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Ectomycorrhizal status of a mature productive black truffle plantation

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Abstract

The truffle-plantation «Los Quejigares» was planted in 1971 by AROTZ-CATESA company. It is a 600 ha plot of *Quercus ilex* mycorrhizated with *Tuber melanosporum* at 1,250 m a.s.l. on calcareous soil. This plantation is the largest of the world and one of the eldest truffle-plantations of Spain and it is in full production. Knowledge of the mycorrhizal status of a mature black truffle plantation is significant for the improvement of truffle cultivation. Ectomycorrhizae were studied for knowing *T. melanosporum* persistence and diversity and abundance of other ectomycorrhizal types. Roots of 16 holmoaks were sampled, 12 trees produce truffle sporocarps and four did not. It was found a mean of about 35% of *T. melanosporum* ectomycorrhizae in the studied trees, being this significantly higher in the productive trees. Also, 105 more different ectomycorrhizal types were found. In spite of the high number of morphotypes found, it seems that they do not replace *T. melanosporum*, showing that there is a coexistence between species in the fungal community associated to the roots.

Key words: AROTZ-CATESA company, characterization of ectomycorrhizae, fungal diversity, *Quercus ilex*, *Tuber melanosporum,* truffle plantations.

Resumen

Comunidad de ectomicorrizas en una plantación adulta productora de trufa negra

La plantación trufera «Los Quejigares» fue establecida en 1971 por la empresa AROTZ-CATESA. Es una parcela de 600 ha de plantas de *Quercus ilex* micorrizadas con *Tuber melanosporum,* que está situada a 1.250 m de altitud sobre suelo calizo. Esta plantación es la más grande del mundo, además de una de las más antiguas de España, y se encuentra en plena producción de trufa negra. El conocimiento del estado micorrícico de las plantaciones truferas maduras es importante para mejorar las técnicas aplicadas en la truficultura. El objetivo de este trabajo es conocer la persistencia de *T. melanosporum* y la diversidad y abundancia de otras ectomicorrizas que conviven con esta especie. Se muestrearon las raíces de 16 encinas, de las que 12 producían carpóforos de trufa y cuatro no. El porcentaje medio de micorrización por *T. melanosporum* en los árboles estudiados fue del 35%, éste porcentaje fue significativamente más alto en los árboles productores. Además, se encontraron 105 tipos de ectomicorrizas. A pesar del alto número de morfotipos encontrados, parece que éstos no producen el desplazamiento de *T. melanosporum*, mostrando que es posible la coexistencia entre diferentes especies pertenecientes a la comunidad de hongos asociadas a las raíces de los árboles.

Palabras clave: AROTZ-CATESA, caracterización de ectomicorrizas, diversidad de hongos ectomicorrícicos, *Quercus ilex, Tuber melanosporum,* plantaciones truferas.

Introduction

The black truffle (*Tuber melanosporum* Vittad.) is an hypogeous ascomycete endemic to Mediterranean calcareous soils in southern Europe which is found in symbiotic association with roots of deciduous trees, mostly oaks (*Quercus* spp.) and hazelnut trees (*Corylus avellana* L.) (Delmas, 1978; Olivier *et al.*, 2002).

Black truffle is a first-class product, the production of which is clearly standstill after the sudden fall in the middle of 20th century. In the first half of this

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century the forests were given up due to the lack of labour force, changes on rural activity, and the rural exodus to the big cities in the industrialization decade. All of those, and possibly other unknown factors, made that production in wild truffle areas had a dramatic fall, going down from 200 tones in 1950's to less than 50 nowadays (Reyna Domenech, 2007).

Its production limitation and its organoleptic characteristics have made of truffles for centuries an expensive refined food, impassioned by writers and poets and destined for an elite of consumers. The frequent presence of truffles in wealthy and tycoons tables has made them a desired product that corresponds with its mysterious origin (Ciani *et al.*, 1992).

