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Inventory of ectomycorrizal fungi associated with a "relic" holm-oak tree (*Quercus rotundifolia*) in two successive winters

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Abstract

Holm-oak (*Quercus rotundifolia*), either as forests or as seminatural stands (the "montado" systems), occupies a vast area of land in Portugal, forming an important ecological and economical reserve that remains very little studied. A «relic» tree of this species, left from a montado stand that was replaced by cereals decades ago and now surrounded by *Eucalyptus globulus*, was visited during two winter carpophore fruiting seasons to scrutinize the ectomycorrhizal species associated with its root system. The sampled sporophores were identified and mapped relative to the oak trunk. Of thirty nine different higher fungi species that have been tentatively identified after four gatherings, twenty nine were problably ectomycorrhizal. *Russula* spp. (a total of ten species) dominated in the first gathering of each season while *Lactarius cremor* and *Helvella lacunosa* dominated in the second of the first and second season, respectively. Practically none of the holm oak-associated mutualistic or facultative species were found in the surrunding eucalyptus plantation. This study will be continued and extended to other locations in the long term, in order to provide data on the ecology of the holm oak ectomycorrhizal associates and to support the molecular identification of fungal isolates obtained from holm oak ectomycorrhizas.

Key-words

Basidiomycota, *Ascomycota*, ectomycorrhizal fungi, *Quercus rotundifolia*, Portugal.

Sumário

A azinheira (*Quercus rotundifolia*), seja em mata ou em montado, ocupa em Portugal uma vasta área, formando uma importante reserva ecológica e económica que permanece pouco estudada. Uma azinheira «relíquia», deixada num montado que foi substituido por culturas arvenses há várias décadas e hoje rodeada de eucaliptos, foi visitada durante dois invernos para recolha de corpos frutíferos de espécies micorrízicas associadas ao seu sistema radicular. Cada carpóforo recolhido foi identificado e a sua posição mapeada relativamente ao tronco. Trinta e nove espécies diferentes de fungos superiores foram identificadas após quatro recolhas, vinte e nove das quais são provavelmente micorrízicas. O género *Russula* (num total de dez espécies) dominou as primeiras colheitas de cada estação enquanto que *Lactarius cremor* e *Helvella lacunosa* dominaram as segundas colheitas da primeira e segunda estação, respectivamente. Praticamente nenhuma das espécies mutualistas ou facultativas associadas à azinheira foram encontradas na plantação de eucalipto circundante. A longo prazo este estudo será continuado e alargado a outras localizações, de modo a fornecer dados ecológicos das associações ectomicorrízicas com a azinheira, e ainda para suporte duma identificação molecular dos isolados fúngicos obtidos a partir das ectomicorrizas da azinheira.

Palavras-chave

Ascomycota, Basidiomycota, fungos ectomicorrízicos, *Quercus rotundifolia*, Portugal

Introduction

Ectomycorrhizal (ECM) fungi are a taxonomically diverse subset of Basiodiomycota and Ascomycota species capable of forming specialized symbiosis structures, called ectomycorrhizas, with wood plant root systems. From these structures they obtain as monosaccharides their main source of carbon, and in exchange provide the host plant(s) with a much improved mineral nutrient acquisition capability (Harley, 1991; Marschner, 1995). Ecologically, either in natural systems or managed plantations, these fungi are known to be essential for growth and survival of conifers and many species of perennial angiosperms, while the presence of suitable hosts is also essential for their establishment (Amaranthus and Perry, 1994).

It is believed, both from conventional sporophore sampling and DNA typing of vegetative mycorrhizal mycelia (Gardes and Bruns, 1996; Dahlberg *et al.*, 1997; Bruns *et al.*, 1998), that natural forests comprise very complex communities of ECM fungi. This complexity is patent in the high number of different species observed in the field, as well as in the diversification of symbiotic behaviours regarding the compatibility with different plants, the periods of activity, rates of growth, spread of each mycelium, patterns of nutrient supply to the plant, etc. (Brownlee *et al.*, 1983; Mason *et al.*, 1983). Complex ECM communities are also observed at the individual tree level (Zak, 1973), thus suggesting that diversification of the symbiotic partners is essentially built at this scale.

