

Changes in ectomycorrhizal diversity in a declining *Quercus ilex* coastal forest

LUCIO MONTECCHIO, ROBERTO CAUSIN, SERGIO ROSSI and SERGIO MUTTO ACCORDI

Dipartimento Territorio e Sistemi Agro-Forestali, Università degli Studi di Padova, Viale dell'Università 16,
35020 Legnaro, Padova, Italy

Summary. Oak decline is generally accepted to be the result of a dynamic interaction between oaks and a mix of abiotic and biotic causes, within which environmental stresses (drought, salinity, frost, low fertility) may be important as predisposing factors. As a result of these interactions, trees gradually begin to show symptoms of general suffering, which below ground consist of functional and anatomical modifications to the rootlets and changes in the ectomycorrhizal status. The present study was performed in a coastal *Quercus ilex* forest, where decline symptoms appeared after heavy land reclamation in the adjoining areas, which caused a rapid lowering of the ground water level and the underground intrusion of seawater from the neighbouring Adriatic Sea into the forest itself. A forest survey including examination of rootlet features from asymptomatic and declining trees suggested that drought and salinity were involved in this decline. The relative frequency of the most recurrent ectomycorrhizal morphotypes distinguished clearly between asymptomatic, weakly declining and strongly declining trees, suggesting that the occurrence and distribution of only a limited number of morphotypes can give an indication of the severity of the decline. Moreover, of all the morphotypes observed only one third were found in all three decline classes, while the remaining two thirds were gradually replaced by others as the proportion of declining trees increased, where the number of morphotypes was greater. The hypothesis of an adaptive response of the ectomycorrhizal community to decline or to the predisposing factors of decline is discussed.

Key words: holm oak, forest decline, root vitality, ectomycorrhizal community.

Introduction

“Oak decline”, which is widespread in Europe, Asia, and in North and Latin America, has a complex aetiology involving dynamic interactions between plants on the one hand, and abiotic and biotic causes on the other. Trees become naturally predisposed to decline when they are weakened by long-term environmental changes (reduction in soil

fertility, air pollution). Decline symptoms appear when more intense, short-term abiotic stresses occur (i.e. drought, frost). Biotic disorders (fungal parasites, defoliating insects) are considered to be contributory factors that become operative at a later stage (Schütt and Cowling, 1985; Manion and Lachance, 1992).

Macroscopically, declining trees display a gradual increase in canopy transparency because of the wilting and falling of leaves, bud abscission, and the reduced and delayed production of new leaves. Growth slows down, the root biomass decreases, the internodes become shorter and many epicormic twigs begin to appear along the trunk and close

Corresponding author: L. Montecchio
Fax: +39 049 8272890
E-mail: montecchio@unipd.it

to the collar. In later stages, the branches begin to desiccate from the distal part, and longitudinal bark cracks sometimes appear. At this point, infections from fungi causing canker, decay, or root rot (*Armillaria* spp., *Collybia* spp., *Biscogniauxia* spp., *Diplodia* spp., *Ganoderma* spp., *Phytophthora* spp.) can appear (Halmschlager, 1998; Bruhn *et al.*, 2000; Jung *et al.*, 2000; Marcais *et al.*, 2000; Anselmi *et al.*, 2002).

Studies on many forest species report that the fine roots of declining trees often show functional and anatomical anomalies and changes of the ectomycorrhizal status as compared with healthy trees (Guillaumin *et al.*, 1985; Perrin and Estival-et, 1989; Thomas and Büttner, 1992; Blaschke, 1994; Causin *et al.*, 1996). Since little is known about holm oak (*Quercus ilex* L.) decline (Fisher *et al.*, 1994; Blaschke *et al.*, 1995; Panaiotis *et al.*, 1997), the main aim of the present study was to examine the main rootlet features (length, extent of ramification, tip vitality) and the composition of the ectomycorrhizal community in holm oak at different stages of decline in a coastal holm oak forest on sandy soil, where both drought and groundwater salinity could be involved in the decline as predisposing factors.

