

Suitability of lime trees for *Tuber melanosporum* culture: mycorrhizae, soil studies, and truffle harvest associated with *Tilia platyphyllos* and *T. x vulgaris*

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Truffles are highly profitable cash crops which grow in forests of many Mediterranean regions. The most important studies on the ecology of *Tuber melanosporum* have been carried out in forests of *Quercus*, *Corylus*, and *Ostrya*. Although it has been reported that this truffle can form mycorrhizae also with *Tilia*, there are no studies on *Tuber melanosporum* fruiting body production associated with lime trees. For this reason, we have carried out a study on mycorrhizae, soils, and fruiting body production in river woods with *T. platyphyllos* and *T. x vulgaris* located in Central Spain. In the study area, we found 13 *Tuber melanosporum* burns associated with *Tilia platyphyllos* and other host plants. Burns associated also with *Tilia* showed less significant production than others with *Quercus faginea*, *Q. ilex*, and *Corylus avellana*. Nevertheless, our studies revealed that the soil properties of these river woods with *Tilia* are very favourable for *Tuber melanosporum*. However, we confirmed that in this region not a single burn had ever been found with *T. melanosporum* production associated exclusively with *Tilia*, without *Quercus* or *Corylus* presence. We conclude that at present *Tilia platyphyllos* and *T. x vulgaris* are of little interest to *Tuber melanosporum* truffle culture, as they hinder fruiting body production.

Keywords: *Corylus avellana*, *Quercus faginea*, *Quercus ilex*, truffle production, Central Spain.

Tuber melanosporum Vittad. (black truffle of Périgord) is a hypogeous ectomycorrhizal ascomycete belonging to the Pezizales order (Tuberaceae family). Due to its intense aroma, it has great culinary and commercial value (more than 1000 € kg⁻¹). The most important studies on the ecology and production of *T. melanosporum* fruiting bodies

have been done in forests and thickets of *Quercus* L., *Corylus* L. and *Ostrya* Scop. located in the Mediterranean areas of France, Italy and Spain. However, it is known that *Tuber melanosporum* can form mycorrhizae with plants of the genus *Tilia* L. (lime trees), but there are no studies of *Tuber melanosporum* association with natural or cultivated *Tilia* populations (Callot *et al.* 1999, Ricard 2003).

The relationship between truffles (*Tuber* Micheli ex Wiggers) and *Tilia* was known in the past (Palenzona & Fontana 1970). Moreover, Gregori & Ciappelloni (1990), Tocci & Lopiparo (1990) and Bencivenga *et al.* (1995) indicate that *Tilia* mycorrhized plants in commercial nurseries devoted to truffle culture. In the last decade, interest in the relationship between *Tuber* and *Tilia* has increased, and has focused on the molecular biology of its mycorrhizae (Amicucci *et al.* 1996, Zeppa *et al.* 2000, Gioacchini *et al.* 2002, Giomaro *et al.* 2002, Polidori *et al.* 2002, Guescini *et al.* 2003, Sisti *et al.* 2003, Menotta *et al.* 2004a, 2004b).

In the Alto Tajo Nature Reserve in central Spain, *Tuber melanosporum* fruiting bodies have been harvested continuously for the last 40 years in mixed river woods with natural formations of *Tilia platyphyllos* Scop. and *T. x vulgaris* Hayne (= *T. x europaea* auct. p. p.) (Fig. 1). This mountainous region shares many similarities with other European habitats in which *Tuber melanosporum* thrives, but it also presents differences with regard to high altitudes and cold climate (García-Montero *et al.* 2005). The southernmost Spanish location of these *Tilia* corresponds to the Alto Tajo Nature Reserve, coinciding with the worldwide southern distribution limit of these species (Browicz 1968, Aedo 1995).