Though a species inventory in almost undisturbed ecosystems offers the "natural" situation from which a reference can be obtained, attention is also directed to situations where the stability of ECM communities is in peril, as it is after forest fires, climate change or human intervention. The "montado" oak grove is a seminatural ecosystem where cork oak (*Quercus suber*) or holm oak (*Quercus rotundifolia*) are dominant, and is maintained for the maximization of economical benefits (Coelho, 1996; Daveau, 1991). Management involves control on the tree spacing, limited livestock nourishment, and sometimes cereal crops. Montado systems occupy in Portugal nearly 10,000 sq. kms. of land with mediterranean climate of variable maritime influence, holm oak preferring the drier, interior areas. The mycological diversity has been the subject of previous field work in continuous areas of montado (Pinho-Almeida and Baptista-Ferreira, 1997). The emergence of sporophores is, in our experience, critically dependent on the climatic fluctuations (rainfall, humidity and temperatures all playing a role) typical of the South of Portugal -- and yet it is unpredictable: some species can be extremely scarce in one year and then very abundant in the next. Therefore, in these systems the species richness is apparent only after many years. The present work reports the first results of extending this characterization to an isolated holm oak tree remaining from a montado that was replaced by agriculture probably in the first decades of this century and now surrounded by tasmanian blue gum (*Eucalyptus globulus*).

Materials and methods

The holm oak tree under study is located on 38º 38' N 7º 30' W at 265 meters above sea level, standing on a lithosoil of graywackes and shists of acidic pH (in October 1995 the pH of horizon-A samples in a sector of the tree root system ranged from 4.3 to 5.6 in KCl, n = 3). The sampling area was a circle up to five meters radius around the trunk, characterized by a thin oak leaf and tiny branch litter coverage with scatttered herb and moss growth which was distinct from the surrounding eucalyptus areas. Only outside this circle living eucalyptus trees and *Cistus* shrubs were present (the nearest eucalypts are noted as dotted small circles in figures 1 to 5). After gathering the sporophores found under the oak tree, a quick exploration across the surrounding eucalyptus stand was also made.

Each separate sporophore collected for analysis was representative of a discrete occurrence in the study area. These sporophores were identified, dried and conserved in a herbarium, according to conventional methods. The classification by Hawksworth *et al.* (1995) was adopted, the species were listed by alphabetical order with basionym as recommended by Courtecuisse and Duhem (1994), and the authors names were updated according to Brummitt and Powell (1992).

The positions of the sporophores in relation to the trunk were recorded, enabling the construction of mapping diagrams designed for a better visualization of the species distributions and for the layout of species succession synthesis tables.

Species lists and mapping diagrams

The sporophores of macromycetes that were found in the area corresponding to the holm oak root system (within a 5 meter radius from the trunk) were collected in four of the eight visits made during the Fall/Winter rainfall seasons of 1996/7/8. These four visits were those that correlated closely with mild to cold temperatures and continuous periods of rain.

A total of 113 specimens were collected, 78 of which enabled a definite identification. The 40 species/varieties identified from the four collections comprised 29 that are considered facultative or obligate mycorrhizal (list 1).

List 1 Species inventoried on the "relic" holm oak (Quercus rotundifolia) root system

i) Ectomycorrhizal or facultative mycorrhizal fungi

Amanita pantherina (Dc.: Fr.) Krombholz
Clavulina cristata (L.:Fr.) Schroeter
Cortinarius obtusus Fr.:Fr.
Cortinarius evernius (Fr.:Fr.) Fr.
Cortinarius hinnuleus Fr.
Hebeloma mesophaeum (Pers.: Fr.) Quél.
Helvella lacunosa Afzel.: Fr.
Inocybe geophylla (Fr.:Fr.) Fr.
Inocybe geophylla var. *lilacina* (Peck) Gillet
Inocybe umbrina Bresadola
Laccaria laccata (Scop.: Fr.) Cooke
Lactarius cremor Fr.
Lactarius subumbonatus Lindgreen
Lycoperdon perlatum Pers.: Pers.
Ramaria versatilis Quél.
Rhizopogon obtextus (Spreng.) S. Rauschert
Russula cyanoxantha Schaeff.: Fr.
Russula drimeia Cooke
Russula fragilis (Pers.:Fr.) Fr.
Russula graveolens Romell
Russula krombholzii R. Shaffer
Russula laurocerasi Melzer