The black truffle is an important part of the culture in countries like France and Italy. Nowadays, and above all in Spain, the truffle has been introduced every time in more wide groups and its consumption is widespread into people. The increase on truffle consumption is also helped by the media, that are doing an important job in the cultural knowledge about truffle in all the countries, not only in the producers but even in the potential consumers.

In this way, the demand is at its very peak, especially from the end of 20th century, so the current average production, estimated below 100 tones per year, is not enough for satisfying the 10% of the market demand (Agrobiotruf S.A., 2007).This is one of the reasons why the cultivation of black truffle has been seriously undertaken in the last 35 years in numerous countries along the Mediterranean region, and also in New Zealand, United States, Canada, Chile or Argentina, looking for increase its production.

The first black truffle plantation in Spain was established in 1968 in the Castellón province. Three years after, Salvador Arotzarena did the first big attempt on black truffle cultivation, «Los Quejigares» plantation. He planted 150,000 trees on more than 600 ha of land (Black, 2006).

Nowadays, the cultivation of *T. melanosporum* attracts an increasing interest as an agricultural alternative in marginal lands and low-income farming regions (De Miguel and Sáez, 2005c). In fact, Soria province, basing on climate, ecological and soil conditions, has about 114,000 ha suitable for *T. melanosporum* production, 86% of them are property of private owners (Martínez-Peña *et al.*, 2008).

Truffle cultivation requires relatively low agricultural inputs, promotes reforestation and economic restoration of rural lands and land-use stability (Bonet *et al.*, 2006). The management of plantations for better black truffle production is an important issue, and over the years a large body of knowledge has been accumulated (Olivier *et al.,* 2002; Wang and Hall, 2004). However, our knowledge of the biology of *T. melanosporum* remains relatively poor, so we still do not know why some *truffières* consistently produce large quantities of truffles, while others in identical conditions do not.

This work studies the *T. melanosporum* symbiotic phase in a mature, productive black truffle plantation with the greater significance in truffle-growing knowledge and the first glance at its below-ground fungal community. The main aims of the present research are to know the *T. melanosporum* mycorrhization percentage and the diversity and abundance of other ectomycorrhizal types presents on the roots as well as to compare the ectomycorrhizal status of different trees of the truffle plantation related with different factors: cultivated/non cultivated areas, productive/non productive trees.

Material and methods

Description of the study site

The study site named «Los Quejigares», is a plantation of holm-oaks (*Quercus ilex* subsp. *ballota* (Desf.) Samp.) mycorrhizated with *T. melanosporum* and established by the company AROTZ-CATESA in 1971 in the municipality of Villaciervos (Soria, Castilla y León, Spain). The former place was a forest with wild black truffle production.

«Los Quejigares» is a 600 ha plot situated between 1,100 and 1,400 m a.s.l., in the Southern slope of the Sierra de Cabrejas (Sistema Ibérico mountain range), on calcareous and well aired soil. Medium annual rain is 780 mm and the medium annual temperature is 8.6°C. The vegetation of the surroundings are savin (*Juniperus thurifera* L.), holm-oak, lusitan-oak (*Q. faginea* Lam.) and Scots-pine (*Pinus sylvestris* L.) stands.

The holm-oaks plantation stake is 6×6 m in 400 ha and 2.5×7 in the rest 200 ha. The establishment of the plantation was doing with mycorrhizated seedlings produced in France and also by the company that manages it. For the plantation maintenance, the following cultivation treatments are doing every year: *soil cultivation*, 10 cm deep, is doing every spring to eliminate grass; *irrigation*, 250 ha are irrigated with doses of

25 L/m^2 every two weeks in July, August and September using sprinkling systems; and *pruning*, every tree is pruning in reverse cone during October and November to increase the soil insolation and make truffle hunting easier (Carbajo, 1999).

«Los Quejigares» is one of the eldest Spanish truffle-plantations and the largest of the world and, nowadays, it is in fully production of black truffle sporocarps. In 1999, the total sporocarp production was 2,500 kg (Carbajo, 1999), nowadays the sporocarp production is estimated in 15-25% of the global black truffle supply, considering the climatic conditions of the year (Black, 2006).