Materials and methods

Decline survey

The study was carried out in a declining *Quercus ilex* L. forest of 1057 ha in Bosco della Mesola (44°47' N, 12°15' E), northern Italy, which borders the Po River delta and the Adriatic Sea (Fig. 1). Heavy drainage of the areas to the east and west of the forest, which were reclaimed about 30 years ago, led to below-ground intrusion of seawater into the forest from the south, as a result of which the soil became arid, and the groundwater level was lowered from -100 to -250 cm and became brackish, with a conductivity that often exceeds 1600 $\mu\text{S cm}^{-1}$ at 18°C, particularly in the southern portions (Ministero Agricoltura e Foreste, 1984; A.R.P.A., Emilia-Romagna Region, unpublished data).

A decline survey, of 1787 *Q. ilex* trees in 33 circular areas (150 m²) and 5 transects (10×50 m) was undertaken from May to August 2000. In order to draw a detailed decline map, the decline survey was integrated with a walk-through sur-

vey, in which each tree was assigned to a defoliation class (class 1, defoliation <30%; class 2, 30–70%; class 3, >70%) using a photographic atlas (Economou *et al.*, 1994). When further major decline symptoms (dead twigs and branches, epicormic twigs, shorter internodes) were detected in a tree (Causin *et al.*, 1996), the tree was assigned to the next higher defoliation class, which represented its decline class. Trees in decline class 1 were considered asymptomatic. All trees with epigeous damage due to parasites, silvicultural practices or weather events were excluded.

Plot description and sample collection

A 2 ha study plot was chosen in an area showing the most evident signs of decline (star in Fig. 1). The plot was on average 1 m a.s.l., with alluvial sandy soil (98.5% sand), organic litter 3–6 cm deep, modern-type humus, and a soil pH of 7.2. Average annual rainfall was 694 mm and the mean monthly temperature 13.1°C. The plot was in a typical *Q. ilex* forest with poorly represented *Fraxinus ornus* and *Crataegus monogyna*.

In May 2001, five 45–55-year-old trees in each decline class were randomly selected, with each selected tree being at least 5 m apart from its neighbour. Six sampled soil cubes (10 cm³) were collected at a distance of 150 cm from each tree and stored in plastic bags at 4°C in the dark. The 90 samples so obtained were subjected to quantitative and qualitative analysis within 10 days.

Quantitative analysis

In each of the 6 soil samples per tree, 15 fine roots were randomly chosen, selecting the distal portion (fragment) including 15 undamaged and fully developed mycorrhizal tips. On each fragment, the total length (TL) and the number of ramifications (NR) were determined. All tips (1,350 per tree) were classified as 'non vital' (NV, scurfy surface and easily detachable cortex, with or without ectomycorrhizae); 'vital unmycorrhized' (NM, well-developed, inflated and turgid tip, mantle lacking); or 'vital ectomycorrhized' (EM, as above, but with a well-developed mantle) according to Baar and De Vries (1995). Damaged and not fully developed tips were excluded. Each ectomycorrhizal system was counted as one unit, irrespective of the extent of ramification. The probability of finding non-vital, vital unmycorrhized or vital ectomycorrhized tips