Russula pectinatoides Peck
Russula queletii Fr.
Russula sororia (Fr.) Romagn.
Russula vesca Fr.
Scleroderma bovista Fr.
Xerocomus chrysenteron (Bull.) Quél.

ii) Saprobic fungi

Agaricus bisporus (J. E. Lange) Singer
Bovista plumbea Pers.: Pers
Collybia butyracea (Bull.: Fr.) Quél.
Laccaria laccata (Scop.:Fr.) Berk. & Broome
Laccaria ohiensis (Mont.) Singer
Lepista nuda (Bull.: Fr.) Cooke
Marasmius rotula (Scop.:Fr.) Fr.
Mycena crocata (Schroad.:Fr.) Kummer
Mycena leucogala (Cooke) Sacc.
Mycena sanguinolenta (Alb. & Schwein.:Fr.) P.Kummer
Peziza cf. *badioides* Donadini
Tubaria furfuracea (Pers.:Fr.) Gillet

The sporophores found in the surrounding eucalyptus stand produced a species list very different from the holm oak-associated list (list 2).

List 2 Species inventoried on eucalyptus (Eucalyptus globulus) root system

i) Ectomycorrhizal or facultative mycorrhizal fungi

Cortinarius brunneus (Person.: Fr.) Fr.
**Laccaria laccata* (Scop.:Fr.) Cooke
Pisolithus arrhizus (Scop.) S. Rauschert

ii) Saprobic fungi

Clitocybe obsoleta (Batsch.:Fr.) Quél.
**Collybia butyracea* (Bull.: Fr.) Quél
Laccaria bicolor (Maire) P.D. Orton
**Laccaria ohiensis* (Mont.) Singer
**Lepista nuda* (Bull.: Fr.) Cooke
**Mycena leucogala* (Cooke) Sacc.
Panellus stypticus (Bull.:Fr.) P. Karst
Psathyrella candolleana (Fr.) Maire
**Tubaria furfuracea* (Pers.:Fr.) Gillet
Tubaria hiemalis Romagnesi ex Bon

* species also present in list 1

The results for the first continuous season are summarized in figure 1 / table 1 and figure 2 / table 2, corresponding to the first and second gatherings, respectively, spaced by a thirty eight days interval. In the second gathering, close to the end of the main rainfall period that winter, only one species was repeated in relation to the first — mostly there was a succession for different species, and the distribution seemed independent from the previous gathering. The sporophores were predominantly distributed to southeast of the trunk and the dominant species was *Lactarius cremor*, covering intensely the northeast, southeast and southwest quadrants but also present in the northwest quadrant. *Lactarius cremor* is normally associated with fagaceous trees and is very frequent in nude soils (Pinho-Almeida and Baptista-Ferreira, 1997), as in the present case.

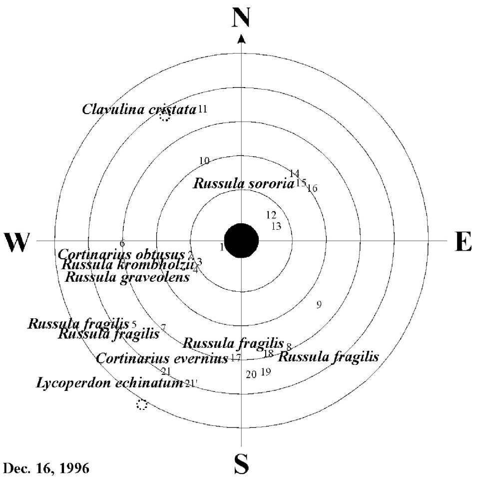

Figure 1 Diagram representing the distribution of specimens gathered on Dec. 16, 1996, over the four geographic quadrants.
The shaded circle in the center is the trunk of the tree, each outward circles represent one meter spacing each. The specimens are indicated by numbers indicating the order in which they were collected, with the taxonomic names for those that could identified. The southwest quadrant showed greatest diversity, *Russula fragilis* was the dominant species.