Sampling and study methods

For this study, four contiguous zones, 10.5 ha in total, were selected in the 6 × 6 m plantation area. Three of these are cultivated every spring while only one remain as control zone, not being cultivated. In every zone, four trees were randomly chosen, three black truffle producers and one non-producer, so 16 holm-oak trees were studied: four non black truffle producers and 12 producers.

Two annual soil samplings were doing using a soil borer, one in Spring and another in Autumn, following the global method (Verlhac *et al.*, 1990), which is adequate to evaluate mycorrhizae percentage and diversity in truffle plantations regardless its age. Two samples per tree were collected, picking 1 kg of soil each, in the superficial part of the soil (10-20 cm) near the tree, without damaging it. As indicate Verlhac *et al.* (1990), the two samples were studied as a single one. Samplings were performed in two consecutive years: from Autumn 1999 to Spring 2001. At each sampling time, 32 samples were collected, so, a total of 128 samples were analyzed.

After the extraction, the samples are preserved in a cold chamber (4°C) for less than two months until use. The samples were then gently washed and introduced in an ultrasonic bath for 15 minutes, set aside for 24 hours and procedure repeated again. If it was necessary, washing procedure was completed with needles and brushes.

Root types were counted under the stereomicroscope, separating non-mycorrhizal roots and roots mycorrhizated with either *T. melanosporum* or other fungal species. Some ectomycorrhizal tips were preserved in formol-acetic-alcohol (FAA) fixative, (Agerer, 1986) for later characterization.

Ectomycorrhizae classification method

In general, all anatomical features that include hyphae can be applied to characterize ectomycorrhizae (Agerer, 1987-2006; Agerer, 1991; Agerer and Rambold, 2004-2009) but only four anatomical complexes are informative for identification of the symbiotic fungus (Agerer, 2006): (a) structure of outer mantle layers as seen in plan view, (b) structure of rhizomorphs, (c) shape of cystidia, and (d) features of emanating hyphae. Some additional, non-anatomical informative characters may occur, for example, (e) chemical reactions and (f) colour of the ectomycorrhizae. The sequence from (a) to (f) indicates decreasing taxonomic and systematic importance (Agerer, 2006).

The ectomycorrhizal morphotypes were characterized by the following items: mantle type, rhizomorph type, cystidia, emanating hyphae, exploration type, hydrophilic or hydrophobic character and colour of ectomycorrhizae.

The mantle types were classified by the distinctive structures of outer mantle layers as seen in plan view according to Agerer (2006). The rhizomorphs were classified according to Agerer (1999) and cystidia according to Agerer (2006). The emanating hyphae features were focused on the clamps presence or absence. The exploration types of ectomycorrhizae were determined according to Agerer (2001). Hydrophilic or hydrophobic character was determined with the statements of Agerer and Rambold (2004-2009).

Statistical analysis

Mycorrhizal percentage was calculated as mycorrhizal tips divided by total of short roots. *Tuber melanosporum* percentage was calculated as *T. melanosporum* mycorrhizal tips divided by total of mycorrhizal tips.

The effect of cultivation on mycorrhization percentage and *T. melanosporum* percentage was analyzed only on black truffles productive trees. In addition, differences on mycorrhization percentage and *T. melanosporum* percentage between productive and non-productive trees, were studied as well in cultivated area. The obtained percentage data did not met the assumptions of a *t*-test. The Mann-Whitney-Wilcoxon non parametric test was used instead. Statistical analysis was performed using the NPAR1WAY procedure of the SAS statistical package (SAS, 2000).

