to consider nature in its entirety. I haven't heard anyone speak at this Conference about "godmother" plants which I have read in various texts are important for encouraging the production of truffles. I wanted

to know whether the introduction of other species of trees or grasses is something that is still done, or whether this practice has been abandoned.

Pierre Sourzat - I will simply allude, if I may, to the biodiversity of flora and fauna. We have these famous godmother plants **t**hat contribute to biodiversity and I will give you a concrete example: in 2003, in France, we had a drought and a heat spell, and despite these unfavourable climatic conditions, we know of plantations where production was nonetheless still at 50 kilos, as opposed to the 70 kilos of previous years.
Production was particularly felicitous because of lavender plants planted in association with the truffle plants; in this particular case, the truffles increased their resistance to drought.

Virgilio Vezzola - I will talk about an experience I had near Lake Garda. In collaboration with the local Comunità Montana (local authority dedicated to the conservation of the mountain territory) we created an establishment in the 90's on terrain suitable for the precious black truffle, using trees mycorrhized with *Tuber melanosporum*: and we got good results, in some cases, excellent, with 100% of the plants productive. Since Lake Garda is a popular area for tourists, we realized that it would be a good idea to have truffles available during the summer; we created summer truffle plantations. We are now beginning to reap the benefits of the plantations created in 1990 -1995, one establishment in particular began to produce summer truffles after five to six years; in its 8th year it produced 8 kilos, in its 9th year, 17, this year, 30. We're dealing here with a mixed plantation of hornbeams, hazels and oaks; in all there are 120 trees set up at short intervals because the summer truffle thrives in a fairly shady environment. Of the three species, the oak began to produce a little later. We created other establishments at altitudes of 1000 and of 300 meters with a truffle which is normally harvested at 100 meters; then we made plantations at both altitudes with truffles normally harvested at 1000 meters. What happened was this: the truffle harvested at 100 meters produced at both altitudes, while the one harvested at 1000 meters did not produce at all at the lower altitude. I'll describe another experience: a summer truffle plantation of about one hectare, after 8 years, had still not produced, in its ninth year, only a few black hornbeams produced; when we went to see the truffle orchard we realized that the establishment had been set up with plants planted too deep and the root system was very deep; this is probably what slowed production down.
Plantations made using only hazels mycorrhized with autochthonous truffles of the same altitude yielded excellent results with 100% of the plants productive. I would like to point out that when we talk about plants mycorrhized with summer truffles, it is important to use truffles gathered locally and at the same altitude.

Jean Claude Pargney - I firmly believe in the ideas presented by Sourzat with regards to the importance of biodiversity in the setting up of truffle orchards, because it encourages the development of flora and fauna which are very important for the nutrition of the truffle. I believe that plants introduced into the truffle plant ecosystem can contribute to the root system and provide refuge zones for fauna. Shady zones are also created in this way, more or less humid, which encourage the retention of water in the soil and help to meet the hydric needs of the plants. Furthermore, we must not underestimate the influx of organic substances and as a result of humus which then becomes a part of the modification of the soil locally, where the truffles develop.

Marcello Raglione - In the course of a trip to survey terrains in natural truffle orchards present in our territory, I was able to make a series of observations. Above all, in reference to the marked decrease in production documented both in France and in Italy, although some people affirm the contrary, the number of truffle hunters is continually on the rise.
Last year, for example the Province of Rieti held five exam sessions for the granting of licenses; at each session, there were 20 aspiring truffle hunters; as a result, 100 licenses were granted in a territory of 150,000 inhabitants. This has repercussions: it means that an excessive number

of people pass through the territory – so much so that the mines themselves are worked to their depths; it even seems that tractors with plows turned over the soil; some may claim that working the soil this way is indispensable but I can assure you that in these cases even big roots are exposed to the air. Another problem is the activities of wild animals, like wild boars.
I also observed that the new truffle orchards, in particular, those with black summer truffles, are all found in what is referred to as low-grade woods, that is near isolated trees that grow in abandoned terrains that were once cultivated either as olive groves, orchards, or vineyards; subsequently, little by little, as the woods advance, no longer regulated by the presence of man, the shadiness increases and the truffle orchards are exhausted. This is demonstrated by the fact that as one enters the woods, one can see that there were old truffle-beds with truffle plants still evident but more or less overgrown with grass and unproductive. So, the depopulation of the countryside, the decrease in pasture lands which restricted the development of bushes, the excessive, indiscriminate and irrational harvesting, not to mention minimal regulation, have all brought about a reduction in production. Therefore, the abandoning of terrains has two contrasting aspects, one positive which results in the increase of natural truffle orchards but the other, given the lack of control over the vegetation, and its consequent densification, results in the reduction of truffle orchards. Therefore, our natural truffle orchards need to become humanly tended truffle orchards so that there is a rational harvest, by which I mean, a harvest entrusted to groups of people who preserve this patrimony just as it is.

Moderator - Thank you, Marcello, this morning I heard a talk given by Spanish researcher who showed us how the thinning of a woods could preserve the natural habitat of the truffle. These sorts of things become difficult in Italy because regulations appropriate for the forest are often in conflict with actions that should be taken for the benefit of the truffle orchards.

Marcello Raglione - What you've just said has made me remember something I forgot to mention earlier. Truffle orchards should be catalogued, because another way to protect them is to know exactly where they are so that we can plan for their correct managing. For example, we found a truffle orchard of about three hectares of *Tuber magnatum* which produced twenty to thirty kilos of truffles each year and as a result thousands of euros. Well, last year, it was reallocated as woodlands; I would like to know how a few hundred euros derived from wood can compensate for the loss of tens of thousands of euros provided by truffles. This also happens in woods where there are *Tuber aestivum* and *T. melanosporum.* Cataloguing should ensure a cutting down of woods aimed at preserving truffles.

Leonardo Baciarelli Falini - I would like to share my experiences as a technician who has worked on many truffle orchards I would like to respond to Vezzola who reported that the oak trees in truffle orchards of *Tuber aestivum* produced later than the black hornbeams. My experience has been different; people say that walnut trees and hornbeams produce sooner than oaks, but, in both *Tuber melanosporum* and in *Tuber aestivum* truffle orchards, both the holms and the oaks have almost always produced earlier than the hornbeams and the hazels which have formed truffle beds which are very evident, yes, but the production itself has been limited and often late.

Moderator - These experiences demonstrate how in different environments the same symbiont plants behave in different ways and the same agricultural interventions can have different effects.

Ian Hall - I think that New Zealand and Australia have something to offer. When we introduced *Tuber melanosporum* and *Tuber borchii,* we did not import insects like springtails or anything else that cohabits with the truffle in your countries. We have a great opportunity for experimentation in our countries because we do not have these insects nor the mushrooms that are present in your soil; if these insects had been important, we would have had no production, but we did produce truffles. As a result, the suggestions being made are not relevant to us.

Moderator - Hall should explain to us how to get a good yield of *Tuber melanosporum* in sub-acid terrain.

Ian Hall - Some of the soils we use are totally unsuitable, they are too humid, the ground cover, the porosity – everything is different from yours, however we obtain enormous quantities of truffles. It's incredible. I think that there is a huge potential in our plantations in New Zealand, and I believe that if we had the financial backing, we would really be able to understand what is happening, by drawing comparisons with Europe. I believe that the study of plantations in these new countries could be useful, because better control of various factors is possible.

Moreno Moraldi - I am the director of UmbraFlor, a nursery that produces truffle plants. I am not here to publicize the nursery but to describe a cultivation experience. In 1990 a group of English oaks (*Quercus robur*) mycorrhized with *Tuber aestivum,* which had not been sold were used by us instead to replant a woods on 7,000 square meters of land, all completely flat, with a high clay content (49%, the rest was silt**)** and absolutely no rocks. The trees were planted very close together, because we intended to use the trees subsequently for the lining of streets. Now the trees are 8-10 meters high, some dominant, some dominated, and an undergrowth of hawthorn, dogwood**,** and other species has formed. When we walked through the plantation, we saw that there was absolutely no grass, as if the truffle beds were all inter-connected; we thought this was caused by the shade created by the trees. Last year, for the first time, 20-30 kilos of summer truffle were found; this year 100 kilos. I would like an explanation for these results because if a client comes to talk to me about making a truffle orchard on an analogous type of soil and with trees planted this thickly, I would advise against it. I give my clients completely different advise, in keeping with what the bibliography says.

Moderator - We're used to planting truffle orchards in the worst possible terrains, those not suitable for ordinary agriculture. This is a mistake caused by the fact that we imitate what we observe in nature; we find natural truffle orchards in inaccessible environments where in the past it had not been possible to deforest and clear land to obtain land for agriculture. We have studied the ecology of the truffle in protected areas and there's no saying that the best agrarian terrains are not also the best truffle plant terrains.

