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## **A preliminary study on the ectomycorrhizae of *Quercus ilex* subsp. *ballota* (Desf.) Samp. in Navarra (Spain)**

### **Abstract**

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This study aims to describe the different ectomycorrhizal morphological types which occur in natural evergreen oak (*Quercus ilex* subsp. *ballota* (Desf.) Samp.) stands located in Navarra (Spain). The descriptions of the nine ectomycorrhizal morphotypes found are provided, as well as the distribution of each morphotype represented by their occurrence as a percentage of the total ectomycorrhizal tip occurrence.

### **Introduction**

The evergreen oak (*Quercus ilex* L.) is an autoctonous species which constitutes a typical formation of Mediterranean vegetation, thus being widely distributed in the Mediterranean area of Navarra. Despite the increasing interest in *Tuber* sp. pl., arising from the implantation of truffle culture with *Q. ilex* L. (Granetti 1995, Meotto & al. 1995, Miguel & Sáez 1997), little is known about the natural ectomycorrhizal associations in which this species is involved..

### **Materials and methods**

In order to describe the different ectomycorrhizal morphological types which occur associated with evergreen oak, four trees were randomly chosen and studied in March 1998 in a *Q. ilex* subsp. *ballota* (Desf.) Samp. wood (in a *Spiraeo obovatae-Querceto rotundifoliae* S. formation) located in Peña Unzué (Navarra, Spain). Samples of 500 g of soil containing roots were removed at a distance of 1 m from the tree trunk, at three orientations: SW, S and SE, and at two different depths from the surface: 10 cm and 20 cm. This made a total of 6 samples per tree with 24 samples overall.

In the laboratory, samples were washed and mycorrhizal tips were separated by size using two sieves (1.7 mm and 0.7 mm respectively). Mycorrhizal tips were then observed and identified using a stereomicroscope and a light microscope.

## Results and discussion

The preliminary descriptions of nine ectomycorrhizal morphotypes, identified according to different features of the mycorrhiza such as colour, branching system, emanating hyphae and mantle surface, which have been the basis for the differentiation of morphotypes, are provided (Agerer 1994, Donnini & Bencivenga 1995, Ingleby & al. 1990, Voiry 1981). Five of the nine morphotypes have been identified, while three morphotypes remain still unidentified due to the lack of any mycorrhizae reported in the literature matching with our descriptions. The photographs of their most striking features are shown in Fig. 2a, 2b & 2c. A fourth unidentified morphotype, the SB type (spinules buclées; Giraud 1988), has been cited before in the literature, but little is known yet about the identity of the fungus which forms the symbiotic association. The percentage abundance of the nine morphotypes is shown in Fig. 1.

These morphotypes have been described as:

*Cenococcum geophilum* Fr. (Ingleby & al. 1990): Black, short, scarcely branched mycorrhiza, with abundant dark-coloured emanating hyphae, which are straight and distinctly septate. There are abundant sclerotia, but never directly connected to the mycorrhiza.

*Tuber brumale* Vitt. (Meotto & al. 1995): Pale-brown mycorrhiza, with pseudoparenchymatous puzzle-like mantle and abundant straight unbranched setae.

*Tuber albidum* Pico (Meotto & al. 1995): Pseudoparenchymatous puzzle-like mantle with acute straight unbranched setae, longer than those of *T. brumale*.

*Hebeloma* Type (Sourzat & al. 1993): Long, white, hairy mycorrhiza, sometimes pinnately-branched, with a transparent plectenchymatous mantle. Abundant long transparent hyphae, with clamp-connections at the septa.

*Hymenogaster* Type (Donnini & Bencivenga 1995): Pseudoparenchymatous polygonal mantle. Long emanating hyphae, branched at an angle of 90°, with clamp-connections at the septa.