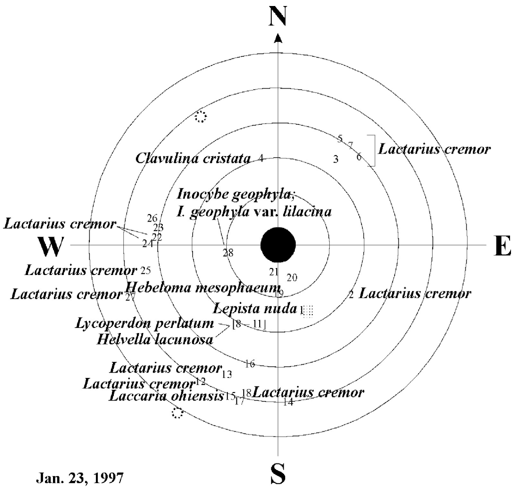

Figure 2 Same as fig.1, second gathering (Jan. 23, 1997). *Lactarius cremor* was the dominant species and occurred in all quadrants.

In the third gathering (figure 3 / table 3), the sporophores were scattered over all quadrants, but clustering predominantly southwest of the trunk. The most prominent species was *Russula sororia*, together with *Russula laurocerasi* and *Russula pectinatoides*, all three belonging to the same section and sub-section *Ingratae/Foetentinae* (Romagnesi, 1967). They are characteristic of *Quercus* spp. woods and well adapted to poor, clayish, heavy and wet soils, appearing frequently on openings.

The fourth gathering (Figure 4 / table 4) was made only after another sixty six days, again with scattering of the sporophores that were found, with greater relevance for the northwest quadrant. Now the dominant species was *Helvella lacunosa*, a facultative mycorrhizal species already present in the second gathering.

Table 1 Distribution of ECM species by different taxonomic groups. First continuous season, December 16, 1996. Average temperature: 12.1 ºC. Rainfall: 15.4 mm. Relative humidity: 96.4 %. Notice the predominance of agaricoid fungi. The genera *Russula* and *Cortinarius* are the only representatives of this group. Mycorrhizal species, m; facultative mycorrhizal species, m/f (*dominant species)

BASIDIOMYCOTINA					
APHYLLOPHOROMYCETIDAE		AGARICOMYCETIDAE			
CLAVARIALES		AGARICALES		RUSSULALES	
CLAVARIACEAE		CORTINARIACEAE		RUSSULACEAE	
Clavulina cristata (L.: Fr.) Schroeter	m/f	*Cortinarius evernius* (Fr.:Fr.) Fr.	m	*Russula krombholzii* R. Shaffer	m
		Cortinarius obtusus Fr.:Fr.	m	**Russula fragilis* (Pers.: Fr.) Fr.	m
				Russula graveolens Romell	m
				Russula sororia (Fr.) Romagn.	m

Table 2 - Distribution of ECM species by different taxonomic groups. First continuous season, January 23, 1997. Average temperature: 9.0 ºC. Rainfall: 0.2 mm. Relative humidity: 82.7%. Notice the predominance of agaricoid fungi. The genus diversity is more marked in this gathering. (* dominant species)