Table 1. Total of counted short roots in the four samplings

Sampling	Short roots	Ectomycorrhizae	*Tuber melanosporum* ectomycorrhizae
Spring 1999	24,316	15,649	9,741
Autumn 1999	12,973	9,885	9,684
Spring 2000	10,190	5,176	3,613
Autumn 2000	16,856	10,902	5,334
Total	64,335	41,612	28,372

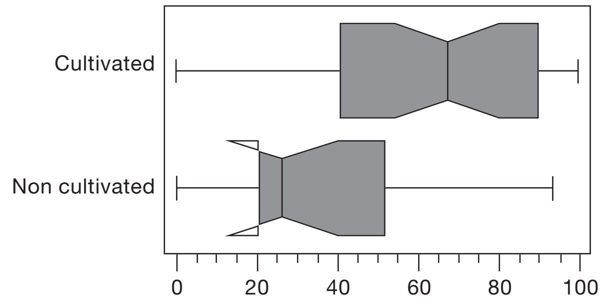

Figure 1. Box and Whisker plot. Total mycorrhization percentages differences for productive trees between cultivated and non-cultivated areas.

Results

Total of counted short roots in each sampling for estimation of total and relative ectomycorrhizal percentages is compiled in Table 1.

The mean total mycorrhization percentage for all the studied zones in the four samplings is near 50%, ranged from 14 to 75% depending on the sampling. A relatively high percentage of *T. melanosporum* mycorrhizae, near 35%, was found in all the studied trees and areas, even in those that are not considered producers of black truffles (Table 2).

Cultivated areas and non-cultivated areas had statistically significant differences in total mycorrhization percentage ($p = 0.0126$), with a medium percentage over 66% in the cultivated areas and over 34% in the non-cultivated areas (Fig. 1). There were no statistically significant differences for the percentage of *T. melanosporum* ($p = 0.0873$), although this was also higher in cultivated areas, being over 46%, and near 22% in non-cultivated areas.

Total mycorrhization percentage has no significant differences between productive and non-productive trees in the cultivated areas ($p = 0.1035$). As could be expected, at the same trees, *T. melanosporum* mycorrhization percentage is significantly higher in the productive trees than in the non-productive trees ($p = 0.0157$) (Fig. 2).

Doing the thoroughgoing morphological study, a total of 106 ectomycorrhizal types have been described.

Table 2. Mycorrhization percentages in cultivated and non-cultivated areas and in trees that produce and not-produce *Tuber melanosporum* sporocarps in the samplings of Autumn 1999 (A99), Spring 2000 (S00), Autumn 2000 (A00) and Spring 2001 (S01)

	Sample	Total mycorrhizae (%)	*T. melanosporum mycorrhizae* (%)	Other mycorrhizae (%)
Cultivated areas	A99	60.54	58.97	1.57
	S00	62.21	46.45	15.76
	A00	74.85	33.92	40.93
	S01	68.12	43.22	24.90
Non cultivated areas	A99	51.18	50.42	0.76
	S00	21.90	12.76	9.14
	A00	30.39	17.80	12.59
	S01	33.76	8.73	25.01
Productive trees	A99	41.91	34.57	7.34
	S00	49.60	37.00	12.60
	A00	66.77	37.75	29.02
	S01	61.47	41.82	19.65
Non productive trees	A99	13.82	6.91	6.91
	S00	43.95	10.86	33.09
	A00	53.68	14.64	39.04
	S01	69.24	17.25	51.99

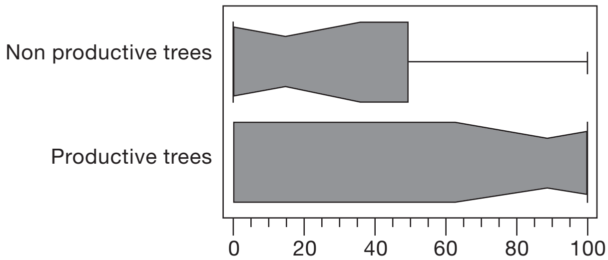

Figure 2. Box and Whisker plot. *Tuber melanosporum* mycorrhization percentages differences for cultivated areas between productive and non-productive trees.

Ectomycorrhizae classification method used in this study has a very conservative character and further molecular analyses should be needed to ratify this classification. Regrettably, conservation of tips in FAA do not allow to do molecular analysis due to DNA degradation.