Based on the current knowledge outlined above, the objective of the present study was to check whether or not *Tuber melanosporum* forms mycorrhizae with *Tilia platyphyllos* and *T. x vulgaris*, and if it is thereby producing fruiting bodies in significant numbers. To determine this, 100 burns of *Tuber melanosporum* were monitored to follow the harvest of fruiting bodies over a period of 7 years. A further aim of this study is to verify the presence/absence of *Tilia* as a symbiotic host in the 100 *Tuber melanosporum* burns in order to ascertain whether this truffle has the capacity to generate fruiting bodies associated with *Tilia* alone, without other symbiotic plants. The interest of these studies lies in helping to elucidate the utility of *T. platyphyllos* and *T. x vulgaris* in *Tuber melanosporum* truffle culture.

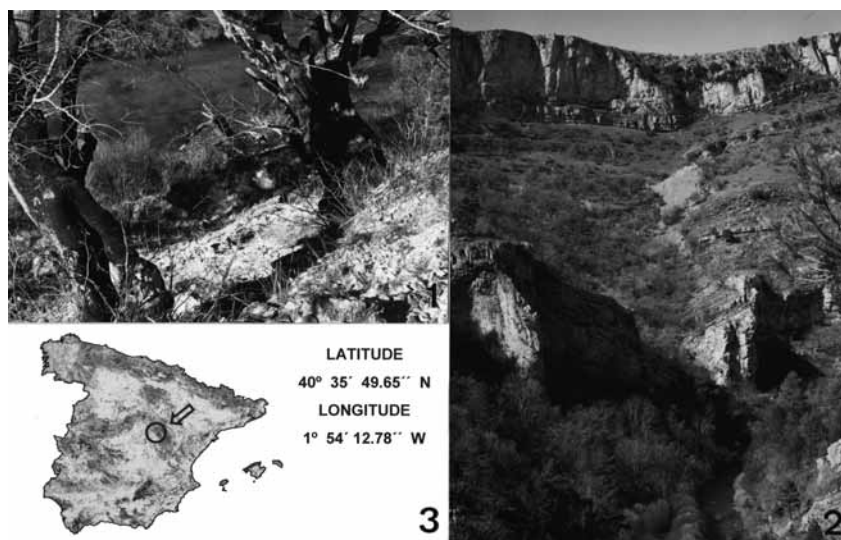
Materials and Methods

Study area

The study area is situated in an adjoining series of ravines and deep gorges dug by the Tajo River (Fig. 2), located within the Alto

Tajo Nature Reserve, on the border between the Belvalle tract and the municipal district of Peralejos de las Truchas (geographic coordinates: 40°35'49.65" N; 1°54'12.78" W) (Fig. 3). This study area is situated in a mountainous region, at an altitude ranging between 1200 m and 1500 m, in a supra Mediterranean bio climatic belt, with a sub humid, shady climate. Average annual precipitation is 797 mm, with low average yearly temperatures (9.7 °C) and very cold winters (coldest month with minimum temperature mean between -7 and -4 °C).

In this area we located 100 *Tuber melanosporum* burns in three habitat types sited close together and sharing similar topographic and mesoclimatic conditions (five km radius). Burns describe spots where *T. melanosporum* grow and produce fruiting bodies. The name 'burn' refers to its phytotoxic capacity and ability to create clearings in the vegetation where its fruiting bodies bear fruit (Fasolo-Bonfante *et al.* 1971, Papa 1992). The three neighbouring habitats were defined according to their vegetation, symbiotic plants, physiography, and stoniness. For a detailed description of the study area and its habitats see García-Montero *et al.* (2005).



Figs. 1-3. Study area: 1. *Tuber melanosporum* burn associated with two *Tilia platyphyllos* specimens and other symbiotic plants. 2. Three studied habitats located on the slopes of deep gorges dug by the Tajo River. 3. Geographic location.

Habitat I

River woods of *Corylus avellana* L., *Tilia platyphyllos* and *Quercus faginea* Lam. The habitat contains riparian formations hosting *Tuber melanosporum* in areas situated at the base of the slopes of ravines along the Tajo River. The woods belong to the

Astrantio-Coryleto avellanae S. geobotanical association. Inventories were established for 13 burns with the presence of *Tilia platyphyllos* and other symbiotic plants.