Guy Rethore - I am a young truffle-farmer from Lot-et-Garonne in France; in 2000, I created a plantation on a calcareous terrain with characteristics held to be favourable to the black truffle; three hundred mycorrhized trees, of which half were oaks, half holm trees, were planted in a hectare of land. In the first three years, the soil was subjected to weeding and after that a mixtures of graminacee at a 1.5 kilos/hectare was sown. After the seeding, we noticed that there was no germination around the plants, and therefore the area had no grass. This year, for the first time, I harvested the first truffles, both beneath the oaks and beneath the holm trees; some say that the holm is more precocious; that was not the case on my plantation.

Gian Luigi Gregori - I am the director of an Experimental Truffle Cultivation Center in Sant'Angelo in Vado. First a reflection, and then I will describe two experiences. It seems to me that the philosophical approach has changed from the 1980's to the present; it has become clear, at different levels and in different fields, that action must be taken with a view to cultivation as an integrated system, in which all components play an important role. As Just as Bragato said that we must first choose our experimental model and the ways in which we want to represent and schematize data, so, too, in cultivation, we must first choose what system we want to use so as to achieve production, and then the route to follow; enough on that subject. I would like to describe with regards to the micelium of *Tuber melanosporum*'s capacity to withstand vegetation, an experience in a truffle orchard set up with a density of 5x5 on suitable terrain; I want to emphasize that the environment both from the climactic and pedologic points of view was suitable for the precious black truffle. After the trees had been planted, they were

completely abandoned because their owner was obliged to emigrate. About 10 years later, a new owner, who knew there was an old truffle orchards decided, as a start, to clean it up. He went in with a hedge trimmer; he took out all the brush and much to his surprise, in a period of three years, beneath the trees which were still alive (180 out of 300), truffle beds began to appear. The owner began to weed the truffle beds to encourage their growth; when the truffle beds were well-established, he stopped weeding them weeding only the circle of expansion. Well, in the space of three or four years after these activities, the truffle orchard had become productive which testifies to the vegetative potential of the mycelium which stayed alive in conditions under which it could not bear fruit.
The second experience I would like to describe involves the valuable white truffle about which there was a great deal of discussion in Aix-en-Provence in 88; less today unfortunately, we are talking about ecology rather than the mechanisms with which the fruit-bearing body is formed, what it needs, in other words, how it is cultivated. The productive results that I am going to describe are clearly inferior to those of the valuable black truffle, but the significance of having obtained these results must be emphasized; it seems to me rather like this: once a path has been made it is important to understand how the individual stages come about, but the path has been made. To be precise, 20 plantations in Central Italy were involved, however I am going to discuss just 5 or 6, which are extremely interesting. I want to make clear that in all the places where the trees were planted, as far back as anyone can remember, there had been no wild or natural production of *Tuber magnatum*. The trees used were obtained through techniques of mycorrhization used for the black truffle starting from selected fruit-bearing bodies. After the trees has been planted, it was expected that in the space of 6-7 years, they would begin to produce truffles. But there was no production, and the plantation were abandoned, or just the grass was cut. All these truffle orchards or at least the ones that survived, went through a phase of production of strange truffles belonging to the *Tuber borchii* family; however, at a certain point, the *T. borchii* production was exhausted and they began to produce (and continue to produce today) in quantities indicated earlier by Professor Bencivenga, carpophores of *T. magnatum.* This result strikes me as very interesting, and I hope that there will be a return to the study of the white truffle and to research on mycorrhization, because one plant produced in a lab is not enough; we need a series of plants to be able to do new experiments and understand a bit more about the whole subject.

Giovanni Pacioni - Over these last few days, we have heard a lot of talk about terrains suitable for the black truffle and we have heard about terrains all rich in stone, however, after more than a decade, of allowing fields to lie fallow, of biological agriculture, of the abandoning of farms, etc a series of truffle orchards have been created on arable hill lands where there had been vineyards and even irrigation systems because vegetables used to be grown there; these projects involved vast extents of land **i**n the regions of Friuli, Veneto, Romagna, Abruzzo, and Lazio as well as argillaceous terrains and they have, for years, produced truffles of notable dimensions.
Probably, as Bencivenga suggested just a little while ago, we have misinterpreted the ecology of the truffle; we thought the ecology of the black truffle was a residual ecology, of marginal terrains, because that's where truffles had been relegated by man who did everything he could to obtain fields he could cultivate; truffles also grow well on optimal agricultural lands; obviously one has to be careful, above all, with irrigation, because if truffles are provided with water, they will mature way ahead of time. Therefore, terrains we have never considered productive for truffles, are, on the contrary, highly productive.

Moderator - That's a very valid point.

Gabriella Di Massimo - I am an agronomist, and in my technical capacity I am responsible for numerous black truffle plantations and I know quite a lot about what is happening at the moment, in nature. I consider *Tuber aestivum* a species which is expanding because it is

colonizing abandoned lands, but I have not observed the same phenomenon when it comes to *Tuber melanosporm.* I am afraid that truffle orchards realized in environments which are probably not their normal, natural ones are plantations in which production is destined to come to an early end. I would like to know if any one has any experiences in this area. With regards to mycorrhizal plants, we are really making it a point to choose plants realized with local truffles and autochthonous plants; this isn't a choice based on scientific criteria, but experience teaches us that plants well-mycorrhized with local truffles and local seeds, permit us to reduce greatly the period of non-productivity. The non-productive phase used to last for 10 years; now it has been reduced to 4-5 years. I would like to know if anyone has any relevant experiences in this area as well. Now, as for the white truffle, I think a lot of studying still needs to be done, before we can talk about production. We have to dedicate ourselves again to the ecological studies which have been abandoned, because if we don't save the natural truffles and just wait for research to make it possible for us to cultivate this species, we run the risk, at least in central Italy, which is the area I know best, of ending up without any production at all.

Virgilio Vezzola - I'd like to respond to what Gabriella just said. In my area, a farmer got a hundred French trees and planted them in land extremely well-suited to the black truffle. Five years later, a Comunità Montana (local authority dedicated to the preservation of mountain areas) plan offered farmers plants at half-price. The farmer, since he still had some land free, decided to do a second planting and we supplied him with some plants produced locally with autochthonous material. After twelve years, the French trees, which were still perfectly mycorrhized with *T. melanosporum,* had not yet begun to produce, whereas the plants that we had produced with autochthonous seeds had begun production, some as early as the fourth years, others in the fifth, and by the seventh year, almost all of them were productive. Subsequently, we harvested the "fioroni" (truffles which mature early) under the productive autochthonous plants and we inseminated them in the French trees; two years after insemination, the French trees had also begun to produce.

Alberto Cretoni - I am someone who is trying to do truffle cultivation. This is my experience: in 1990, I planted truffle-inoculated trees in two fairly different pieces of land, one highly suited to the black truffle and one less so; a year after the first plantation was set up, it was subject to extreme traumas because of a hail storm; the trees were almost completely destroyed and as a result the plantation was not cultivated regularly after that. This plantation did not yield good results even though recent monitoring has demonstrated that mycorrhizae of the truffle are still present, so there are grounds for hope. The other plantation was set up with 300 plants of which 260 were of black truffle and 40 summer, which were planted, by mistake, in the midst of the plants mycorrhized with the black truffle. At the beginning, the plants were irrigated to facilitate their survival, and 99% of them took; then they were pruned so as to eliminate the small branches, from ground level up to a meter; there was some weeding, too and after the third or fourth year, we did nothing else except for the occasional nonwoven mulching to combat excessive drought. This truffle orchard, although located in an less suitable environment, began to give good results including pieces over 300 grams – therefore, more than satisfactory.
In the areas where the plants mycorrhized with summer truffles had also been planted, production began and, this year, in which we had a relatively rainy spring, we got encouraging results: in fact 125 kilos of truffle were harvested from less than 40 plants. Water, however, is very important. It would be a good idea if agricultural techniques like irrigation were further investigated, because this is really what we need; we have a lot of good will, and we're ready to make a lot of sacrifices, but don't abandon us, because otherwise we won't be able accomplish anything. As long as we have good plants, suitable lands, and technicians with the right suggestions, as well as good weather, of course, we will very likely continue to eat truffles.

----------In 2000, a neighbour of mine in the south of France, planted on land totally unsuited to truffle because degraded and with a pH content of about 6, about 50 non-autochthonous

holm trees from Austria. The farmer cultivated them and took care of them as if it were a fruit orchard but without any results. Two years ago, on a suggestion from Sourzat, he treated the truffle orchard with lime (2 kilos of calcium carbonate under the foliage of each tree) and last winter he had his first production; a few days ago, we went past his plantation and we noticed that production is again near at hand.