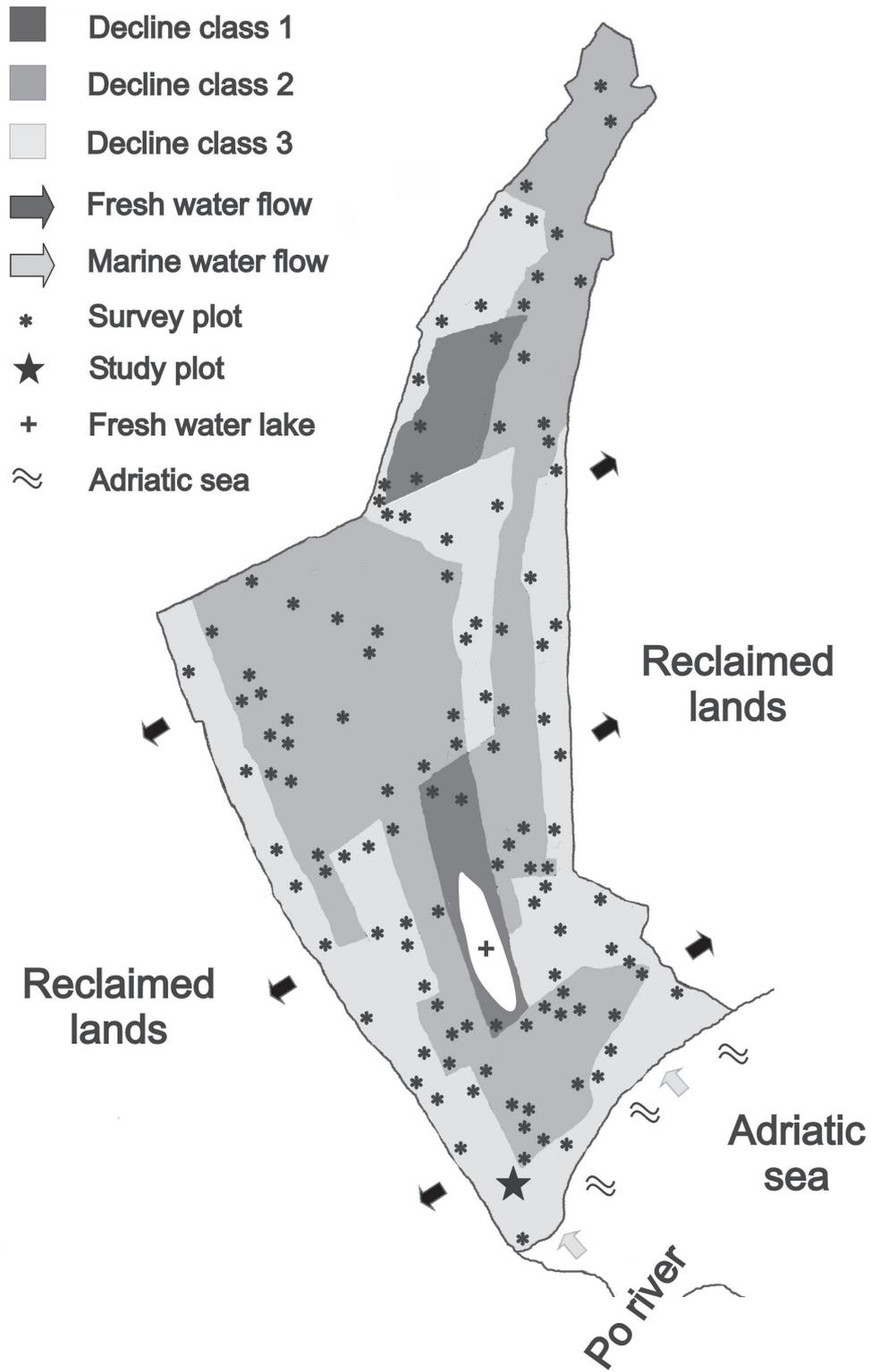


Figure 1. Decline distribution map. The decline classes indicated identify the dominant decline class observed. Decline class 1 identifies asymptomatic trees.

(PNV, PNM, PEM respectively) was calculated as a percentage of the total recovery frequency (Cassusi *et al.*, 1986). Relationships between TL, NR, PNV, PNM and PEM were determined by the Spearman correlation ($P < 0.05$). Statistical differences between decline classes were determined with the Kruskal-Wallis non-parametric test ($P < 0.05$), followed by pairwise tests between these classes using the Mann-Whitney U-test ($P < 0.05$).