Habitat II

Habitat with *Tuber melanosporum* burns located in mono-species *Quercus faginea* formations of the *Cephalanthero longifoliae-Querceto fagineae* S. geobotanical series. This habitat contains open woods situated halfway up the slopes of ravines along the Tajo River. Inventories were established for 65 burns.

Habitat III

Habitat with *Tuber melanosporum* burns located in stands of *Quercus ilex* L. subsp. *ballota* (Desf.) Samp. This habitat contains open woods of the *Junipero thuriferae-Querceto rotundifoliae* S. geobotanical association, situated at the top of slopes along Tajo River ravines. Inventories were established for 22 burns.

Study of truffle production

Tuber melanosporum has been harvested in the study area since the end of the 1960s. The local county councils regulate harvesting and restrict the number of collectors. Truffles are harvested with the help of trained dogs. This study was based on collaboration with five truffle collectors between 1994 and 2000. These five collectors indicated the 100 burn sites with the greatest truffle production in the three habitats of the study area. The exact geographic coordinates of these burns are not given to maintain the secrecy of the *T. melanosporum* locations.

For our statistical studies, we used the harvesting data of the five gatherers who regularly exploited most of the 100 selected burns. The maximum annual production of these burns has been calculated using the following procedure (García-Montero 2000): Between 1994 and 2000, each of the five collectors noted their monthly collection of fresh fruiting bodies, in grams, from each of the 100 burns. Throughout these 7 years we made a series of systematic field trips with the 5 collectors every 15 days during the *Tuber melanosporum* collecting season. On these trips, we confirmed the collection data for the ascomata on that day, and we checked the monthly collection records of the five gatherers. At the end of each year, we added up the data of the 5 collectors to obtain the total yearly yield of ascomata for each of the 100 burns. After 7 years of observation, we determined the maximum annual *T. melanosporum*

fruiting body production/yield for each burn. Only the data for the maximum annual production of each burn was used in the statistical analyses. This method enabled us to standardise the production data for the 100 burns, and to minimise the effects of annual microclimatic variations, random effects of actions by fauna, and any collection errors made throughout the 7 years of the study.

Quantitative analysis

We carried out a statistical analysis to determine whether or not there were significant production differences between the three different habitats of *Tuber melanosporum*. This was done by choosing the logarithm of annual maximum production of each burn as the dependent variable. The statistical treatment was performed with the Statistica Program v. 6 (StatSoft, Inc., Tulsa, Ok, 1999). Normality was checked using the Shapiro-Wilks' Test. The homogeneity of variance of the ANOVA test was checked by using the Levene test. The independence was checked by using the Barlett test.

Study of the host plants and the mycorrhizae

We checked for the presence/absence of *Tilia* as compared to the occurrence of *Quercus* and *Corylus* trees as symbiotic partners of *Tuber melanosporum* in the 100 burns. Lastly, we interviewed 14 truffle gatherers in the Alto Tajo Nature Reserve. Our aim was to discover whether or not there were any burns with fruiting body production under *Tilia platyphyllos* and/or *T. x vulgaris* alone, without *Quercus* and *Corylus*.

The existence of natural symbiosis between *Tilia platyphyllos* and *Tuber melanosporum* was verified in *Tilia platyphyllos* burns. This was done by sampling five roots of *T. platyphyllos* in the interior of productive burn sites. Sampling was done in accordance with the procedures of Di Massimo *et al.* (1996) and Bencivenga *et al.* (1995), and mycorrhizae from these samples were studied. The mycorrhizae were identified using a stereoscopic microscope (Leica WildMZ8) and a transmitted-light microscope (Leica LeitzDMRB) following Verlhac *et al.* (1990), Bencivenga *et al.* (1995), and Granetti's (1995) descriptions and recommendations.