ASCOMYCOTINA		BASIDIOMYCOTINA									
		APHYLLOPHORO MYCETIDAE		AGARICOMYCETIDAE						GASTERO MYCETIDAE	
PEZIZALES		CLAVARIALES		AGARICALES				RUSSULALES			
HELVELLACEAE		CLAVARIACEAE		CORTINARI ACEAE		TRICHOLOMAT ACEAE		RUSSULACEAE		LYCOPERD ACEAE	
Helvella lacunosa Afzel.:Fr	m/f	*Clavulina cristata* (L.:Fr.) Schroeter	m/f	*Hebeloma mesophaeum* (Pers.: Fr.) Fr.	m	*Laccaria laccata* (Scop.: Fr.) Cooke	m	**Lactarius cremor* Fr.	m	*Lycoperdon perlatum* Pers.:Pers.	m/f
				Inocybe geophylla (Fr.:Fr.)	m						
				Inocybe geophylla var. *lilacina* (Peck) Gillet	m						

Table 3 Distribution of ECM species by different taxonomic groups. Second continuous season, November 11, 1997. Average temperature: 14.8 ºC. Rainfall: 0.2 mm. Relative humidity: 87.1%. Notice the predominance of agaricoid fungi. *Russula* and *Lactarius* were the most representatives genera. *Russula sororia* was the dominant species. (* dominant species)

BASIDIOMYCOTINA									
AGARICOMYCETIDAE								GASTEROMYCETIDAE	
BOLETALES		AGARICALES				RUSSULALES		SCLERODERMATALES	
BOLETACEAE		AGARICACEAE		CORTINARIACEAE		RUSSULACEAE		SCLERODERMATACEAE	
Xerocomus chrysenteron (Bull.) Cluéll.	m	*Amanita pantherina* (DC.:) Fr. Kromboholz	m	*Cortinarius evernius* (Fr.:Fr.) Fr.	m	*Lactarius cremor* Fr.	m	*Scleroderma bovista* Fr.	m/f
				Cortinarius obtusus Fr.:Fr.	m	*Russula cyanoxantha* Scheff.: Fr.	m		
				Cortinarius hinnuleus Fr.	m	*R. drimeia* Cooke	m		
						R. fragilis (Pers.: Fr.) Fr.	m		
						R. laurocerasi Melzer	m		
						R. pectinatoides Peck	m		
						R. queletii Fr.	m		
						**R. sororia* (Fr.) Romagn.	m		
						R. vesca Fr.	m		

Table 4 Distribution ECM species by different taxonomic groups. Second continuous season, January 18, 1998. Average temperature: 8.6 ºC. Rainfall: 0.8 mm. Relative humidity: 97.8%. The agaricoid fungi were not predominant, the dominant species was *Helvella lacunosa*. (* dominant species)

ASCOMYCOTINA		BASIDIOMYCOTINA									
		APHYLLOPHOROMYCETIDAE				AGARICOMYCETIDAE				GASTEROMYCETIDAE	
PEZIZALES		CLAVARIALES				AGARICALES		RUSSULALES		SCLERODERMATALES	
HELVELLACEAE		CLAVARIACEAE		RAMARIACEAE		CORTINARIACEAE		RUSSULACEAE		SCLERODERMATACEAE	
**Helvella lacunosa* Afzel.:Fr.	m/f	*Clavulina cristata* (L.:Fr.) Schroeter	m/f	*Ramaria versatilis* Quélet	m/f	*Cortinarius evernius* (Fr.:Fr.) Fr.	m	*Lactarius cremor* Fr.	m	*Rhizopogon obtextus* (Spreng.) S.Rauchert	m
						Cortinarius obtusus Fr.:Fr.	m	*Lactarius subumbonatus* Lindgreen	m		
						Inocybe umbrina Bresadola	m				

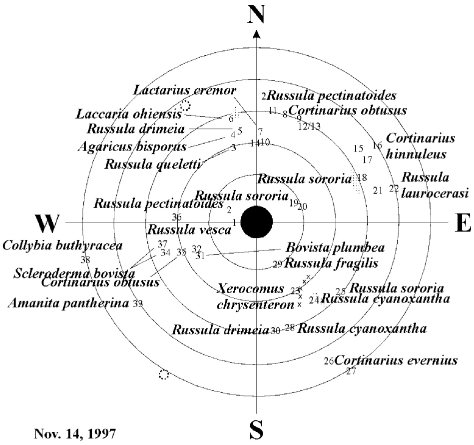

Figure 3 Same as fig.1, third gathering (Nov. 14, 1997). Sporophores were more prevalent on the southwest quadrant, and *Russula sororia* was the dominant species.