Moretti - I come from Umbertide; my family has a long tradition of white truffle hunters. This has been our experience of 130 years as truffle hunters: we are destroying the truffle orchards by the savage chopping down of trees. Trees are chopped down like the *Salix caprea*, whose wood has scant value, just as sick trees are chopped down, though they are the ones that produce truffles. Another problem is the number of truffle-hunters. In Città di Castello, there used to be 60 truffle diggers; now, between Umbertide and Città di Catello, which are about 20 kilometers apart, we have no less than 4,200 truffle diggers, and the harvesting zones are becoming more and more restricted. Now we are hindered in very possible way, because everywhere we go we find signs saying no crossing allowed, no truffle or mushroom harvesting allowed. It seems that the owners of terrains who didn't even know there were truffles on their property, have become the best truffle-hunters of all. Seventy percent of the territory, what with off-limits area, protected oases, limited funds, and no hunting allowed, is closed; this is really shameful. It's time for the Region to decide to free up harvesting.

Gilberto Bragato - I don't have any experience as a truffle-hunter or of truffle cultivation. With reference to this Conference, I want to emphasize the fact that we have among our participants a series of researchers who come from European countries different from the traditional truffle-producing ones. It's very interesting to hear about production experiences, at the moment, only natural, of species we have always considered of secondary importance, as, for example the summer truffle, or the *T. macrosporum.* It's also interesting to hear about experiences involving the white truffle, which we find above all along the shores of the Danube. All these experiences from other countries could be useful for the organization and planning of truffle orchards not only in those countries but above all here in Italy, because in my opinion, these natural situations which we have lost, are precisely the ones we should study. Moreno Moraldi's remarks particularly struck me, and I connected them to the slides on Hungarian summer truffle orchards where the truffle coexists in symbiosis with the English oak tree. The same holds true for the Swedish experience where once again the English Oak is always present in truffle orchards. I think it would be a good idea to investigate these environments in which *T. aestivum* interacts with organic substances because English Oak produces a thick leaf litter and as result a lot of organic substances which in turn enrich the first layers of the soil. Therefore, it would be important to investigate this aspect further and, in particular, the biological activity which it brings about and which can be used to increase productivity both in future truffle orchards and in those which are already productive. I am particularly interested in those areas in which the bark creates a truffle bed with low visibility because in these cases there is yet another factor which encourages the creation of an adequate productive layer. Perhaps it would be a good idea to begin to experiment with these types of procedures.

Moderator - I will also describe my very limited experience. I live in an area where the land has absolutely no stone and the fine earth is 45% clay, 40% lime and 10% sand. In 1990, I planted mycorrhized plants of summer truffle on this land; I just want to point out that this is a terrain remote from any trees, and on which herbaceous crops have always been grown and, as a result, entirely without any symbiont mushrooms which could be competitive with the truffle. A few examinations of the roots revealed the almost exclusive presence of truffle mycorrhizae and a few years ago, production of *Tuber aestivum* began. The carpophores, however, grow very close to the surface, at times they are almost on the surface, and as a result, the quality of the truffles is often poor.

Paul Thomas - I have a very brief comment from England. We have a plantation with just over 3,000 trees. It is only two years old, but we have had a truly good level of brulé formation. And the brulé of *Tuber aestivum* had already formed after just 9 – 12 months.
But the extremely interesting thing is that the owner of these plantations, who lives nearby, and knows a lot about truffle orchards, noticed that when the trees were planted in summer, a lot of cockroaches appeared. I don't have any hard data on this, it's just an observation. But the whole site was covered with very small cockroaches who went all over the plantation and in particular on the brulé at the base of the trees. I thought perhaps that this might be an interesting observation with regards to the activities of insects in plantations.

Silvano Fabrizi - My history with the truffle goes back to the 90s, when because of my work, I became involved with the Experimental Center for Truffle Cultivation in Sant'Angelo in Vado, and I came into contact with the first nine plants which were given to me as a present. I planted the nine trees and subsequently, in 1991, I acquired other plants and began cultivation. In the first years I continually weeded the plants. I pruned them, as had occurred naturally when there were goats in my area; in short, I treated them very badly and then I abandoned them. In the ninth year, I happened to stop by the truffle orchard and my dogs literally went crazy; it was February 2nd, 2000. and from that day on, I have had a good production; I have harvested as much as 40 kilos of truffle from three hundred plants. This year, I started to harvest early because by the end of October, the truffles were mature and I harvested them so that they wouldn't go bad the way they had in previous years. In fact, last year, I found beautiful truffles towards the end of October, but I didn't harvest them, in the hopes that I would be able to harvest them in the period when it was legal to sell them. But, I never did find those truffles, and for that reason, this year I chose to harvest early.

Moderator - Mr Fabrizi, did you notice any difference between the irrigated part and the dry part, in particular, with regards to the period of maturation?

Silvano Fabrizi - This year I had rain on July 27th and then on the 29th, and as a result I didn't notice anything. Last year, as opposed to the year before, in which I harvested 40 kilos, I had only 8 kilos, all from the irrigated plants.

Moderator - Your experience is important because in drought years, if one doesn't intervene with irrigation, one has no production. Irrigation, however, can also be harmful, if it is done irregularly. If there is anyone here in the room who can report on experiences with irrigation, it would be helpful to hear about their experiences; it would be useful to know what type of irrigation was used, if above or below the foliage, on what kind of terrain, etc.

Truffle farmer from the Marches Region - I come from the Marches, near Roccafluvione, in the province of Ascoli Piceno, one of the best areas in Italy for the production of black truffles. I have about 10 hectares of truffles; I began planting orchards in '94 on an agricultural terrain and not on marginal terrains, as had been the rule in the past. Some said the terrain wasn't appropriate because the previous crops had been fertilized and weeded: both of which damage mycorrhizae. But that hadn't happened; the truffle orchard was a great success. Production was good above all in the past when the weather was favourable; we had enough rain. About 80% of the plants were productive; some produced 5-6 kilos of truffles; the terrain was rich in lime and also had a 30 – 40%b clay content and some rocks. Clay is necessary because it manages to keep some water in the terrain. Subsequent plantations made on agricultural terrains are also giving good results, even though, in the last few years unfortunately, the climate hasn't been on our side.

Moderator - This experience, along with others I've heard during the course of the conference, suggests that we should eliminate the danger of competing mushrooms. The best results are

obtained on terrains where there have not previously been woody plants with ectomycorrhizae because otherwise the truffles present in the roots of our trees have to overcome the competition of mushrooms present in the soil, and these latter are often aggressive because well-adapted to the local environmental conditions. This is why we are getting our best results on agricultural terrains far from woods and where there are not trees with ectomycorrhizae**.** I think this is an important point to make here at this conference. In addition to woody plants, it's important to be careful of other shrub tree species like *Cistaceae* (gen. *Helianthemum*, *Cistus*) which live in symbiosis with ectomycorrhizal mushrooms and which we commonly find on uncultivated lands. Unfortunately in Italy, and above all in Umbria, a region rich in hills and mountains, truffle orchards are often made on little pieces of abandoned land and where vegetation, including some *Cistaceae,* has certainly developed.

Pierre Sourzat - I listened to the previous farmer who planted a truffle orchard on terrain on which corn had previously been grown and to the talk of the gentleman who said that in a forest environment he has the problem of contamination from competing mushrooms. I must say that the general rule is not to plant in woody environments or in environments where pesticides have been used which could block the biological action of the soil, a biological action favourable to *T. melanosporum.* Nonetheless, we can find situations in woody environments where the production of *T. melanosporum* dominates competing mushrooms as well as situations in which mycorrhized plants planted in terrains where grains had previously been grown begin to produce late. In situations of this kind it is possible to observe a modification of the mycorrhized system into black winter truffle or summer truffle. This means that we still have problems understanding what blocks the truffle plant ecosystem and what doesn't block it. In other words, it is important to learn more about both what works and what doesn't work, and I believe that studying what doesn't work could give us excellent material to work with to improve truffle plant production.

Moderator - Thanks, Pierre, it is certainly true that there are other conditioning factors that affect the results in different environments such as woods and fields, where pesticides have been used. I would like to bring to everyone's attention the fact that various people have described experiences of good production on terrains with a 40 -50% clay content. Marcello Raglione explained to us that different types of clay exist which behave in different ways. If Marcello could go into a bit more detail on the subject, I think everyone would be pleased.