Soil analysis

Soil samples, each about 2 kg, were taken from four burns located at the river wood sites with *Tilia platyphyllos* (Habitat I): two samples inside burns from which the greatest number of *Tuber melanosporum* fruiting bodies had been extracted in recent years,

and, at a distance of ca. 50 m, two reference samples inside burns without recent ascomata production. Soil analysis and classification was carried out according to the FAO (1990). Only the first 30 cm of each soil profile was studied because *Tuber melanosporum* ascomata are usually formed in this soil zone (Verlhac *et al.* 1990). The following determinations were made: pH, total organic carbon (TOC), percentage of total calcium carbonate (equivalent carbonate), granulometric analysis, cationic exchange capacity (CEC), following the methods of the ISRIC (1995); total nitrogen was analysed with the variant of the Bouat & Crouzet (1965) method. The determination of exchangeable cations of Ca^{2+} and Mg^{2+} was done using AAS (Philips UP9100x), and K^{+} was assayed with a flame photometer (Sherwood 410).

Taxonomic studies

Tilia platyphyllos Scop. Specimens studied: three samples; *loc.* Belvalle, Beteta (Cuenca, Spain); June 1998; *leg.* L. G. García-Montero, G. Di Massimo & D. Moreno; voucher specimens obtained from the *Tuber melanosporum* burns in the study area were conserved in the AH herbarium (AH 27003). The taxonomic identification was confirmed based on Aedo (1995) and Browicz (1968).

Tilia x vulgaris Hayne. Specimens studied: three samples; *loc.* Belvalle, Beteta (Cuenca, Spain); June 1998; *leg.* L. G. García-Montero, G. Di Massimo & D. Moreno; voucher specimens were conserved in the AH herbarium (AH 27002). This tree presents typical hybrid characteristics, which are intermediate between *T. platyphyllos* and *T. cordata* (Browicz 1968, Aedo 1995).

Tuber melanosporum Vittad. Specimens studied: three samples; *loc.* Belvalle, Beteta (Cuenca, Spain); February 2002; *leg.* D. Moreno & L. G. García-Montero; the fruiting bodies from burns in the study area were conserved in the AH Herbarium (AH 35542, AH 35543, AH 35544).

Results

It has been shown that *Tilia platyphyllos* spontaneously forms mycorrhizae with *Tuber melanosporum* in the study area: microscopic studies of the roots' morphological characteristics allowed a clear identification of *T. melanosporum* mycorrhizae.

We carried out an ANOVA to determine whether the variations in production of *Tuber melanosporum* between the 13 burns with *Tilia platyphyllos* in Habitat I matched those of the 87 burns of the other habitats. This test revealed significant differences ($F = 11.0825$;

$p < 0.0001$) indicating that each yield of *Tuber melanosporum* could be associated with different micro-environments and with the different symbiotic plants in the three habitats. Table 1 shows the average values for fruiting body production in each habitat type. To analyse differences in the averages, another *post hoc* test was done (LSD Tukey test), the results of which indicated that production was significantly lower in the 13 burns of habitat I with *Tilia platyphyllos* (LSD test, probabilities for post-hoc tests; habitat I vs. habitat II: $p = 0.00015$; habitat I vs. habitat III: $p = 0.045$; habitat II vs. habitat III: $p = 0.085$).

In addition, we compared the tree diversity (*Quercus* spp./*Corylus* and *Tilia* spp. or *Tilia* alone) at *Tuber melanosporum* burns: no burn was found with *Tilia x vulgaris*, and none of the 100 burns was associated exclusively with *T. platyphyllos* (Tab. 1). Interviews conducted with 14 truffle gatherers revealed that during 40 years they have not seen a burn with fruiting body production that was associated exclusively with *T. platyphyllos* or *T. x vulgaris* (without *Quercus* and *Corylus*) in the Alto Tajo Nature Reserve.