Description and identification of ectomycorrhizas have evolved greatly following the systematic studies by Agerer (1986, 1987-2006, 1991) and mainly by molecular characterization (Horton and Bruns, 2001). Although there are some descriptions for mycorrhizas, most are not precise (De Román *et al.*, 2005), so conclude that one morphotype belongs to a species is difficult. Only 11 of those 106 types have been identified due to their anatomical and morphological characters (Table 3).

The most frequent ectomycorrhizal types found were *T. melanosporum*, Type *Hebeloma* (Fr.) P. Kumm., *Quercirhiza quadratum* (Águeda *et al.*, 2008) and *Cenococcum geophilum* Fr., being Type *Hebeloma* the second most abundant morphotype in the non-productive trees.

Only 43 types were present in a percentage over 0.1% along the four samplings. From them, 19 types belong to the short-distance exploration type (Agerer, 2001) and 12 to the medium-distance smooth subtype (Agerer, 2001). Six types belongs to the medium-distance fringe subtype and four to the medium-distance mat subtype (Agerer, 2001). The less represented exploration type is long-distance (Agerer, 2001), with only two types. Contact and pick-a-back exploration types (Agerer, 2001) are not present.

Table 3. Identified ectomycorrhizal morphotypes encountered in «Los Quejigares» plantation, with their percentage of presence along the four samplings and their presence in trees that produce (P) or not (NP) *Tuber melanosporum* sporocarps

Identified ectomycorrhizal morphotype	Presence percentage	P / NP
Cenococcum geophilum Agerer, 2006	2.5	P / NP
Hymenogaster cf. *citrinus* Bencivenga *et al.,* 1992	0.1	NP
Pisolithus tinctorius Agerer, 2006; Agerer and Rambold, 2004-2009	2.3	P
Quercirhiza quadratum Águeda *et al.,* 2008	2.7	P / NP
Tuber aestivum Agerer, 2006; Müller *et al.,* 1996; Zambonelli *et al.,* 1995	0.6	P / NP
Tuber brumale Agerer, 2006; De Román and De Miguel, 2005; Fischer *et al.,* 2004	0.1	P
Tuber melanosporum Agerer, 2006; Rauscher *et al.,* 1995	69.7	P / NP
Type *Cortinarius* Agerer, 2006	0.1	NP
Type *Genea* Agerer, 2006	0.4	P / NP
Type *Hebeloma* Agerer, 2006	5.1	P / NP
Type *Scleroderma* Agerer, 2006	1.2	NP

As for the identification of the different ectomycorrhizal types, only one type could belong to the Bolete clade, in spite of the clamps presence in its hyphae and the rhizomorphs absence. Those characters are not very common for this fungus clade, but do fit with the fungi belonging to the Gomphidiaceae (Agerer, 1999) and also with some species of the *Suillus* having dotted hyphae and clamps (Yamada *et al.*, 2001).

Twenty-five of the 43 morphotypes present over 0.1% have characters relating them to the Telephoroid clade. Mycorrhizae of the Thelephoroid clade are very diverse, but they are recognised by their frequently brown or brownish colour and because they may possess blue granules that become greenish in KOH or hyphae can simply turn green in KOH, indicating the presence of thelephoric acid, also have thelephoroid rhizomorphs and emanating hyphae with or without clamps (Agerer, 2006; Kõljalg *et al.*, 2000). Most of the types found in this work share some of those characters to identify them as Thelephoroid, or, at least, to recognize their Thelephoroid nature.