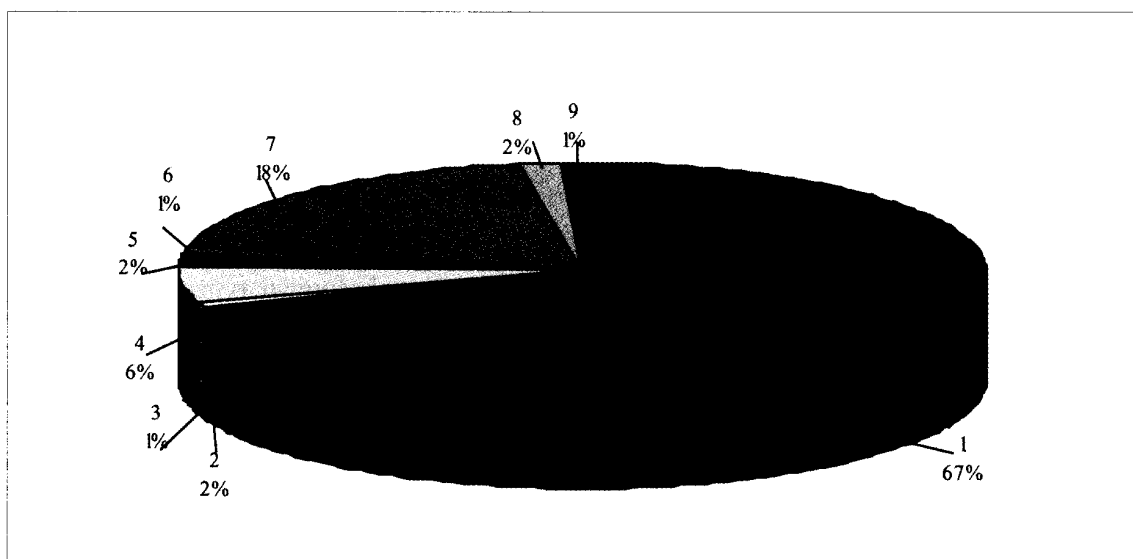


Fig. 1. Percentage of abundance of ectomycorrhizal morphotypes; 1: *Cenococcum geophilum*; 2: *T. brumale*; 3: *T. albidum*; 4: *Hebeloma*; 5: *Hymenogaster*; 6: SB type; 7: Type 1; 8: Type 2; 9: Type 3.

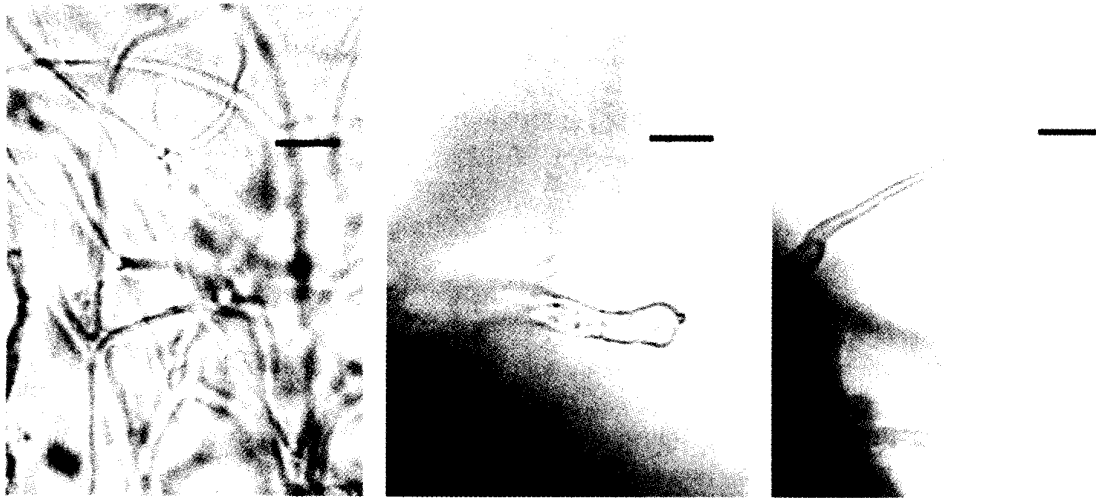


Fig. 2. Unidentified morphotypes of ectomycorrhizae (bars= 0,01 mm); a: Type 1; b: Type 2; c: Type 3.

SB Type (Giraud 1988): Pseudoparenchymatous puzzle-like mantle with frequent yellowish setae bearing clamp-connections at the septa.

Type 1: Pseudoparenchymatous puzzle-like mantle. Long thin transparent hyphae, very frequently divided in two or even three branches always at an angle of  $<90^\circ$ . Fig. 2a.

Type 2: Pale brown mycorrhiza with a pseudoparenchymatous polygonal mantle. There are sparse hyaline cystidia with thickened walls, ending in a distinctive rounded shape. Fig. 2b.

Type 3: Dark brown mycorrhiza, with a pseudoparenchymatous puzzle-like mantle. Abundant dark-red setae, with a broad base and slightly curved in the apex. Fig. 2c.

## Conclusions

This examination of the ectomycorrhizae formed on evergreen oak has identified 9 different morphotypes. The most abundant mycorrhizae found were *Cenococcum geophilum* representing 67 % of the total found and Type 1 representing 18 % of the total found. Despite seven other morphotypes being identified, only a small proportion of the total number of ectomycorrhizal tips found belonged to these morphotypes.

Although ectomycorrhizae of two species of *Tuber* have been found - *T. brumale* and *T. albidum* - there was no evidence of *T. melanosporum* mycorrhizae, which produce the edible truffle, associated with these trees.

The examination and identification of ectomycorrhizal fungi, associated with *Q. ilex* in the forests of Navarra, is to be continued. This will provide a greater understanding of the ecology of these natural forests which may in turn increase the potential for *Q. ilex* to be used in the production of edible truffles in this area.

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