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ECTOMYCORRHIZAL MORPHOTYPES IDENTIFIED IN TWO SITES (BURNED AND NON-DISTURBED) IN A *QUERCUS ILEX* L. SUBSP. *BALLOTA* (DESF.) SAMP. FOREST IN NAVARRA (SPAIN).

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SUMMARY

DE ROMÁN, M.; DE MIGUEL, A.M. & ETAYO, M.L. (1999). Ectomycorrhizal morphotypes identified in two sites (burned and non-disturbed) in a *Quercus ilex* L. subsp. *ballota* (Desf.) Samp. forest in Navarra (Spain). *Pub. Bio. Univ. Navarra, Ser. Bot.*, 12: 45-57.

Since 1998, a field study has been undertaken in a *Quercus ilex* L. subsp. *ballota* (Desf.) Samp. forest located in Nazar (Navarra, Spain). This study aims to establish a qualitative and quantitative comparison between the ectomycorrhizal morphotypes which have been collected, described and identified in a burned site and in a non-disturbed one within this forest. Thus, this study aims to contribute to the knowledge of the ectomycorrhizae associated with evergreen oak in field conditions, because most of the studies on evergreen oak ectomycorrhizae are related to truffle growing or artificial mycorrhization in the nursery.

Key words: *Quercus ilex*; ectomycorrhizae; identification; morphology; *Tuber melanosporum*; forest fire.

RESUMEN

DE ROMÁN, M.; DE MIGUEL, A.M. & ETAYO, M.L. (1999). Morfotipos de ectomicorrizas identificadas en dos zonas (quemada y no quemada) de un bosque de *Quercus ilex* L. subsp. *ballota* (Desf.) Samp. en Navarra (España). *Pub. Bio. Univ. Navarra, Ser. Bot.*, 12: 45-57

Desde 1998, se ha llevado a cabo un estudio de campo en un bosque de *Quercus ilex* L. subsp. *ballota* (Desf.) Samp. situado en Nazar (Navarra, España). El objetivo de este estudio es la comparación cualitativa y cuantitativa de los tipos de ectomicorrizas que han sido descritos e identificados en una zona quemada y una zona control de este carrascal. Así, se pretende paliar la escasez de conocimiento en el tema de las ectomicorrizas asociadas con la carrasca en condiciones naturales, ya que la mayoría de los trabajos publicados están relacionados con la truficultura o la micorrización artificial en vivero.

Palabras clave: *Quercus ilex*; ectomicorrizas; identificación; morfología; *Tuber melanosporum*; incendio forestal.

INTRODUCTION AND OBJECTIVES

In the last ten years, the studies on ectomycorrhizae have increased considerably. Nevertheless, most of them have been done in boreal or temperate forests in North America (DANIELSON, 1983) and in North and Central Europe (DAHLBERG *et al.*, 1997) and are mainly based on coniferous trees. Moreover, most of them deal with the artificial mycorrhization of seedlings in the nursery (TIMONEN *et al.*, 1997), or with ecological studies of fruit bodies from ectomycorrhizal fungi in field conditions (BAAR, 1996). Thus, there is a lack of knowledge on the ectomycorrhizae associated in the field with Mediterranean tree species such as the evergreen oak (*Quercus ilex* L. subsp. *ballota* (Desf.) Samp), although more and more Spanish research groups are leading an important research on the topic (CARTIÉ *et al.*, 1996; DUÑABEITIA *et al.*, 1996; FERNÁNDEZ DE ANA MAGÁN & RODRÍGUEZ, 1992; HONRUBIA *et al.*, 1992; MANJÓN *et al.*, 1994; RODRÍGUEZ BARREAL *et al.*, 1997).

On the other hand, the studies on truffle growing have developed quickly since 1970, when the first experimental plots were planted with oak (*Quercus faginea*), evergreen oak (*Quercus ilex*) and hazel (*Corylus avellana*) artificially mycorrhized in the nursery with black truffle (*Tuber melanosporum* Vitt.) in France (CHEVALIER & DELMAS, 1976) and Italy (LUPPI & FONTANA, 1977). At the end of the 80's, the influence of the Italian and French experiences reached Navarra and other surrounding regions, and in 1993, the Department of Botany of the University of Navarra, in collaboration with the ITGCereal (now ITGAgricola), started a research project aimed to delimit the potential truffle-occurring area of black truffle in Navarra (SÁEZ & MIGUEL, 1995). A further aim of this project was the monitoring of several truffle plantations in order to record data on the evolution of the mycorrhization and on the appearance and possible competition of other ectomycorrhizal species in the development and occurence of the black truffle (MIGUEL & SÁEZ, 1997).