Systematic sporocarp samplings have not been done in the study area but, at ectomycorrhizae sampling, sporocarps of *Hebeloma sinapizans* (Paulet: Fr.) Gillet, *H. crustuliniforme* (Bull.: Fr.) Quél., *Cortinarius* spp., *Scleroderma* spp. and cleistothecia of *Cenococcum geophilum* (Fernández-Toirán and Águeda, 2007) have been found. Also, sporocarps of the *Inocybe* genus were present, although its mycorrhizae have not been found. Thelephoroid sporocarps have not been found, likely due to the difficulty on their tracking down and also because the elimination of wood debris, but, by the moment, in Soria province ten species (*Pseudotomentella tristis* (P. Karst.) M. J. Larsen, *Tomentellopsis echinospora* (Ellis) Hjortstam, *Tomentella asperula* (P. Karst.) Höhn & Litsch., *T. badia* (Link) Stalpers, *T. botryoides* (Schwein.) Bourdot & Galzin, *T. bryophila* (Pers.) M. J. Larsen, *T. ellisii* (Sacc.) Jülich & Stalpers, *T. galzinii* Bourdot, *T. neobourdotii* M. J. Larsen, and *T. subclavigera* Litsch.) are quoted (Tellería, 1990). The lack of information about the anatomical characteristics of Thelephoroid mycorrhizae (De Román *et al.*, 2005) limits the identification at species level.

Discussion

The results obtained show that *T. melanosporum* mycorrhizae persists in the roots of the host trees after more than three decades. Culture practices carrying out along the years, and not only the ecological factors, promote the stay of *T. melanosporum* mycorrhizae, and, consequently, the black truffle production.

Total ectomycorrhizal percentages are significantly higher in cultivated areas. Breaking of roots by spring soil cultivation favours grow of new tips, capable of form new ectomycorrhiza that maintain mycorrhization percentage or even increase it. This grow is favoured by pruning too. Soil cultivation also removes the herbaceous plants, that compete with fungi for water and other mineral nutrients. Management practices in truffle plantations are focused, mainly, in three parameters: maintenance of soil humidity, elimination of competitor plants and maintenance of soil insolation (Colinas and Reyna Domenech, 2007) being these variables key questions in the development of ectomycorrhizal fungal communities in Mediterranean forests (Richard *et al.*, 2004).

Tuber melanosporum percentage is significantly higher in productive trees. It has been observed that in most mature truffle-plantations there are one or more trees that are unable to produce truffles when they are rounded by others that produce sporocarps. Those trees grow on the same ecological conditions and are subjected to the same cultivation treatments than productive ones. The answer to this could be related in a great way to the maintenance minimum threshold of black truffle mycorrhization in the roots. This fact, combined with the presence or absence of some particular ectomycorrhizal species could be related to the triggering of the truffles production (Murat *et al.*, 2008). Truffle plantations are doing nowadays in areas that were before agricultural lands, so it is very important maintaining a minimum threshold of mycorrhizal percentages of the introduced fungus in the seedlings (Bonet *et al.*, 2006; Samils *et al.*, 2008).

Two years data suggest that trees keeping its ectomycorrhizal fungal community through the time. Species that form a mycorrhizal fungal community evolve as forest species evolve (Allen, 1991). Truffle plantations have continuous severe man-made disturbances, the cultural practices, that do not let evolve the ectomycorrhizal fungal community through the time. Data offered here are not enough to support this affirmation, so a study over a longer time period will be needed.

In most of the ectomycorrhizal fungi, there is a poor correspondence between those that appear dominant as sporocarps *vs.* those that appear dominant on roots (Horton and Bruns, 2001). In the case of *T. melanosporum* it seems that the investment in sporocarps formation is the same than in root tips colonization, whereas other Tuberales like *T. magnatum* invest more in sporocarps formation than in root tips colonization (Murat *et al.*, 2005). In fact, here *T. melanosporum* mycorrhizal percentage in non-productive trees is lower than in productive ones.

The diversity of ectomycorrhizal fungi species is higher than it could be expected before doing this study, this fact may be related to the age of the trees, near 40-years-old, and with the soil inocula associated to the forest that was before doing the plantation. Nevertheless, molecular characterization should be carried out in further analysis to confirm these data.

Cenococcum geophilum is one of the most abundant competitor mycorrhiza in all the samplings and coexists with *T. melanosporum* mycorrhizae even in plants with a high colonization by the last species. *Cenococcum geophilum* is absent in other Spanish and Italian black truffle plantations (Bacciarelli-Falini *et al.*, 2006; De Miguel and Sáez, 2005; De Miguel *et al.*, 2001). All those truffle plantations were established in plots which had agricultural management, to avoid the native ectomycorrhizal inoculum, whereas the study area was established in a cut down forest stand (De Miguel *et al.*, 2006).