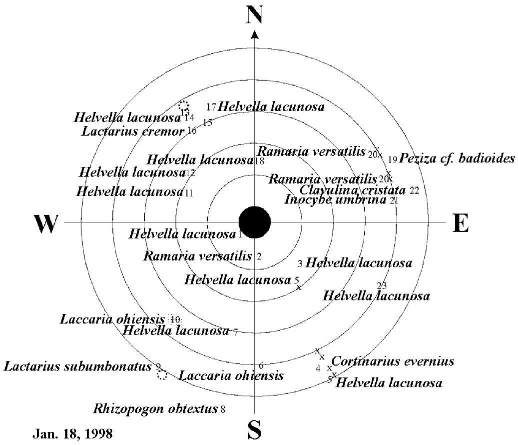

Figure 4 Same as fig.1, fourth gathering (Jan. 18, 1998). The dominant species was *Helvella lacunosa* that occurred in almost every quadrant.

Comparisons

In figure 5 and table 5 we have collected the information, from the four gatherings of the present study, on the ECM fungi distributions in space and time associated with the holm oak tree. These fungi comprise all dominant species found. Usually a frequent or dominant species in one gathering was repeated in the next gathering, albeit at much lower abundance (table 5). However, though each season appeared to be initiated by a certain diversity of the *Russula* genus (figures 1 and 3), at its end (figures 2 and 4) it was totally absent. In *Quercus* sp. woods the occurrence of *Russula* sp. sporophores usually a short period of time, limited by their intolerance to cold temperatures. The sections here represented were mainly *Ingratae*, *Piperinae* and *Heterophila*, all of them known to interact with *Quercus* sp. and well adapted to clayish soil (Pinho-Almeida and Baptista-Ferreira, 1997).

Table 5 Overlay chart for the mycorrhizal species common in the two continuous periods. The frequent or dominant species in one period passed for the next period. Time and positions according to the four quadrants. (x present; *** dominant)

	Sample				Quadrant			
Mycorrhizal (m) or facultative (m/f) species	Dec. 16, 1996	Jan. 23, 1997	Nov. 14, 1997	Jan. 18, 1998	N-E	E-S	S-W	W-N
Clavulina cristata (m/f)	x	x		x	•			•
Cortinarius evernius (m)	x		x	x		•	•	
Cortinarius obtusus (m)	x		x	x	•		•	•
Helvella lacunosa (m/f)		x		***	•	•	•	•
Lactarius cremor (m)		***	x	x	•	•	•	•
Russula fragilis (m)	***		x		•	•	•	
Russula sororia (m)	x		***		•	•		