In 1998, we have started a project on the diversity and the abundance of the ectomycorrhizae associated with evergreen oak in field conditions, due to the importance of this tree species in the Mediterranean area (DE ROMÁN & DE MIGUEL, 1998). Since forest fires are a common hazard in the Mediterranean area (TORRES & HONRUBIA, 1994), we have established one experimental plot in an evergreen oak stand burned five years ago, while another plot has been set in a control non-disturbed stand within the same forest.

This study aims to compare the diversity and abundance of ectomycorrhizae in both plots, in order to know more about the evolution of a burned forest. A possible application of this study is the use of mycorrhized plantlets in reforestation purposes as an alternative to traditional reforestation, once we know which mycorrhizal species are most suitable for the optimum development of the tree.

MATERIALS AND METHODS

The study site is a natural *Quercus ilex* L. subsp. *ballota* (Desf.) Samp. forest located in Nazar, within the potential truffle-occurring area in Navarra (Spain). Part of this forest was burned five years ago, but most of it remains undisturbed. Thus, we have established a burned and a control study sites.

Five trees from each site were randomly chosen and sampled in November 1998 and March 1999. Samples of soil containing roots were taken with a 10cm-deep soil corer at a distance of 1m from the tree trunk and at three different orientations around it.

In the laboratory, each sample was divided into two subsamples: 150 gr for the quantitative and 300 gr for the qualitative approach. Mycorrhized tips were washed and separated using two sieves (1.7mm and 0.7mm respectively).

The quantitative study was done following the gridline intersect method (BRUNDRETT *et al.*, 1994), and the results are given in percentage of mycorrhized root tips. On the other hand, different features of the mycorrhiza, such as colour, emanating hyphae, mantle surface and mantle edge were the basis for the description and identification of ectomycorrhizal morphotypes (AGERER, 1994; BENCIVENGA *et al.*, 1995; DONNINI & BENCIVENGA, 1995; INGLEBY *et al.*, 1990; VOIRY, 1981).

RESULTS

The most important features of the 18 ectomycorrhizal morphotypes described in both study sites are given in Table 1, including identified and unidentified (non- described in the literature) morphotypes. Fig. 1 shows photographs of the most striking features of six unidentified morphotypes.

Table 1.- Brief description of the 18 ectomycorrhizal morphotypes found.

Type	Macroscopic description	Mantle type	Emanating hyphae	Clamp connections	Rhizomorphs	Cystidia or setae	Sclerotia
***Cenococcum geophilum* Fr.**	Black, short, scarcely branched	Plectenchymatous, hyphae arranged star-like	Dark, straight and distinctly septate	No	Absent	Absent	Very abundant black sclerotia
***Sphaerosporella brunnea* (Alb. & Schwein.) Svrcek&Kubicka**	Dark brown, simple to monopodial pinnate	Psedoparenchymatous poligonal	Scarce; thick but sharply narrowed at the septa	No	Absent	Absent	Absent
***Tuber brumale* Vitt.**	Pale brown	Pseudoparenchymatous puzzle-like	Absent	No	Absent	Abundant straight unbranched setae	Absent
***Tuber mesentericum* Vitt.**	Brown woolly	Pseudoparenchymatous poligonal	Very long and straight	No	Absent	Absent	Absent
***Thelephora terrestris* (Ehrh.) Fr.**	Long sinuous, simple to irregularly pinnate	Pseudoparenchymatous with long irregular cells	Only their first septum bears a clamp connection	Yes	Absent	Absent	Absent
***Hebeloma*-like**	Long, white, hairy, sometimes pinnately-branched	Transparent plectenchymatous	Abundant, long and transparent	Yes	Present	Absent	Absent
***Hymenogaster*-like**	Dark brown, irregularly pinnate	Pseudoparenchymatous poligonal	Long, branched at an angle of 90°	Yes	Absent	Absent	Absent
***Scleroderma*-like**	White cottony	Plectenchymatous	Tortuous hyphae forming ring-like structures	No	Present and very abundant	Absent	Absent

Table 1 (continuation)