It is also remarkable the presence, in low proportion, of two other mycorrhizae belonging to the genus *Tuber*. *Tuber aestivum* appeared in a non-productive tree and in another productive tree, and *T. brumale* in a productive one. Those two species also appear together with *T. borchii* Vittad. in Italian non-productive black truffle plantations (Bacciarelli-Falini *et al.*, 2006). None of the two species seem to cause here the displacement of *T. melanosporum*, contrary as happen in other French or Italian plantations (Bencivenga, pers. com.; Granetti *et al.*, 2005; Souzart, 2004).

During the years, *Hebeloma* has been considered like a fungal genus that displace *T. melanosporum* ectomycorrhizas in truffle-plantations (Olivier *et al.*, 2002). Its presence, mainly in non-productive trees, would accord this statement.

Comparing with the results in young truffle-plantations that produce or not black truffles (Bacciarelli-Fallini *et al.*, 2006; De Miguel and Sáez, 2005; De Miguel *et al.*, 2001), there is evidence that mycorrhizal diversity increases with the age of the plantation. The ectomycorrhizal fungal community characterized in «Los Quejigares», although it is dominated by *T. melanosporum*, is similar to those in forests stands, formed by many different species that are represented in very low proportion. Very similar ectomycorrhizal types as those found in this study have been reported in Spanish native holm-oak stands, black truffle productive and non-productive (Águeda *et al.*, 2003; De Román, 2003). This fact shows the similarity between the ectomycorrhizal community of the truffle plantations and the wild surrounding stands.

When mycorrhizated seedlings are placed in a site, the invasion of other ectomycorrhizal species on their roots occurs from inoculum of surrounding areas, and there exists a natural mechanism of competition (Allen, 1991). The presence of competitive species adapted to local conditions could displace the introduced fungus on the seedlings (Sourzat, 2004). The cultural management of those seedlings would determine and favour the persistence of the introduced species (Colinas and Reyna Domenech, 2007; Verlhac *et al.*, 1990). Unfortunately, all the factors favouring the dominance of a particular fungal symbiont are not well known yet (Allen, 1991; Smith and Read, 2008; Varma, 2008).

Colonization strategy of ectomycorrhizal species could be an important factor on their development (Agerer, 2001). The low presence of long-distance exploration types, could be related to culture practices, like soil cultivation, irrigation or pruning, that limited their presence and favoured the short-distance exploration type.

Thelephorales order belongs to the subclase Agaricomycetidae and is formed by two families, that include 18 genus and 177 species (Kirk *et al.*, 2001). Nowadays, it is known that, more or less, one hundred of the Thelephoroid species are able to form ectomycorrhizae, the most of them of the *Tomentella* genus and less of the *Pseudotomentella* and *Tomentellopsis* genus. Thelephoriod ectomycorrhizae seem to be constant, diverse and abundant in the fungal community associated to broadleaves stands in the continental template areas (Jakucs *et al.*, 2005; Tedersoo *et al.*, 2006). The lack of information about the anatomical characteristics of Thelephoroid mycorrhizae limits its identification at species level. The abundance of Thelephoroid types is a common trait in the quantification and characterization of ectomycorrhizae in Spanish plantations and wild black truffle productive stands (Águeda *et al.,* 2003; De Miguel and Sáez, 2005b; De Miguel *et al.,* 2001; De Román, 2003; Etayo, 2001). The presence of this group of fungus is common in black truffle productive areas, where do not seem to have influence in its fructification.

Nowadays, «Los Quejigares» is 38-year-old and the sporocarps production is increasing every year (Ruíz

Barbarín, pers. com.). How long will it last this increasing production it is not know, but its keeping involves a hope for truffle cultivation future, that, until now, has been developed more for feeling than for science. The accumulated knowledge during the years in «Los Quejigares» and in other plantations suggests an encouraging future for truffle cultivation and offers an alternative resource for marginal and inland areas with low incomes.

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