Marcello Raglione - Anyone who was here for my talk heard me say that a 40% clay content is acceptable, however it's important to be aware of the type of clay involved, i.e. whether we are talking about expandable clay or stable clay. Then there's something else to keep in mind; the tests to ascertain clay content are of the sieve type, and as a result, all particles which are smaller than 2 microns are nonetheless included. That means that in the soil textural class of clay, other composites are included like carbons; therefore the amount of real clay present may actually be much less. I'll take advantage of this occasion to pick up on what the truffle farmer who spoke just before me said about herbicides. I'll give another example. In Acqualagna, along a ditch, there was *Tuber magnatum* production, along the right bank, as well as along the left.
On the right bank, a meadow continued to be present, whereas on the left, sunflowers, corn, etc began to be grown. On the right side, the truffle orchards survived; on the left side they did not, because of herbicides. In this case, herbicides were distributed every year and transported from the water up to the bank where the truffle mycelia which had been damaged were located. This situation is different from the situation of the field where herbicides used to be used; the negative effects of herbicides last for just a few days or a few weeks, so that in that case, when the plants were planted, enough time had gone by and the herbicides had decomposed and lost their effect. So I have reassuring news for truffle farmers – if a terrain has been subjected, in former years, to potent herbicides, by the time the plants are planted, the herbicides will no

longer have any effect; and they will actually have cleared the field of all the other mushrooms that could have been in competition with the truffle.

Moderator - Thank you for repeating this extremely useful information; this evening many truffle-farmers are present who didn't hear the talks given during the Conference.

Gérard Chevalier - I am not a truffle farmer, but I have a wide-ranging experience and 40 years of observation under my belt. As far as truffle-producing trees are concerned, I can state that they are now no longer a hypothesis, but a certainty. At the moment, truffle plants are produced from seeds, from acorns, which are heterogeneous and as a result produce heterogeneous plants which can give excellent results, with a 100% of plants productive. When mycorrhizal plants are produced, once realizes that no plant resists mycorrhization; all plants mycorrhize, but they do not all produce at the same rate. Under good conditions, precocious production occurs 3.5 years after planting. We made a mistake when we used the hazel tree for which we had such high hopes. It was said that the hazel has many mycorrhizae and produces earlier than the oak, but this turned out not to be true, because holms can have precocious production, too. At the moment, the holm is the truffle-producing tree most widely used in France; it is a good producer, and once planted, produces after just three and a half years. What's important is the selection of the plants and I agree that it is better to use the local ecotype; However, I believe that cloning will permit a better selection of mycorrhizal plants, the production of much more homogenous plants with more regular production and with root systems both better developed and more adapted to mycorrhization in part because they do not have taproots and, as a result, have roots which are more superficial.
Recent attempts at cloning have demonstrated that clones are only better than seedlings in certain environments. As opposed to what many nursery-owners claim, we simply can't say that one clone is best under any circumstances, because one can yield good results in i.e. environmental conditions best suited to it, while another will prove better in other conditions. What we need to do is to produce clones (pairs of plant and truffle) for every region, and these will be better than heterogeneous pairs obtained from seeds. This is as far as mycorrhizal plants are concerned. With regards to soils suitable for truffles, in Auvergne, where I work, there are many plantations on a wide variety of soils, and it has been observed that the physical-chemical conditions of the soil influence the production of truffles. In some cases, we have *T. melanosporum* production, in others *T. brumale*. We've realized that a key factor is the softness of the soil, and as a result I believe that we can build up artificial soils when we have only unsuitable soils at hand. It is possible to modify soils, by adding limestone or mineral elements like volcano ash (pozzolana) to make the soils lighter and to build them up from the physical or chemical point of view; we've made a few trials but as yet we have no results.
Third, I would like to talk about truffle-producing establishments that exhaust their production precociously. In fact, there are plantations that don't last very long. It is said that production terminates because the mineral elements in the soil are exhausted. This is not entirely true, because the production of truffles is relatively small, i.e. doesn't involve the tons involved in the growing of agricultural products, which do indeed deplete many elements; the truffle extracts very little from the soil. The black truffle wears down roots, it lives on roots, on wood tannins and polyphenols, etc, so that to obtain more truffles, one constantly needs more sprouts: when the truffle no longer has any sprouts, it disappears. We need to find systems which encourage the continual formation of young roots: how to procure young roots for the truffles to eat? There are two solutions, the first is the pruning of trees; this is an indirect solution because when we prune trees we indirectly encourage the regeneration of the base system; the second solution is to till the soil with appropriate tools so as to break some roots, thereby encouraging the regeneration of the base system.
I believe that in the future truffle plantations will have to be tilled with tools appropriate to truffle cultivation; at the moment we do have machines suitable for this purpose, so the tool of the future will not be the hoe but rather light tractors and light-weight tools which aerate the soil

and break up the base system thereby regenerating the roots; in my opinion, this is what we need to work on in the future.

Moderator - This afternoon, no one has discussed the distribution of spores on the field to keep mycorrhization alive and encourage the survival of truffle orchards. I have experience in the area; in fact, replenishing the stores of truffle spores in truffle orchards that are productive or that are beginning to exhaust production has allowed me to improve production and, in some cases, to bring an exhausted truffle orchard back. The reasons for this are not hard to find: in the past, truffle hunters were few in number; their dogs were not expert, and as a result, all the truffles were not gathered and truffle orchards could preserve themselves for a long time. Now, truffle-hunters are numerous, the dogs are professionals, and absolutely nothing is left in the soil, and all this creates problems for truffle orchards' survival.

Moreno Moraldi - I'd like to go back to what Chevalier said about clones. I agree with Gérard about the possibility of using autochthonous clones in particular areas where one uses them because, in that case, it is the only way to get excellent results. While we are on this subject, I would like to remind everyone that the use of clones is not permitted in woods; the European Community has issued a directive that forbids the use of cloned plants in woods. So, we have to be careful when we talk about clones because we are talking about plants that cannot be used in truffle cultivation in the context of forestation. The stricture is justified because of the risk of the spreading of diseases; clones are a series of absolutely identical subjects, and any pathogen could get out of control.

Gabriella Di Massimo - I am about to say something that I probably would never have dreamed of saying: "I don't agree with Chevalier" for the same reasons that Moreno Moraldi has given and above all because we are heading into a period of climatic variations and as a result we have to ensure that our plantations have a certain genetic variability which will guarantee us a greater hardiness. I firmly believe in the importance of autochthonous material and as a result we have chosen to proceed along similar sisterly lines in the Matriarch project we are collaborating on with the Comunità Montana (local authority responsible for the conservation of mountain areas) from Spoleto (the Matriarch project was the subject of my talk yesterday). The use of clones, whether they be of plants or of truffles is, I believe, necessary to create conservation fields which would guarantee us a store of germplasm for the future. We need this because natural truffle orchards are becoming rarer and rarer. With regards to mycorrhizal trees, I would like to clarify one thing; yesterday we spoke about certification and someone said that we do not yet know what the perfect mycorrhizal plant is, and how many mycorrhizae it should have; this is a very dangerous remark; mycorrhizal plants must be abundantly mycorrhized, and fortunately, the Italian, Spanish and French nurseries I know of all produce excellently mycorrhized plants; this is a very important point, and we must not turn back now. As far as spore -inoculation in the field is concerned, we have, in effect, saved some truffle orchards whose production was in decline, but this result has to be evaluated over time because we don't know whether in fact it was the adding of spores that worked or simply the tilling we did when we put them in the soil. The difficult part for those of us who work in the field is precisely the multiplicity of the parameters that we have to bear in mind when we evaluate results.

Moderator - Thank you, Gabriella. As you referred to my talk yesterday on how many mycorrhizae a plant should have to qualify as valid for the purposes of truffle cultivation, I want to clarify that we really don't know, and the method used in Italy, which calls for a minimum of 30% of mycorrhization, came out of the observations a group of researchers made on production in the best nurseries and, to be exact, on one-year old plants, rather than coming from experimental trials with plants planted with different degrees of mycorrhization. It's clear that the higher the percentage of mycorrhization, the better the plant and the better the results that plant can give us. I just don't want my remarks yesterday to be misinterpreted.

William Saenz - I would like to comment on two points. With regards to inoculation in the field with truffle spores, an example comes to mind: in Lot, there is a natural truffle orchard that has been producing for more than thirty years; it faces north and the harvesters always stopped harvesting in mid-January because after that date, the truffles were always low quality. As a result, many truffles stayed in the terrain, and therefore, there was a lot of inoculation, and perhaps for this reason, the truffle orchard has had such a long lifespan.
I'd also like to discuss another point, the influence of former cultivations on the success of the truffle orchard. The territory in Lot is full of woods now, and there are many truffle orchards which are, by now, unproductive, and few terrains are still being cultivated. In this situation, in recent years, many people have tried to establish new truffle orchards on terrains that once were part of the woods, or in exhausted truffle orchards, and the results have not been bad. I want to make clear that these plantations are created on terrains which, once the woods have been cleared, have been well worked and prepared with tools which make it possible to eliminate the greater part of the roots.