Type	Macroscopic description	Mantle type	Emanating hyphae	Clamp connections	Rhizomorphs	Cystidia or setae	Sclerotia
AD type	Woolly	Pseudoparenchymatous poligonal	Branched at a right angle	No	Absent	Absent	Absent
SB type	Simple, pale orange	Pseudoparenchymatous puzzle-like	Absent	Yes	Absent	Frequent yellowish setae	Absent
Type 1 Fig. 1a	Simple, pale brown	Pseudoparenchymatous puzzle-like	Long, thin, divided always at an angle of<90°	No	Absent	Absent	Absent
Type 2 Fig. 1b	Pale brown	Pseudoparenchymatous poligonal	Absent	No	Absent	Cystidia with thickened walls and rounded-shape endings	Absent
Type 4 Fig. 1c	Simple, dark brown	Tightly plectenchymatous	Absent	Yes	Abundant	Straight brownish setae parallel to the mantle edge	Absent
Type 5	Simple, white cottony	Pseudoparenchymatous puzzle-like	Thin, hyaline; very abundant	Yes	Abundant	Absent	Abundant white sclerotia
Type 6 Fig. 1d	Dark, usually in contact with Cenococcum	Plectenchymatous poligonal, hyphae arranged star-like	Long and tortuous	Yes	Present	Sparse cystidia with thickened base, bottle-like	Absent
Type 7 Fig. 1e	Simple, dark	Pseudoparenchymatou, puzzle-like	Long and tortuous	No	Absent	Absent	Absent
Type 8 Fig. 1f	Simple, pale brown	Pseudoparenchymatous puzzle-like	Absent	No	Absent	Deeply curved cystidia, very abundant	Absent

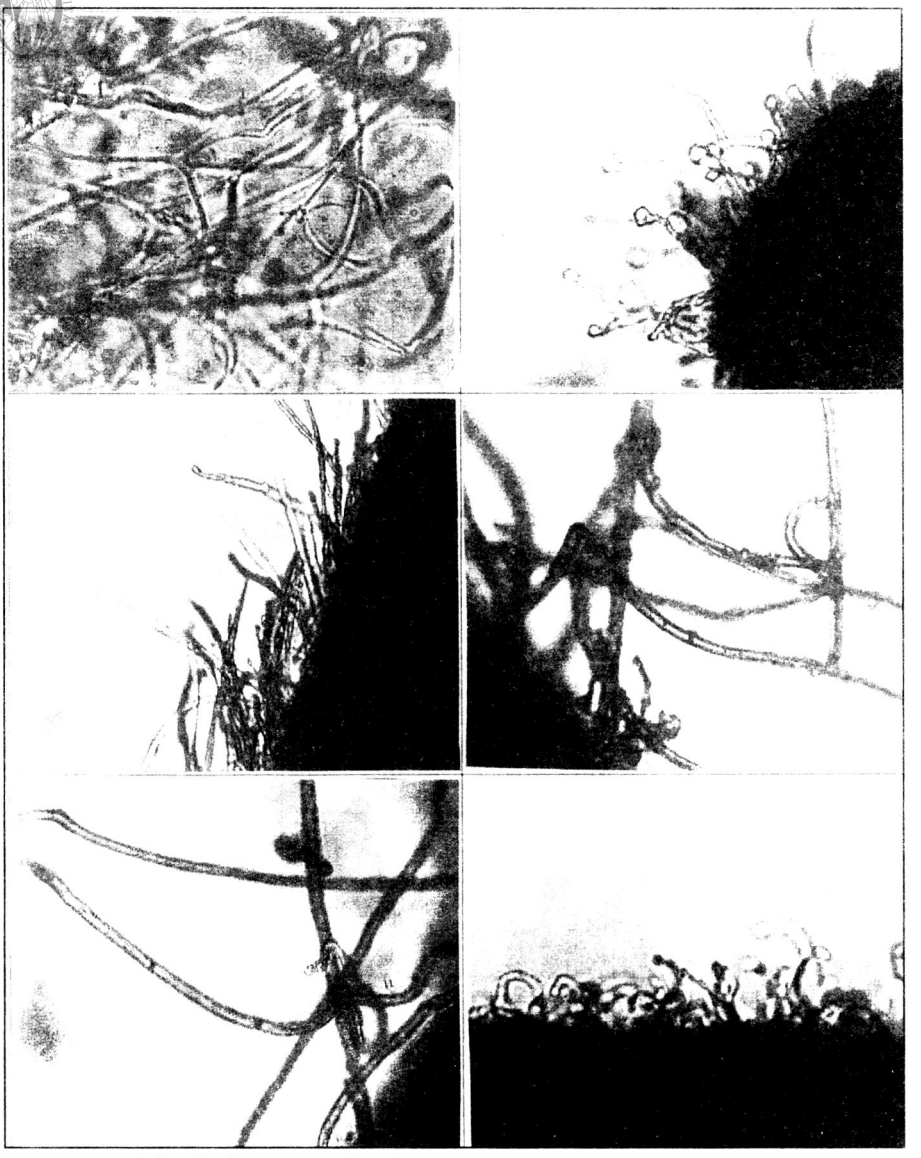

Figure 1.- Unidentified ectomycorrhizal morphotypes. a) Type 1, thin hyphae divided into two or three branches (100X); b) Type 2, cystidia ending in a rounded shape (100X); c) Type 4, straight setae running parallel to the mantle edge (40X); d) Type 6, long tortuous hyphae with clamp-connections (100X); e) Type 7, long tortuous hyphae without clamp-connections (100X); f) Type 8, deeply curved cystidia.