Qualitative analysis

All the vital ectomycorrhizae were morphotyped, recording colour, type of ramification and features of mantle surface, type of outer mantle, occurrence and type of emanating hyphae, rhizomorphs, cystidia and laticifers (Agerer, 1991; Tedersoo *et al.*, 2003). Within 2 months, new samples were collected from the same trees so as to have sufficient mycorrhized tips to assign the most frequently occurring types to a fungal family, genus, species or to already described "unidentified" (*sensu* Agerer, 1991) ectomycorrhizae (Agerer, 1987–2002; Agerer, 1996–2002; Agerer and Rambold, 1998; Goodman *et al.*, 1996; Cairney and Chambers, 1999; Montecchio *et al.*, 1999a; Montecchio *et al.*, 1999b; Montecchio *et al.*, 2001a; Montecchio *et al.*, 2001b; Montecchio *et al.*, 2002; Montecchio *et al.*, 2004). Two undescribed morphotypes, *Lactarius chrysorrhoeus* and *Leccinum lepidum*, were classified by both morphological (Agerer, 1991; Agerer and Rambold, 1998) and molecular (White *et al.*, 1990; Gardes and Bruns, 1993) characteristics. Molecular classification was by comparing PCR amplification and restriction of the ITS region of the ribosomal DNA of the ectomycorrhizal mantles of the two morphotypes, with amplifications from the carpophores of the two species collected in the same forest. All specimens were preserved in FEA solution (formaline 90%, ethanol 5%, acetic acid 5%) and stored in the herbarium of the TeSAF Dept., University of Padova, Italy.

To guard against overestimating the number of morphotypes due to poor vitality or the paucity of distinct features, all morphotypes with a total frequency of less than 0.1% in all classes were grouped together as "minor morphotypes".

Multivariate analysis of the relations between decline classes and morphotype occurrence was performed using canonical discriminant analysis

(CANDISC procedure, SAS Institute, 1990), grouping the data by soil sample ($n=90$) and considering only the morphotypes with a recovery frequency greater than 1% in at least one decline class ($n=17$). Mahalanobis squared distance was performed to compare the means of each pair of decline classes.

Results

Decline survey

Decline symptoms were widespread throughout the forest. Trees in severe decline (class 3: white areas in Fig. 1) were mainly found far away from the internal canal network, in denser stands, in older stands, on higher ground, closer to the sea and along the forest boundary. Asymptomatic trees (class 1) were prevalent in only two areas (black areas in Fig. 1), one with young trees, the other with a small lake whose level was kept stable artificially. Trees in class 2 (medium decline: grey areas in Fig. 1) were found mainly in the remaining areas. Within the 2 ha study plot, 6% of trees were in decline class 1, 31% in class 2 and 63% in class 3.

Quantitative analysis

The Spearman correlation showed that TL was positively correlated with PEM ($r = 0.57$) and negatively with PNV ($r = -0.63$). NR was positively correlated with PNV ($r = 0.57$) and negatively with PNM ($r = -0.64$). The other correlations were not significant.

In the Mann-Whitney U-test, PNM decreased significantly from asymptomatic to severely declining trees, while PNV and NR increased significantly. No significant differences were found for TL or PEM (Table 1).

Table 1. Mean \pm standard deviation of PNM, PNV, PEM, TL (mm) and NR in each decline class. Values followed by the same letter within rows are not significantly different (Mann-Whitney U-test; $P < 0.05$).

	Class 1	Class 2	Class 3
PNM	0.32 \pm 0.10 a	0.20 \pm 0.12 ab	0.03 \pm 0.01 b
PNV	0.41 \pm 0.12 a	0.57 \pm 0.16 a	0.81 \pm 0.12 b
PEM	0.25 \pm 0.08 a	0.22 \pm 0.08 a	0.15 \pm 0.11 a
TL	15.17 \pm 2.05 a	13.80 \pm 1.42 a	11.89 \pm 2.06 a
NR	1.25 \pm 0.25 a	1.43 \pm 0.76 a	3.23 \pm 1.34 b