The results of our soil analyses indicate that soil properties of burns and woods with *Tilia platyphyllos* and *T. x vulgaris* in this area favour the production of *Tuber melanosporum* [Verlhac *et al.* (1990), Callot *et al.* (1999), and Ricard (2003)] (Tab. 2). Moreover, the properties of the four *Tilia* soils are very similar to that of 30 soils from five *Tuber melanosporum* habitat types in this region (García-Montero 2000). Most of these 34 soils have a moderately basic pH and high percentages of carbonates. Their levels of organic carbon are moderate and their C/N ratio is close to 10, which indicates good humidification. They have comparatively high values of exchangeable cation complex. The soil structure is good with a granular tendency and abundant pores, and the texture tends to be sandy clay loam, all of which give the soil good permeability and aeration.

Tab. 1. – One hundred burns with *Tuber melanosporum* fruiting body production in three habitats with or without *Tilia platyphyllos* in several ravines of the Tajo River. Production = annual production of fruiting bodies per burn (fresh weight); N = sample size; SD = standard deviation.

Habitat code	Host trees	Number of burns (N)	Mean production (g/y)	SD	Minimum production (g/y)	Maximum production (g/y)
I	<i>Q. faginea</i> , <i>C. avellana</i> , and <i>T. platyphyllos</i>	13	319.23	305.98	30	1200
II	<i>Q. faginea</i>	65	1149.23	1130.01	100	6000
III	<i>Q. ilex</i>	22	841.82	906.90	60	3000

Tab. 2. – Description of four soil samples from Habitat I (river woods with *Tilia platyphyllos*). TOC = total organic carbon; N = nitrogen; CEC = cationic exchange capacity; CaCO₃ = percentage of total calcium carbonate; SD = standard deviation.

Sample code	pH _{H2O}	CaCO ₃ (%)	TOC (g kg ⁻¹)	N (g kg ⁻¹)	Ca ²⁺ (cmol kg ⁻¹)	Mg ²⁺ (cmol kg ⁻¹)	K ⁺ (cmol kg ⁻¹)	CEC (cmol kg ⁻¹)	Sand (%)	Silt (%)	Clay (%)
1	7.90	10.21	2.55	0.27	50.53	1.48	0.29	29.26	82	8	10
2	8.17	13.38	2.86	0.38	51.31	0.78	1.03	20.33	37	38	25
3	7.85	0.21	9.17	0.74	38.80	6.50	0.66	30.65	62	17	21
4	7.00	18.44	4.32	0.38	19.24	1.54	1.10	18.66	64	10	26
Mean	7.73	10.56	4.73	0.44	39.97	2.58	0.77	24.72	61	18	21
SD	0.51	7.69	3.06	0.20	14.96	2.64	0.37	6.11	19	14	7

Discussion

This study revealed significant differences in truffle production in three habitat types located in the ravines of the Alto Tajo. The results indicate that *Tuber melanosporum* yields (biomass of ascomata) from burns greatly vary with micro-environmental conditions and symbiotic plants. In the same area, production rates from burns associated with *Tilia platyphyllos* were much lower than those from burns with *Quercus* and *Corylus*. Nevertheless, the soil properties found for these *Tilia* woods are similar to that of the *Quercus/Corylus* stands in this area. Thus, these *Tilia* soils should be quite suitable for *Tuber melanosporum* without posing any notable obstacle for the development of this truffle.

Although we could confirm that *Tuber melanosporum* actually forms mycorrhizae with *Tilia platyphyllos* in the study area, no burn has ever been found with *Tuber melanosporum* production when the host trees were exclusively *Tilia* species. Bencivenga M., G. Di Massimo and G. L. Gregori (pers. com. 2004) have stated that, in Italy, they had never found *Tuber melanosporum* ascomata associated with *Tilia*, whereas Granetti *et al.* (2005) indicate that *Tuber melanosporum* might produce fruiting bodies with *Tilia* spp. under certain ecological conditions.