---------- I am a truffle farmer. I would like to add something, with regards to the last two comments. First of all, with regards to precocious harvesting, before coming to the Conference, I sold ten kilos of good quality black truffles and I harvested another three kilos of putrefied truffles that I preferred not to leave in the soil. Now I've understood that I should have left the rotten truffles in place. When I go back home after the Conference, I expect to harvest more or less the same amount of truffles that I harvested before coming to Spoleto. I get good results, but I don't understand what I action to take to improve or conserve production.
I listened to your talk at the opening of the conference when you said that researchers spend a lot of their time in labs, and should spend more time in the fields. Truffle farmers aren't able to analyze or to draw conclusions so as to identify a suitable model for planting and growing; they would be happy to have researchers available who are able to analyze and synthesize. I hope that the next generation of researchers will go out on the field, listen to truffle farmers, go to their houses, see what is happening and study on site. There are lots of truffle orchards that are working well and so I think it's important that their productions be known and their farming practices, too, so that by comparing the different ways of planting and growing truffles, methodologies can be developed that guarantee successful production in truffle orchards.

Moderator - Since no one else has asked to speak, I declare this round table closed; it has provided us all with a great many valuable suggestions which I am sure will be of great use to both farmers and researchers alike.

Cerimonia di chiusura

Mattia Bencivenga
In qualità di presidente della Commissione scientifica, mi corre l'obbligo di fare alcune considerazioni su queste quattro giornate che si stanno chiudendo. Innanzitutto esprimo la mia grande soddisfazione per l'ottima riuscita del Congresso che ha visto la partecipazione di oltre 300 iscritti provenienti da 23 nazioni dislocate in tutti i continenti del globo. La soddisfazione scientifica è dimostrata dall'elevato livello delle relazioni e delle comunicazioni; per ragioni di tempo abbiamo dovuto costringere molti iscritti a presentare le loro comunicazioni sotto forma di poster la cui importanza scientifica non è certamente inferiore alle comunicazioni orali.
Altro motivo di soddisfazione è la constatazione che la ricerca sui tartufi e sulla tartuficoltura è in forte crescita. Nell'ultimo ventennio nuovi settori scientifici si sono dedicati allo studio del tartufo, come ad esempio la genetica che, applicando le ricerche biomolecolari, ha consentito una migliore caratterizzazione dei corpi fruttiferi e delle micorrize ed infine di conoscere il genoma del tartufo nero. Sono aumentate le ricerche applicate alle coltivazioni mostrando un'inversione di tendenza. In passato venivano privilegiate le ricerche di laboratorio perché consentono di ottenere risultati più rapidi rispetto alle ricerche di campo che sono generalmente più lunghe e con risultati incerti per l'impossibilità di controllare i diversi fattori ambientali, ed inoltre sono meno quotate rispetto alle ricerche di base. In questo Congresso le comunicazioni relative alle coltivazioni sono il doppio rispetto a quelle delle altre sessioni.
Dal 1988 ad oggi l'interesse del tartufo e della tartuficoltura ha valicato i confini europei: sono state individuate altre aree di produzione naturale (Cina, Marocco, Romania, ecc.), la coltivazione è stata tentata con successo in altri Continenti (Nuova Zelanda, Australia), però la produzione naturale è ovunque in regressione.
Ecco l'importanza delle ricerche di base e applicate al tartufo, che potranno consentire di conservare le tartufaie naturali sopravvissute e di migliorare le tecniche di coltivazione.
Da questo Congresso, interpretando il pensiero di tutto il Comitato scientifico, viene fuori un'esortazione verso i giovani affinché intraprendano ricerche, integrate da più competenze, sul tartufo e sulla tartuficoltura; il confronto continuo tra gruppi di ricercatori consentirà di affrontare e risolvere i numerosi problemi biologici e colturali ancora irrisolti.
Con questa esortazione spero, se l'anagrafe me lo consentirà, di vedere risolti i problemi appena accennati durante il Quarto Congresso Internazionale di Spoleto sul Tartufo che, secondo quanto ha deciso il Comitato organizzatore, si terrà qui a Spoleto esattamente tra 20 anni (2028) istituzionalizzando un incontro ventennale.
Senza aspettare il 2028 un incontro più ravvicinato (tra 5-6 anni) verrà organizzato in Spagna durante il quale verranno presentati i risultati di un grosso progetto di ricerca che coinvolge la maggior parte dei ricercatori spagnoli. Nel frattempo l'attività intorno al tartufo dovrà essere mantenuta viva mediante la costituzione di commissioni, così come è stato deciso negli incontri di questi giorni:
una commissione costituita da poche persone dei paesi produttori di tartufi che prenda in esame ed elabori un metodo di analisi e certificazione delle piante tartufigene che possa essere utilizzato in tutta l'Europa;
un'altra commissione dovrà prendere in considerazione la legislazione sul tartufo che possa equiparare le norme tra i paesi produttori di questi funghi.
Dal Congresso perciò partono queste iniziative che dovranno affrontare e risolvere i problemi appena accennati oltre a quelli scientifici.

Vincenza Campagnani - Presidente della Comunità Montana del Monti Martani e del Serano
La parte tecnico-scientifica è stata discussa da Bencivenga, a me il compito di tirare le somme della parte politica ed organizzativa del congresso. Non so se questo sia un momento felice o triste: momento felice perché siamo nella fase conclusiva di un incontro ben riuscito, è un momento infelice perché con molti di voi ci stiamo salutando. Voglio esprimere la mia felicità per il congresso tutto, perché credo sia stata una grossa ventata di positività portata nel nostro

Ente, che in questo momento sta attraversando la fase di riforma. Da qui a poco la sottoscritta sarà sostituita per cui chiudo in bellezza, è stato tutto positivo è stato bello vedervi qui e vedervi attenti e scambiarvi le idee; sicuramente ognuno di voi ha migliorato le proprie conoscenze, vale anche per me, che faccio un altro mestiere, ed ho appreso molte cose: sono appassionata di tartufi perché vengo da un piccolo paese di montagna dove tutti vanno a raccogliere i tartufi, per cui dal punto di vista pratico li conosco bene, ma dal punto di vista scientifico solo quello che ho appreso durante le poche ore che gli impegni mi hanno consentito per ascoltare le relazioni.
Voglio trasmettervi la mia positività affinché ognuno di voi, tornando nel vostro paese, possa ricordare Spoleto nel suo complesso oltre al Congresso, e rimanga qualche cosa di positivo nei vostri cuori.
Per la Comunità Montana è stata una prova d'esame, non è stato un punto di arrivo e tanto meno un punto di partenza. La Comunità Montana ha una lunga storia sul tartufo che origina nel 1968, quando il Comune di Spoleto organizzò il primo congresso Internazionale sul tartufo, continua nel 1988 quando fu l'Ente organizzatore, e speriamo di continuare in modo che Spoleto diventi un incontro ventennale.
Mi preme sottolineare che tutta questa positività, che questa sera riesco ad esprimere, la devo a tutti coloro che hanno collaborato per la realizzazione di questo evento e vi assicuro che è stato faticoso affinché tutto funzionasse bene e dare a tutti gli intervenuti la migliore ospitalità; laboriosa è stata l'organizzazione precongressuale che per noi è iniziata nel 2006.
Sicuramente qualche lacuna c'è stata: in questo ventennio abbiamo migliorato le lacune del 1988, nel 2028 miglioreremo quelle di questo congresso.
Come ho sentito da molti congressisti credo che l'organizzazione sia stata veramente eccellente e se non abbiamo avuto grossi problemi lo devo a Fabrizio Gentili che ha guidato la segreteria organizzativa, ai ragazzi dell'Istituto Alberghiero che hanno mostrato grande professionalità e alla tipografia Severini: a tutti un grazie di cuore.
Un altro ringraziamento va all'amministrazione comunale che ci ha ospitato in questa bella cornice, altro grazie doveroso all'Università di Perugia, a tutti i dipendenti della Comunità Montana dei Monti Martani e del Serano e in particolare ad Alvaro Paggi. Il grazie va esteso agli interpreti che ci hanno permesso di capire le relazioni presentate in altre lingue ed infine a tutti i congressisti perché senza di loro il congresso non avrebbe avuto senso.

Alvaro Paggi - Permettetemi soltanto di associarmi ai ringraziamenti fatti dalla Presidente. Vorrei fare due considerazioni: con la prima vorrei far notare l'età giovanile delle persone chiamate sul palco e che hanno lavorato all'organizzazione del congresso, siamo abituati a considerare in modo superficiale i giovani, che in questa occasione ci hanno dimostrato grande professionalità e dedizione. La seconda si riferisce alla pubblica amministrazione, permettetemi di dire che quando la pubblica amministrazione si impegna può fare molte cose buone. Per ultimo non posso non ringraziare tutti i congressisti e permettetemi un ringraziamento particolare all'amministrazione della Comunità Montana e alla nostra Presidente.