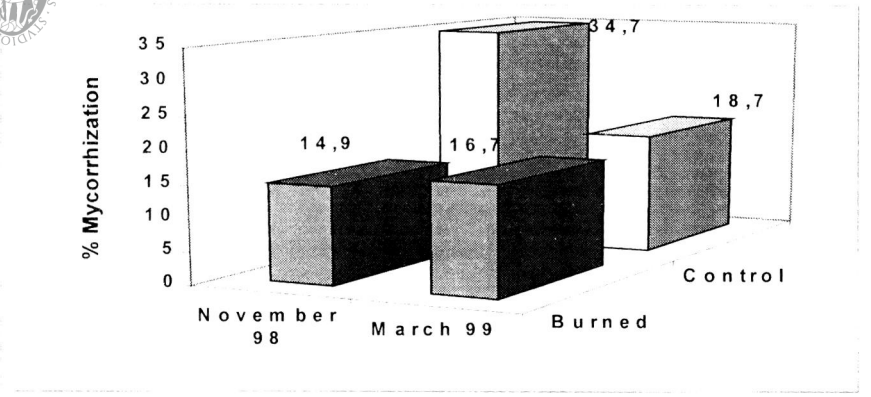

Figure 2.- Percentage of mycorrhization in both stands and in both samplings

	Burned		Control	
Ectomycorrhizal types	**16/11/98**	**15/03/99**	**16/11/98**	**15/03/99**
Cenococcum geophilum				
Type 6				
Type 1				
Sphaerosporella brunnea				
Tuber brumale				
Hebeloma-like				
Tipo 7				
Hymenogaster-like				
Tuber-like				
Type 8				
Thelephora terrestris				
Type 2				
SB type				
AD type				
Type 4				
Type 5				
Scleroderma-like				
Tuber mesentericum				

Table 2.- Ectomycorrhizal morphotypes in decreasing order of occurrence and abundance.

Abundances			
	>40%		1-5%
	10-40%		<1%
	5-10%		Absent

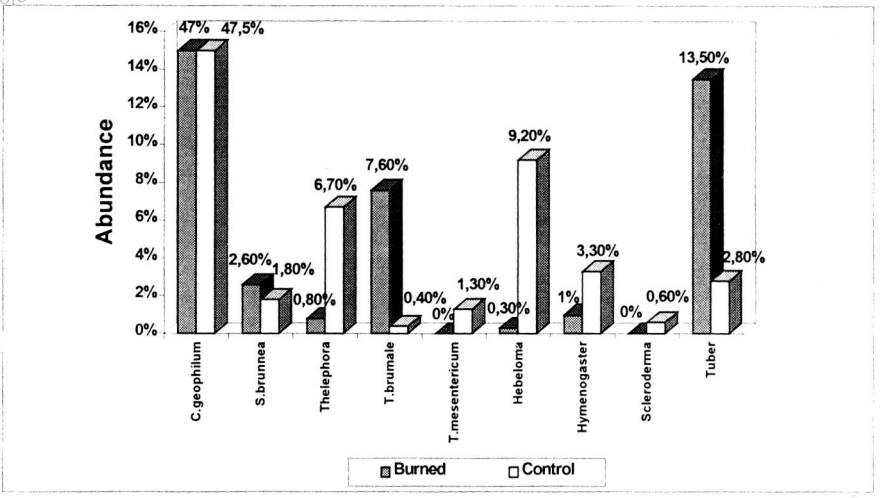

Figure 3.- Abundance of identified ectomycorrhizal morphotypes in burned and control areas.