We conclude that *Tuber melanosporum* does not produce fruiting bodies in significant numbers when associated with isolated *Tilia platyphyllos* and *T. x vulgaris* trees. However, Pacioni (1992) stated that *T. cordata* is a common species in cool, deep soils that are suitable for *Tuber magnatum* fruiting body production; Granetti *et al.* (1995) pointed out the importance of *Tilia* spp. in the yield of *Tuber magnatum* in Italy. Like *T. magnatum*, *Tilia* spp. inhabit relatively humid and shady areas.

For these reasons, we conclude that *Tilia platyphyllos* and *T. x vulgaris* do not stimulate *Tuber melanosporum* fruiting, and their

microenvironments are not appropriate for the development of *T. melanosporum* ascomata. These results and data from the available bibliography suggest that *Tilia* is of little interest in *Tuber melanosporum* truffle culture. Consequently, for the time being, the commercial exploitation of *Tilia platyphyllos* and *T. x vulgaris* seedlings mycorrhized with *Tuber melanosporum* is not recommended.

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References

- Aedo C. (1995). *Tiliaceae*. In: *Flora ibérica: plantas vasculares de la Península Ibérica e Islas Baleares* (eds. Castroviejo S., Aedo C., Cirujanos S., Laínz M., Montserrat P., Morales R., Muñoz-Garmendía F., Navarro C., Paiva J., Soriano C.), Real Jardín Botánico C.S.I.C., Madrid: 186–189.
- Amicucci A., Rossi I., Potenza L., Zambonelli A., Agostini D., Palma F., Stocchi V. (1996) Identification of ectomycorrhizae from *Tuber* species by RFLP analysis of its region. *Biotechnology Letters* **18**: 821–826.
- Bencivenga M., Donnini D., Tanfulli M., Guiducci M. (1995) Tecnica di campionamento delle radici e degli apici radicali per la valutazione delle piante micorrizate. *Micologia Italiana* **24**: 35–47.
- Bouat A., Crouzet C. (1965) Notes techniques sur un appareil semi-automatique de dosage de l'azote et de certains composés volatils. *Annales Agronomiques* **16**: 107–118.
- Browicz K. (1968) *Tilia*. In: *Flora europaea* (eds. Tutin T. G., Heywood V. H., Burges N. A., Valentine D. H., Walters S. M., Webb D. A.), Cambridge University Press, Cambridge: 247–248.
- Callot G. (1999) *La truffe, la terre, la vie*. INRA, Versailles.
- Di Massimo G., García-Montero L. G., Manjón J. L., Díez J. L. (1996). Hongos micorrícicos competidores de *Tuber melanosporum* Vitt. presentes en ecosistemas naturales y cultivos del centro de España. *Boletín de la Sociedad Micológica de Madrid* **21**: 189–199.
- FAO (1990) *Guideline for soil description*. FAO, Roma.