Premiazione dei ricercatori che hanno dedicato la propria attività scientifica al tartufo e alla tartuficoltura

Il Presidente della Comunità Montana consegna una targa ricordo a ricercatori, pensionati o prossimi alla pensione, che si sono distinti per le loro ricerche sul tartufo e la tartuficoltura:

Anna Fontana: A colei che ha ideato e prodotto la prima pianta tartufigena. Da quella prima sintesi micorrizica (*Pinus strobus* L. x *Tuber maculatum* Vittad.) e dalle sue ricerche sulle micorrize è nata la moderna tartuficoltura.

Gérard Chevalier: A colui che ha dedicato la propria vita allo sviluppo della tartuficoltura francese. Mediante il suo carisma e la sua opera di scienziato multiforme ha introdotto e migliorato la coltivazione di *Tuber melanosporum* e di *Tuber uncinatum* in diverse località tartufigene francesi.

Bruno Granetti: Al primo ricercatore umbro che ha effettuato ricerche di base ed applicate sul tartufo e sulla tartuficoltura. Il suo entusiasmo e la sua meticolosità hanno permesso la conclusione di numerose ricerche e la realizzazione di numerose tartufaie produttive.

Mario Palenzona: Al ricercatore che, per attività istituzionale e per hobby, ha approfondito le ricerche di base e applicate condotte dai ricercatori italiani e francesi consentendogli di avviare la coltivazione razionale dei tartufi in Piemonte ed in altre Regioni Italiane.

Closing Ceremony

Mattia Bencivenga
As president of the Scientific Commission, I have the task of making some general remarks about these four days that are now drawing to a close. Above all, I would like to express my great satisfaction at the wonderful success the Conference has had, with the participation of more than 300 participants from 23 countries and all the continents of the globe. That the conference was a success, too, scientifically, is demonstrated by the high level of the talks and written communications; because of time constraints, we were forced to ask many participants to present their remarks in the form of posters; their scientific contribution was, of course, no less significant than that of the talks.
Another reason for my satisfaction is the realization that research on truffles and truffle cultivation is clearly increasing. In the last twenty years, new scientific sectors have dedicated themselves to the study of the truffle, for example, genetics which, by applying bio-molecular research, has allowed us to better define fruit-bearing bodies and mycorrhizae and to understand the genome of the black truffle. Applied research in truffle cultivation has also increased, and revealed a new tendency. In the past, laboratory research was preferred because it allows one to obtain results more rapidly than research on the field, which generally takes more time, and gives uncertain results because of the impossibility of controlling various environmental factors, **a**nd what's more this type of research is also less well-paid than basic research. But in this conference, the number of talks related to planting and growing has doubled in comparison with previous sessions.
Since 1988, the interest in truffles and truffle cultivation has gone beyond the borders of Europe. Other areas of natural production have been discovered (China, Morocco, Romania, etc), cultivation has been successful on other continents (New Zealand, Australia), however, natural production is regressing everywhere.
This is why basic research and applied research are both so important for the truffle, because they will make it possible to preserve the natural truffle orchards that have survived and to improve techniques of truffle cultivation.
Out of this Conference, and here I express the thoughts of the entire Scientific Committee, comes forth an exhortation addressed to all our young colleagues; we need research, interdisciplinary where possible, on the truffle and on truffle cultivation. Dialogue among groups of researchers will make it possible to take on and solve numerous biological and agricultural problems, as yet unresolved.
This exhortation will, I hope, the office of vital statistics permitting, mean that the problems I have just referred to will be resolved during Spoleto's Fourth International Conference on the Truffle which, in accordance with what the Organizational Committee has decided, will be held in Spoleto in exactly twenty years (2028), making a meeting every twenty years an institution.
However, well before 2028, in 5-6 years, another meeting will be held in Spain during which the results of a huge research project, involving the majority of Spanish researchers, will be presented. In the mean time, activities involving the truffle must be kept alive through the creation of commissions, in keeping with what was decided in our meetings over the last few days:
one commission made up of just a few people from the truffle-producing countries which will examine and create a system of analysis and certification for truffle plants that can be used throughout Europe;
another commission will consider legislation on truffles which could standardize regulations among truffle-producing countries.
As a result of this Conference, then, we have undertaken to deal with and solve these problems, in addition to the scientific ones.

Vincenza Campagnani: President of the Comunità Montana (local authority responsible for the conservation of mountain areas) of the Martani Mountains and of Serano
Bencivenga has discussed the technical-scientific aspects of our Conference, it is my task

now, to sum up the political and organizational ones. I don't know whether this is a happy or sad moment: it is a happy one because we are at the conclusion of a meeting which has been very successful; it is an unhappy moment because now we must say good-bye to many of you. I want to express my happiness about the whole Conference because I think it has really been a breath of fresh air, and very positive for our Organization which is, at the moment, going through a period of reform. In just a little while, I myself will surrender my position to someone else; my term, then, draws to an end on a happy note; it has all been positive, it has been wonderful to see you all here and to see your attention, the exchange of ideas; I am sure each of you knows more than you did when you arrived; I myself have learned, even though I am in a different line of work, so many things. I am passionate about truffles because I come from a little town in the mountains where everyone hunts truffles, so that from a practical point of view, I know them well, but from a scientific point of view, I know only what I was able to learn from the talks here, in those few hours when my work allowed me to be present.
I want to transmit my positivity so that every one of you, when you go back to your own countries, can remember Spoleto in its entirety, in addition to the Conference, and so that something positive can remain in your hearts.
For the Comunità Montana (local authority responsible for the conservation of mountain areas), this has been a test, rather than a point of arrival or a point of departure. The Comunità Montana has a long history with the truffle which began in 1968 when the municipality of Spoleto organized the first International Conference on the Truffle; it continued on in 1988 when we became the organizing body of the second International Conference and we hope to continue this tradition, so that an International Conference is held in Spoleto every twenty years.
It is important for me to underline that I owe all the positivity which I am able to express this evening to everyone who worked together to make this event possible, and I assure you a lot of work went into both making everything run smoothly and giving the best possible hospitality to all the participants. The pre-conference organization was also quite an undertaking; we began preparations in 2006. Of course, I am sure there were some lacunae; in these twenty years we were able improve on the lacunae of the 1988 conference; and in 2028, we will improve on those of this conference.
I believe, and many Conference participants have said as much to me over the last few days, that the organization of the Conference this year was really excellent, and if we indeed had no significant problems, we owe that to Fabrizio Gentili, who oversaw all the secretarial aspects, and to the Hotel Institute students, who were always extremely professional, and to the Severini Typography; I thank you all from the bottom of my heart.
My thanks go, too, to the municipal administration, who welcomed us into this space, and must go, as well, to the University of Perugia, to everyone who works for the Comunità Montana and, in particular, to Alvaro Paggi. I also extend my thanks to the interpreters who allowed us to understand the talks given in other languages, and finally to all the Conference participants, without whom this conference would never have existed.

Alvaro Paggi: Allow me simply to add my thanks to those of the President. I would like to share two reflections with you. First of all, I would like to call everyone's attention to the young age of the people called up to this stage, who worked to organize the conference; we are used to thinking superficially of young people; on this occasion, they demonstrated to us all their great professionalism and dedication. My second reflection concerns the public administration. Allow me to say that when the public administration works hard for something, it is able to get a lot of good done. Last, I cannot neglect to thank all the Conference participants; and allow me to express my particular thanks, too, to the administration of the Comunità Montana and to our president.

Awarding of prizes to researchers who have dedicated their scientific activities to the truffle and to truffle cultivation.

The President of the Comunità Montana gives a commemorative plaque to retired researchers or those near retirement who have distinguished themselves through their research on truffle and on truffle cultivation.

Anna Fontana: To she who first had the idea and produced the first truffle plant. From that first mycorrhizal synthesis (*Pinus strobus* L. x *Tuber maculatum* Vittad.), and from her research on mycorrhizae, modern truffle cultivation was born.

Gérard Chevalier: To he who dedicated his very life to the development of French truffle cultivation. Through his charisma, his wide-ranging work as a scientist, he introduced and improved the cultivation of *Tuber melanosporum* and *Tuber uncinatum* in various truffle-producing areas in France.

Bruno Granetti: To the first Umbrian researcher to do both basic and applied research on the truffle and on truffle cultivation. His enthusiasm and his meticulousness have made it possible both for numerous research projects to be successfully completed and for numerous productive truffle orchards to be created.

Mario Palenzona: To the researcher who, through his institutional activities and hobbies, took the basic and applied research of Italian and French researchers to another level, making it possible to begin a rational cultivation of truffles in Piedmont and in other Italian regions.