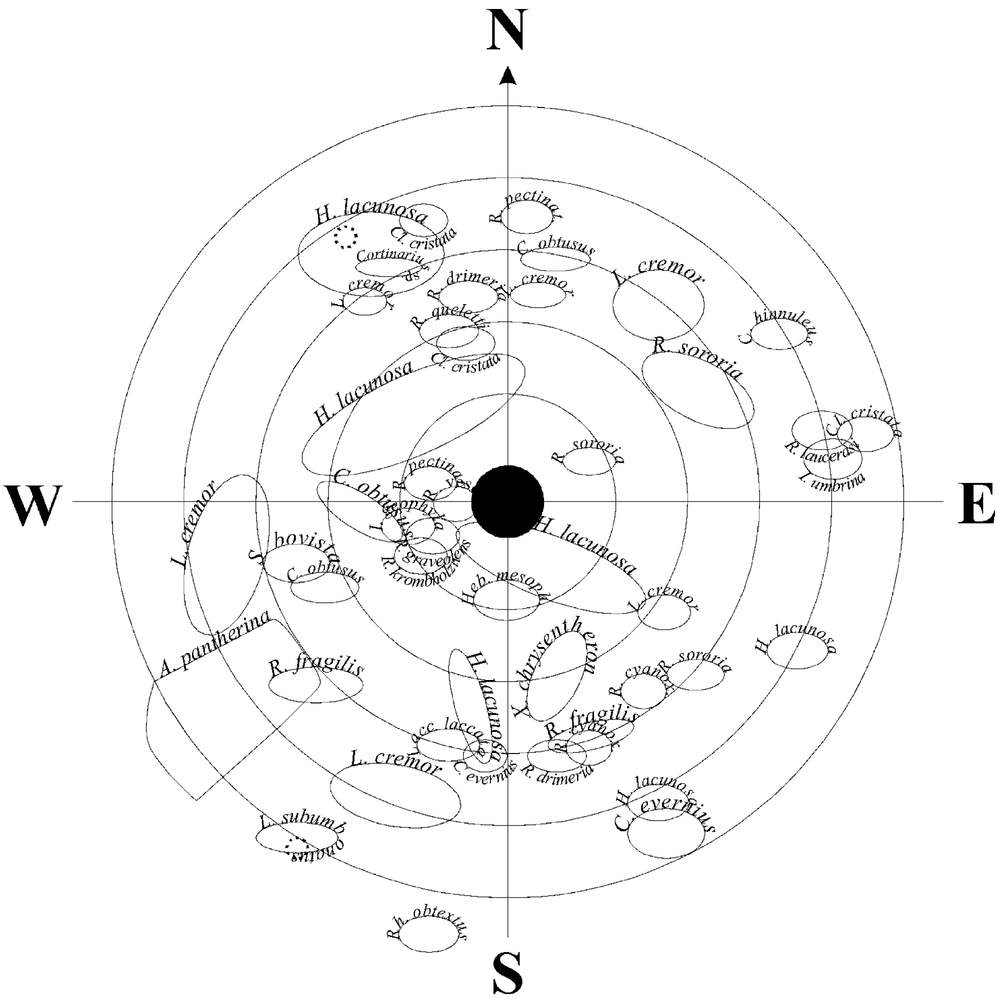

Figure 5 Summary diagram of the holm oak-associated ectomycorrhizal fungi identified in the present study.

The *Cortinariaceae* family was present in almost all gatherings on a wider spectrum of time; here they were represented by the *Telamonia* sub-genus, showed an hydrophilic tendency according to the habitats occupied at the time of basidiome formation (Moser, 1978).

Holm oak-associated saprobiont species were less than half in number compared to the ECM species, perhaps due to the soil being almost nude; organic litter or mosses were not abundant on the surveyed area. In these microecosystems the ubiquitous branch- wood- and soil- *Mycena* spp. mycobionts were common and were also found associated with the eucalypts. The appearance of one *Agaricus bisporus* may cause a little surprise due the inexistence of plains or pastures, its usual habitat, however the soil mobilization practices in the surrounding areas could justify the ocurrence of this species.

Discussion

The five-meter radius around the trunk of the holm oak tree corresponded roughly to the area of expansion of its radicular system, since this is normally commensurate with the breadth of the tree crown; it appears unlikely that the ECM fungi sporophores in this area, of species practically absent from the surrounding plantation, were associated with other mycorrhizal species (*Eucalyptus*, *Cistus*) in the neighbourhood.

Sometimes the taxonomic identity of these sporophores was difficult to ascertain, either due to bad preservation of discriminating characters or to variations that are commonplace in certain cases: for example, some close species can look similar in response to environmental conditions, while on the other hand some developmental stages of the sporophores in one species could be so variable as to lead to misinterpretations.

It is generally agreed that the holm oak forest represents the natural climax ecosystem for the interior regions of South Portugal (Ribeiro *et al.*, 1988). The *Quercus rotundifolia* tree that is the subject of the current inventory remains as a relic of this kind of ecosystem: first it became isolated by the extensive replacements by cereal crops in the first decades of the 20th century (Daveau, 1991) and, for the last 12 years or so before this study, confined by an *Eucalyptus globulus* plantation. Thus for several decades this tree has lost its mycelial linkages (Brownlee *et al.*, 1983; Amaranthus and Perry, 1994) to other ectomycorrhizal plants, making its ECM associates, of which list 1 in the present paper surely reveals only a small part, a relic mycological assemblage.