- Fasolo-Bonfante P., Fontana A., Montacchini F. (1971) Studi sull'ecologia del *Tuber melanosporum*. I. Dimostrazione di un effetto fitotossico. *Allionia* **17**: 48–53.
- García-Montero L. G. (2000) *Estudio sobre la trufa negra Tuber melanosporum Vitt. en el centro de España: patrones ecológicos, análisis edáficos e interacciones de micorrizas en la truficultura*. PhD Thesis, University of Alcalá, Spain.
- García-Montero L. G., Di Massimo G., Pascual C., Casermeiro M. A., Manjón J. L. (2005) Habitats of *Tuber melanosporum* in the central Iberian Peninsula (High Tajo Basin). *Mycologia Balcanica* **2**: 197–203.
- Gioacchini A. M., Menotta M., Polidori E., Giomaro G., Stocchi V. (2002) Solid-phase microextraction gas chromatography/ion trap mass spectrometry and multistage mass spectrometry experiments in the characterization of germacrene D. *Journal of Mass Spectrometry* **37**: 1229–1235.
- Giomaro G., Sisti D., Zambonelli A., Amicucci A., Cecchini M., Comandini O., Stocchi V. (2002) Comparative study and molecular characterization of ectomycorrhizas in *Tilia americana* and *Quercus pubescens* with *Tuber brumale*. *FEMS-Microbiology-Letters* **216**: 9–14.
- Granetti B. (1995) Caratteristiche morfologiche, biometriche e strutturali delle micorrize di *Tuber* di interesse economico. *Micologia Italiana* **2**: 101–117.
- Granetti B., Angelini P., Rubini A. (1995) Morfologia e struttura delle micorrize di *Tuber magnatum* Pico e *Tuber borchii* Vitt. con *Tilia platyphyllos* Scop. *Micologia Italiana* **24**: 27–34.
- Granetti B., De Angelis A., Materozzi G. (2005) *Umbria terra di tartufi*. Regione Umbria, Terni.
- Gregori G. L., Ciapelloni R. (1990) *Produzione di piantine micorrizzate con Tuber magnatum Pico*. In: *Secondo congresso internazionale sul tartufo* (eds. Ben-civenga M., Granetti B.), Comunità Montana dei Monti Martani e del Serrano, Spoleto: 211–218.
- Guescini M., Pierleoni R., Palma F., Zeppa S., Vallorani L., Potenza L., Sacconi C., Giomaro G., Stocchi V. (2003) Characterization of the *Tuber borchii* nitrate reductase gene and its role in ectomycorrhizae. *Molecular Genetics and Genomics* **269**: 807–816.
- ISRIC (1995) *Procedures for Soil Analysis*. FAO, Wageningen.
- Menotta M., Amicucci A., Sisti D., Gioacchini A. M., Stocchi V. (2004a) Differential gene expression during pre-symbiotic interaction between *Tuber borchii* Vittad. and *Tilia americana* L. *Current Genetics* **46**: 158–165.
- Menotta M., Gioacchini A. M., Amicucci A., Buffalini M., Sisti D., Stocchi V. (2004b) Headspace solid-phase microextraction with gas chromatography and mass spectrometry in the investigation of volatile organic compounds in an ectomycorrhizae synthesis system. *Rapid Communications in Mass Spectrometry* **18**: 206–210.
- Pacioni G. (1992) *El cultivo moderno y rentable de la trufa*. De Vecchi, Barcelona.
- Palenzona M., Fontana A. (1970) Synthèse des mycorrhizes de *Tuber magnatum* avec semis de *Quercus pubescens*. *Mushroom Science* **10**: 1007–1012.
- Papa G. (1992) Componenti di *Tuber melanosporum* con attività fitotossica. *Micologia e Vegetazione Mediterranea* **7**: 109–110.
- Polidori E., Agostini D., Zeppa S., Potenza L., Palma F., Sisti D., Stocchi V. (2002) Identification of differentially expressed cDNA clones in *Tilia platyphyllos*-*Tuber borchii* ectomycorrhizae using a differential screening approach. *Molecular Genetics and Genomics* **266**: 858–864.
- Ricard J. M. (2003) *La truffe. Guide technique de trufficulture*. Centre Technique Interprofessionnel des Fruits et Légumes CTIFL, Paris.
- Sisti D., Giomaro G., Cecchini M., Faccio A., Novero M., Bonfante P. (2003) Two genetically related strains of *Tuber borchii* produce *Tilia* mycorrhizas with different morphological traits. *Mycorrhiza* **13**: 107–115.

- Tocci A., Lopiparo G. (1990) *Strategia di ricerca sul tartufo*. In: *Secondo congresso internazionale sul tartufo* (eds. Bencivenga M., Granetti B.), Comunità Montana dei Monti Martani e del Serano, Spoleto: 591–602.
- Verlhac A., Giraud M., Leteinturier J. (1990) *La truffe guide pratique*. Centre technique interprofessionnel des fruits et légumes CTIFL, Paris.
- Zeppa S., Vallorani L., Potenza L., Bernardini F., Pieretti B., Guescini M., Giomaro G., Stocchi V. (2000) Estimation of fungal biomass and transcript levels in *Tilia platyphyllos*-*Tuber borchii* ectomycorrhizae. *Fems Microbiology Letters* **188**: 119–124.

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