CLEMENTI XII P O M


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Appendice

Verbale della Commissione
“Metodo europeo di analisi e certificazione delle piante micorrizate”

La riunione si è tenuta presso la sala del Congresso alla presenza di tutti i congressisti.
Il prof. Bencivenga apre la discussione ricordando che a Cordoba, in occasione di “World Fungi” (10-16 dicembre 2007) era emersa la necessità di uniformare, in Europa, il metodo di analisi e certificazione delle piante micorrizate. L’Italia ha un suo metodo, su basi morfologiche, elaborato da una commissione di esperti nel 1985 e rivisto, su invito di nove Regioni italiane, nel 1995. Detto metodo, che poteva essere una base di discussione, fu inviato ai partecipanti alla riunione di Cordoba con l’intento di concludere la discussione qui a Spoleto. Purtroppo gli impegni di ciascuno di noi hanno impedito di portare a termine il compito assegnatoci a Cordoba.
Per la elaborazione del metodo è necessario, innanzitutto, stabilire alcuni parametri minimi che purtroppo non hanno il conforto della sperimentazione scientifica. I parametri da stabilire sono:

1) la percentuale di apici radicali uniti in simbiosi con il tartufo per poter considerare una pianta micorrizata idonea alla coltivazione dei tartufi. Ovviamente più è alta la percentuale di apici micorrizati, migliore è la pianta; va rilevato, però, che non si conosce il numero minimo di apici micorrizati che deve possedere una pianta per produrre i tartufi una volta collocata a dimora. I ricercatori italiani, sulla base delle esperienze personali fatte in anni di controlli delle piante micorrizate, hanno ritenuto di stabilire una percentuale minima di micorrizazione del 30%;
2) la percentuale di piante tartufigene presenti in un lotto omogeneo per essere considerato idoneo ai fini della tartuficoltura. Il metodo italiano prevede che detta percentuale debba non essere inferiore all’80% e che nessuna pianta del lotto sia priva di micorrize.

Dopo aver individuato i parametri sopra accennati si pongono altri interrogativi:

a) come si valuta la percentuale di apici radicali micorrizati sul totale degli apici di una pianta? Basta una stima visiva o è necessario contare tutti gli apici micorrizati e quelli nudi presenti nell’apparato radicale? Nel primo caso l’analisi è speditiva, ma soggettiva e come tale non ha valore legale in caso di controversie. Nel secondo caso si pone il problema di quali e quante radici devono essere esaminate: da lavori fatti in passato risulta che la percentuale esatta può scaturire solo analizzando tutti gli apici radicali;
b) è sufficiente l’analisi morfologica o questa deve essere integrata da un’analisi molecolare?
c) l’analisi deve essere solo molecolare?

E’ a questi quesiti che una commissione di lavoro dovrà rispondere per formulare un metodo di analisi che possa essere utilizzato in tutta Europa.
All’introduzione di Bencivenga prosegue un’ampia discussione dalla quale emerge che la stima visiva, fatta da persone competenti e con buona esperienza, consente di valutare correttamente la percentuale di micorrizazione di una pianta. La stima, inoltre, consente di analizzare decine di piante nell’unità di tempo, mentre la conta degli apici richiede tempi molto più lunghi.
L’esame visivo, che si basa esclusivamente sui caratteri morfologici, non sempre è in grado di garantire l’identificazione delle micorrize in esame soprattutto nei periodi in cui non è presente il micelio peritrofico; in questi casi è necessario affiancare all’analisi morfologica quella molecolare. La Regione dell’Umbria ha apportato alcune modifiche al metodo formulato nel 1995 tra le quali la più importante è la verifica molecolare delle analisi morfologiche: dopo l’analisi morfologica il metodo prevede il prelievo di alcune micorrize da sottoporre ad analisi molecolare. Viene prelevato ed analizzato un campione per ogni morfotipo di micorrize presenti nella pianta in esame.
Il prof. Arcioni non è d’accordo perché ritiene che l’analisi delle piante debba essere fatta con metodi moderni che garantiscono l’assoluta certezza del materiale in esame. L’analisi molecolare condotta su un miscuglio di più micorrize consente di valutare la presenza e

l'abbondanza delle micorrize prodotte dai funghi presenti: l'analisi molecolare rileva una sola micorriza estranea in un miscuglio di 50 micorrize.
La maggior parte dei presenti è d'accordo di utilizzare l'analisi morfologica e di ricorrere all'analisi molecolare quando si ha il minimo dubbio per le seguenti ragioni:

- la possibilità di fare migliaia di analisi in un periodo di tempo limitato a circa un mese, cioè al periodo durante il quale i vivai richiedono la certificazione delle loro piante;
- contenere le spese che i vivaisti devono sostenere per la certificazione delle proprie piante che si traducono in costi maggiori per i tartuficoltori.

A conclusione della riunione viene proposto di nominare una commissione costituita da rappresentanti di istituzioni/enti con comprovata esperienza di analisi e certificazione delle piante tartufigene e da rappresentanti di vivai produttori di piante micorrizate. La commissione dovrà essere costituita da quattro persone per ogni nazione tartufigena: due ricercatori e due vivaisti.

Per l'Italia le persone proposte sono:
Donnini Domizia, ricercatore presso il Dipartimento di Biologia Applicata dell'Università di Perugia e Leonardo Baciarelli Falini, incaricato dal Dipartimento di effettuare le Analisi presso i vivaisti.
Per i vivaisti propongo l'Umbraflor quale struttura a partecipazione pubblica e Raggi Vivai, azienda con consolidata esperienza.

Mattia Bencivenga

Minutes of the Commission
"European method of analysis and certification of mycorrhizal plants"

The meeting was held in the Conference hall in the presence of all the Conference participants.
Professor Bencivenga opens the discussion by reminding everyone that in Cordoba, on the occasion of "World Fungi" (December 10-16, 2007), the need to render uniform the method of analysis and certification of mycorrhizal plants in Europe became evident. Italy has its own method, based on morphology, drafted by a commission of experts in 1985 and revised, at the request of nine Italian Regions in 1995. Said method, which could have provided a basis for discussion, was sent to those participating in the Cordoba meeting, with the intent of concluding the discussion here in Spoleto. Unfortunately, all of our commitments kept us from following through on the task assigned to us in Cordoba.
To define the method in all its details it is necessary, above all, to establish some minimum parameters which unfortunately are not supported by scientific experimentation. The parameters to be established are:

1) The percentage of root tips involved in symbiosis with the truffle needed be able to consider a mycorrhizal plant suitable for the cultivation of truffles. Obviously, the higher the percentage of mycorrhized tips, the better the plant; it must be noted, however, that we do not know the minimum number of mycorrhized tips a plant has to have to be able to produce truffles once it has been planted. The Italian researchers, on the basis of personal experience accrued over years of contact with mycorrhized plants, decided to establish a minimum percentage of mycorrhization at 30%.
2) The percentage of truffle-producing plants present in a homogenous lot needed for the lot to be considered well-suited to the goals of truffle cultivation. The Italian method requires that said percentage be no less than 80% and that no plant on the lot be lacking in mycorrhizae.

After having identified the above-mentioned parameters, other questions arose:

a) How does one evaluate the percentage of mycorrhized root tips with respect to a plant's total tips? Is a visual estimation enough, or is it necessary to count all the mycorrhized roots and all the bare roots present in the root system? In the first case, the analysis is rapid but subjective, and as such has no legal value when there are controversies. In the second case, the question arises as to which and how many roots have to be checked: from past attempts, we have learned that the exact percentage can be obtained only if all the root tips are analyzed.
b) Is a morphological analysis sufficient, or must it be made in conjunction with a molecular analysis?
c) Should the analysis just be molecular?

These are the questions a working Commission will have to answer to formulate a method of analysis which can be used in all of Europe.
After Bencivenga's introduction, a long discussion ensued from which the following consensus emerged: a visual estimate made by well-trained people with solid experience makes a correct evaluation of the percentage of mycorrhization in a plant possible. What's more, the estimate makes it possible to analyze twenty or thirty (or more) plants at a given time, while the counting of tips requires a much longer time frame.
A visual exam, which is based exclusively on morphological characteristics, is not always able to guarantee the identification of the mycorrhizae under examination, above all in periods when the peritrophic mycelium is not present. In these cases, it is necessary to support the morphological analysis with a molecular one. The Region of Umbria made some modifications of the method formulated in 1995, the most important of which is the molecular verification of morphological analyses. After the morphological analysis, the method calls for the removal of

some mycorrhizae for the purpose of molecular analysis. A sample is taken and analyzed of ever morphotype of mycorrhizae present in the plant being checked.