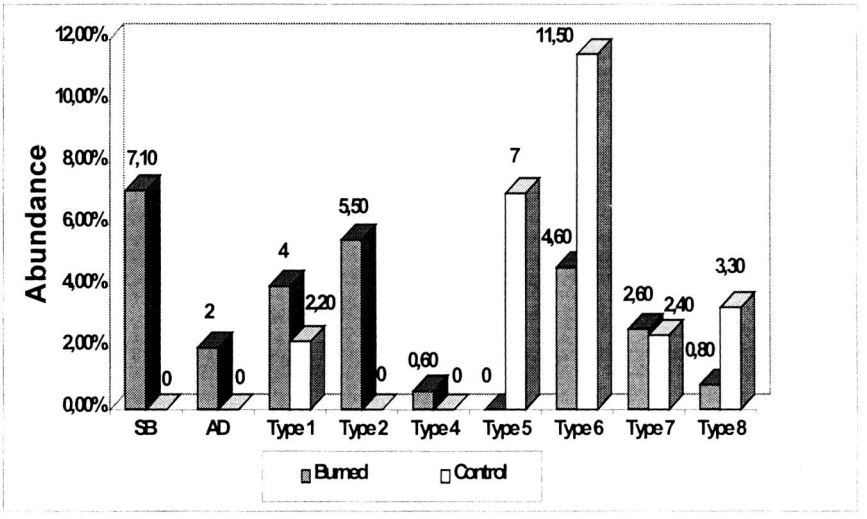

Figure 4.- Abundance of unidentified ectomycorrhizal morphotypes in burned and control areas.

The percentage of mycorrhization in both stands and in both samplings can be seen in Fig. 2, while the average abundance of each morphotype related to the total number of ectomycorrhizal tips found in each plot is given in Fig. 3 & 4. A table showing more detailed data on the evolution of the occurrence and the abundance of each morphotype is also given (Table 2).

DISCUSSION

The first sampling in November 1998 yielded a percentage of mycorrhization which was much higher in the control site (Fig. 2). Therefore, we could postulate that mature forests had an abundant climax mycorrhizal flora, while the mycorrhizal community in the burned site still had to go through several succesional stages until recovering from the disturbance.

Nevertheless, in March 1999 the results were quite different. The percentage of mycorrhization was then practically the same in both areas, which implies mycorrhization had sharply decreased in the control site (Fig. 2). This could be due to a seasonal variation in the percentage of mycorrhization linked to different levels of activity of ectomycorrhizal fungi in burned and control trees at the end of the winter: burned trees seem to be more active than mature ones at this time of the year.

On the other hand, the qualitative study has yielded 18 ectomycorrhizal morphotypes, 11 of which have been found in the literature, while the rest still remain unidentified (see Table 1 and Fig. 1 for the description of morphotypes). Initially, a possible correspondence between our Type 1 and Tuber macrosporum Vitt. (MEOTTO et al. 1995; GIOVANNETTI & FONTANA, 1980-81) had been detected, but after a careful comparison of both types, this hypothesis had to be dropped.

Cenococcum geophilum, Type 1, Type 6 and Sphaerosporella brunnea are the only species which occur in both samplings and in both areas, that is, they always take part in the ectomycorrhizal community of evergreen oak (Table 2).

Cenococcum geophilum is the most abundant morphotype, and can not be considered as a stress indicator since it is equally abundant in the burned and control plots.

There are some ectomycorrhizal types which tend to be associated with either burned or control trees (Fig. 3 & 4): SB type (spinules bucleés; GIRAUD, 1988), AD type (angle droit; GIRAUD, 1988), Type 2, Type 4 and Tuber brumale occur exclusively or nearly exclusively in burned trees, while Type 5, Scleroderma type, Tuber mesentericum and Hebeloma type are also mainly related to non-disturbed trees.

As it was expected, *Sphaerosporella brunnea* is more abundant in the burned area. This type is well-known for being a pioneer species suitable for the colonization of burned areas (MEOTTO & CARRATURO, 1987-88).

CONCLUSIONS

Although further research needs to be done, we may come to a few preliminary conclusions:

1. *Cenococcum geophilum* is one of the few morphotypes that have always been found taking part in the ectomycorrhizal community of evergreen oak, and is extremely abundant (ca. 47%) in both sites and in both samplings.

2. Our results also support that *Sphaerosporella brunnea* could be a pioneer species suitable for the colonization of burned areas, as it has been previously stated in the literature (Meotto & Carraturo, 1987-1988).

3. Although our study site is located within the truffle production area (SÁEZ & DE MIGUEL, 1995), no *Tuber melanosporum* mycorrhizae or carpophores were found yet. Nevertheless, other *Tuber* mycorrhizae have been recorded: *Tuber brumale* in the burned site, *Tuber mesentericum* in the control site, and *Tuber*-like mycorrhizae in both areas (DE ROMÁN & DE MIGUEL, 1999).

4. So far, the diversity of ectomycorrhizae seems to be slightly higher in the burned plot, although as far as the number of samplings increases, the differences in diversity between both sites decrease.

This study will be continued for three years, and more data on the evolution of mycorrhization and the description of ectomycorrhizal morphotypes will be available in future contributions.

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