When matched with the lists of Molina *et al.* (1992), list 1 shows a majority of “broad host range” or angiosperm-restricted ECM fungi (table 6), suggesting that the presence of an eucalyptus plantation — now over a decade old — may be helpful in reconstructing mycelial linkages that allow this mycological community, in particular the early successional ECM species (Mason *et al.*, 1983), to spread. The fact that only *Laccaria laccata* sporophores appeared in association with both species does not mean that with time other fungi have the opportunity to propagate to the eucalyptus. Such spread might in turn promote the welfare of this exotic tree plantation, in which case the sparing of a century-old autochthonous oak tree, even if unwarily, would prove beneficial for the eucalyptus trees.

Table 6 Host range restriction of the ECM fungi in list 1, according to Molina et al. (1992)[a]

Narrow host range	Intermediate	Broad host range
Rhizopogon obtextus	*Russula sororia*	*Amanita pantherina*
— *Pinus* spp.	— Angiosperms	*Cortinarius evernius*
	Ramaria versatilis	*Cortinarius obtusus*
	— Angiosperms	*Hebeloma mesophaeum*
		Inocybe umbrina
		Laccaria laccata
		Lycoperdon perlatum
		Russula cyanoxantha
		Russula vesca
		Scleroderma bovista
		Xerocomus chrysenteron

[a] The following synonyms in Molina *et al.* (1992) were adopted (ref.): *Rhizopogon obtextus* = *R. luteolus*; *Ramaria versatilis* = *R. fumigata*; *Xerocomus chrysenteron* = *Boletus chrysenteron*.

Most of the *Russula* spp., the *Lactarius* spp., *Helvella* spp. and a few others (*Cortinarius hinnuleus*, *Inocybe geophylla* and *Clavulina cristata*) are absent from the lists of Molina *et al.* (1992) — but *Helvella lacunosa* has been found to be mycorrhizal with *Pinus* spp. (Martinez-Amores *et al.*, 1991) and with *Quercus* spp. (Trappe, 1971, cited in Martinez-Amores *et al.*, 1991), thus ranking with broad host range species. Possibly there was not enough information on the ecology of compatibility of those species to define their rank in that review work. Significantly, all dominant taxa in each of our gatherings belonged to this poorly characterized set. No doubt, the availability of efficient methods of identification based on DNA markers (Bruns *et al.*, 1998) will enable an unequivocal determination of the host restrictions for ECM species prevalent in Portugal, bringing other complementary advantages such as detection of species that seldom produce sporophores (Gardes and Bruns, 1996) as well as aiding in the resolution of some conventional identification difficulties.

Laboratories studies have proven that *Russula* spp. and *Lactarius* spp. have high capacity to produce extracellular oxidase and peroxidase (Gramss *et al.*, 1998). These enzymatic activities may be important for long-term biodegradation processes, acting in the remotion of organopollutants (Donnely and Fletcher, cited in Gramss *et al.*, 1998). The ectotrophic mycobionts give a selective advantage to their phytobionts, collaborating in different functions according to the developmental phase, ecological conditions and possibly climatic fluctuations (Malloch *et al.*, 1980; Brownlee *et al.*, 1983; Linderman, 1988). A higher diversity of the mycobionts, especially after reaching an adequate balance, will probably be the most beneficial for the phytobionts. Such balanced interactions require many years to be established, through a series of successions and transformations. The complex spatial distribution of ECM fungi associated with just one tree, as outlined in figure 5, might be an example of a balanced, if fragile, community. Soil mobilization practices used in the eucalyptus plantation nearby have also been made on this tree, and we wonder how long this relic ECM community will resist such practices. Courtecuisse (1997) and other authors recognized the contingency of studying the effects of disturbances that can cause irreversible consequences, urging on a reflection and a set of measures that prevents the repression or extinction caused of certain mycorrhizal taxa (Arnolds, 1989).

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