Professor Arcioni does not agree because he holds that plants must be analyzed with modern methods which guarantee absolute certainty with regard to the material being examined. The molecular analysis of a mixture of mycorrhizae makes it possible to evaluate the presence and the amount of mycorrhizae produced by the fungi present; a molecular analysis is able to detect even one foreign miycorrhiza in a mixture of 50 mycorrhizae.
The majority of those present agree that is it best to use a morphological analysis and to have recourse to a molecular analysis when there is even the slightest doubt about results, for the following reasons:

this makes it possible to do thousands of analyses in a limited period of time (about a month), that is, the period in which nurseries request certification for their plants;

keeps down costs for nurseries that have to certify their trees, and, as a consequence, keeps them down for truffle farmers as well.

At the end of the meeting, the proposal is made to nominate a commission made up both of representatives of experts with documented experience in the analysis and certification of truffle-producing plants and of representatives of nurseries that produce mycorrhizal plants. Every truffle-producing country is to name four members to the commission: two researchers and two nursery people.

For Italy, the people proposed are:
Donnini Domizia, a researcher at the Department of Applied Biology, University of Perugia, and Leonardo Baciarelli Falini, hired by the department to effect analyses at nurseries.
For the nursery representatives, I propose Umbraflor as it is involved in the public sector and Raggi Vivai, a company with a great deal of experience.

Mattia Bencivenga

Riunione Commissione
Coltivazione *Tuber aestivum* Vittad.

La riunione si è tenuta il 25/11/2008 alle ore 18,30 presso una sala del Chiostro di San Nicolò, sede del Congresso di Spoleto.
Erano presenti Bencivenga, Chevalier, De Miguel, Honrubia, Bragato, Pacioni, Gregori, Zambonelli, Wang, Wedèn, Raglione, Donnini, Di Massimo, Baciarelli Falini, Sourzat, Reyna, Estrada, Salerni, Baglioni, Murat, Guerin-Laguette, Bruhn e altri.
Bencivenga apre la discussione ricordando che la riunione attuale fa seguito a quella tenuta a Cordoba in occasione di "World Fungi" (10-16 dicembre 2007).
In quell'occasione era emerso che non esistono esperienze consolidate di coltivazione di *Tuber aestivum* pur essendo la specie più diffusa e consumata in Europa. Bencivenga ricorda che fu proposto un programma di ricerca che prevedeva di impiantare, in Italia, Francia e Spagna, una tartufaia coltivata di tartufo estivo utilizzando materiale autoctono. Si pensò anche di interessare un vivaio a cui mandare semi e tartufi per far produrre, in maniera omogenea, le piante micorrizate da utilizzare nell'impianto delle tartufaie nei tre paesi. Purtroppo i buoni propositi non si sono concretizzati.
Segue un'animata discussione sulla distinzione di *Tuber aestivum* e *Tuber uncinatum* alla quale partecipano quasi tutti i presenti. Alcuni sostengono che si tratti di due entità ecologicamente diverse in quanto in alcune località fruttificano solo in estate ed in altre solo in autunno-inverno. Altri sostengono che esistono piante che producono sia in estate che in autunno-inverno. Altri riferiscono che piantagioni di *Tuber uncinatum* realizzate a bassa quota non hanno fornito produzioni al contrario di quanto si è verificato a quote elevate.
Chevalier fa presente che la coltivazione di *Tuber uncinatum*, in Francia, sta fornendo ottimi risultati ed ha grandi prospettive di sviluppo. Consente una piantagione più densa rispetto a *Tuber melanosporum* e terreni non utilizzabili per questa ultima specie di tartufo.
Al termine della discussione Bencivenga trae le seguenti conclusioni:

- la coltivazione di *Tuber aestivum* è molto importante perché è la specie di tartufo che, avendo un prezzo contenuto, è la più consumata in Europa;
- a causa dei cambiamenti climatici, si stanno riducendo le superfici destinabili alla coltivazione di *Tuber melanosporum* la cui produzione, pur essendo sottoposto a coltivazione, è in declino;
- la produzione naturale di *Tuber aestivum* è ancora sufficientemente elevata, ma è probabile una prossima riduzione per le stesse cause che hanno determinato la riduzione delle specie più pregiate;
- le poche esperienze condotte in Italia dimostrano che la coltivazione di *Tuber aestivum* non rivela troppe difficoltà;
- possono essere utilizzati terreni situati a quote molto diverse purché non siano eccessivamente argillosi o sabbiosi o a reazione sub-acida;
- considerando la grande variabilità degli ambienti dove la specie cresce allo stato spontaneo, si può supporre che nell'ambito della specie siano presenti ecotipi diversi, per cui si ritiene importante impiantare le tartufaie utilizzando piante micorrizate con materiale autoctono;
- nella piantagione è bene rispettare le condizioni di crescita del tartufo spontaneo utilizzato per la produzione delle piante (per es. se il tartufo proviene da piante isolate, è bene utilizzare un'ampia densità di impianto);
- anche nei riguardi delle piante simbionti la specie non mostra particolari esigenze, in natura è stata osservata un'interazione positiva nella consociazione di una conifera con una latifoglia.

Bencivenga conclude auspicando l'intensificazione delle sperimentazioni in campo e una sempre crescente collaborazione tra i ricercatori che si interessano di tartufi e tartuficoltura.

Commission Meeting
Tuber aestivum Vittad. cultivation

The meeting was held the 25/11/2008 at 18:30 in the hall of the Cloister of San Nicolò, the seat of the Spoleto Conference.
Bencivenga, Chevalier, De Miguel, Honrubia, Bragato, Pacioni, Gregori, Zambonelli, Wang, Wedèn, Raglione, Donnini, Di Massimo, Baciarelli Falini, Sourzat, Reyna, Estrada, Salerni, Baglioni, Murat, Guerin-Laguette, Bruhn and others were present.
Bencivenga opens the discussion by reminding everyone that the present meeting is a follow-up on the one held in Cordoba on the occasion of "World Fungi" (December 10th-16th, 2007).
On that occasion, it became clear in discussion that there are no documented experiences on the cultivation of *Tuber aestivum,* although it is the species most widely consumed in Europe.
Bencivenga reminds everyone that a research project was proposed that called for the planting in Italy, France, and Spain, of truffle orchards (one in each country) dedicated to the cultivation of summer truffle using autochthonous material. The idea was to get a nursery involved to which seeds and truffles could be sent so that mycorrhizal plants could be produced in a homogenous manner and then used to set up truffle orchards in the three countries. Unfortunately, these good intentions were never translated into action.
An animated discussion follows on the distinction between *Tuber aestivum* and *Tuber uncinatum* in which almost everyone present participates. Some maintain that we are dealing with two ecologically different entities in that in some locations, they bear fruit only in summer and in others, only in autumn-winter. Others claim that plants do exist which produce both in summer and in autumn-winter. Others report that *Tuber uncinatum* plantations set up at low altitudes had no production, in contrast with what occurred at high altitudes.
Chevalier recounts that *Tuber uncinatum* cultivation in France is giving excellent results and that there are excellent prospects for development. It makes possible a plantation denser than plantations obtained with *Tuber melanosporum* and the use of terrains which cannot be used for the latter species of truffle.
At the end of the discussion, Bencivenga draws the following conclusions:

- the cultivation of *Tuber aestivum* is very important because, as its price is not excessively high, it is the most widely-consumed in Europe.
- because of climate change, the areas which can be used for the cultivation of *Tuber melanosporum* are diminishing; therefore, *Tuber melanosporum* production, although this truffle is being cultivated, is in decline;
- natural production of *Tuber aestivum* is still sufficiently high, but it is likely that production will soon diminish, for the same reasons that have brought about the reduction of the more valuable species;
- the few attempts made in Italy have demonstrated that *Tuber aestivum* cultivation does not present any particular difficulties;
- terrains at different altitudes may be used, as long as they are nor excessively argillaceous or sandy or at sub-acid reaction;
- given the wide range of environments in which the species grows wild, it can be assumed that in the range of the species different ecotypes are present, and for this reason it is important to plant truffle orchards using plants micorrhized with autochthonous material;
- in a plantation, it is best to respect the growth conditions of the wild truffle used in the production of the plants (for example, if the truffle comes from isolated plants, it is a good idea to plant at ample intervals);
- with regards to symbiont plants as well, the species is not particularly demanding; in nature, a positive interaction has been observed in association with.

Bencivenga concludes with the hope that experimentation in the field will intensify and that there will continue to be an ever-growing collaboration among researchers interested in truffles and truffle cultivation.

ECOLOGY AND POPULATION BIOLOGY SESSION

TAXONOMY, BIOLOGY AND ECTOMYCORRHIZAE SESSION

CONSERVATION, COMMERCE AND VALORISATION SESSION

CULTIVATION SESSION

finito di stampare