Qualitative analysis

Sixty-two morphotypes were recovered with a total frequency greater than 0.1%. Of these, 3 were assigned to a family (Cortinariaceae), 13 to a genus (*Amanita*, *Boletus*, *Hygrophorus*, *Inocybe*, *Lactarius*, *Russula*, *Tuber*, *Xerocomus*), 5 to a species (*Amphinema byssoides*, *Cenococcum geophilum*, *Cortinarius ionochlorus*, *Lactarius chrysorrheus*, *Leccinum lepidum*), and 4 to ectomycorrhizae previously described in detail (*Q. araneosa* + *Q. ilex*, *Q. bicolor* + *Q. ilex*, *Quercirhiza cistidiophora* + *Q. ilex*, *Q. incrustata* + *Q. ilex*). The remaining ones remained undetermined.

Asymptomatic trees had a significantly ($c^2=10.5$) lower number of morphotypes (10.6 ± 2.3) than declining trees in class 2 and class 3 (14.4 ± 1.4 and 15.8 ± 0.8 respectively). In total, 34, 45 and 40 morphotypes were detected in classes 1, 2 and 3 respectively. Twenty of the morphotypes were found in all decline classes, while 4, 10 and 14 were found exclusively in class 1, 2 and 3 respectively (Table 2). The remaining morphotypes occurred in two classes: these were always adjoining classes.

Multivariate analysis of variance detected that the 17 most frequent morphotypes (marked

Table 2. Average frequencies of morphotypes (%) in the three decline classes. Asterisks indicate morphotypes used for multivariate analysis. Group 1: Q016; group 2: Q086, Q103, Q018, Q082, Q080; group 3: Q017; group 4: Q055; Q056; Q057; Q058; Q059; Q060; Q061; Q062; Q064; Q065; Q066; Q067; Q068; Q069; Q071; Q072; Q073.

Morphotype	Class 1	Class 2	Class 3	Morphotype	Class 1	Class 2	Class 3
<i>Cortinarius ionochlorus</i> *	0.30	2.71	0.68	Q095	0.09	0.20	-
Q033 Cortinariaceae*	3.38	0.72	0.28	Q076 <i>Russula</i> sp.*	-	1.05	-
<i>Amphinema byssoides</i> *	0.42	2.59	0.37	Q084	-	0.49	-
<i>Cenococcum geophilum</i> *	1.36	0.99	0.01	Q098	-	0.40	-
Q001 <i>Boletus</i> sp.*	0.25	0.09	1.87	Q093	-	0.20	-
Q012	0.86	0.72	0.53	Q104	-	0.16	-
Q015 <i>Amanita</i> sp.	0.40	0.62	0.48	Q079	-	0.15	-
Q039 <i>Hygrophorus</i> sp.*	1.44	0.14	0.73	Q081	-	0.15	-
Q030 <i>Lactarius</i> sp.*	1.26	0.70	0.07	Q075	-	0.14	-
<i>Leccinum lepidum</i> *	1.31	0.20	0.04	Q077	-	0.12	-
<i>Quercirhiza cistidiophora</i> *	1.06	0.05	0.20	Q085	-	0.10	-
Q008 <i>Amanita</i> sp.	0.19	0.43	0.53	Group 2	-	0.26	-
Q026	0.09	0.47	0.17	Q022 <i>Inocybe</i> sp.	-	0.72	0.46
Q070 <i>Russula</i> sp.	0.58	0.07	0.01	Q005 Cortinariaceae	-	0.01	0.23
Q034 <i>Lactarius</i> sp.	0.15	0.23	0.27	Q006	-	0.14	0.01
Q002 <i>Russula</i> sp.	0.37	0.20	0.07	Q036	-	0.01	0.14
Q032	0.04	0.18	0.38	Q045	-	0.09	0.01
Q011 <i>Russula</i> sp.	0.19	0.14	0.06	Group 3	-	0.01	0.01
Q014 <i>Lactarius</i> sp.	0.10	0.07	0.04	Q024 <i>Xerocomus</i> sp.*	-	-	3.12
Q031	0.04	0.07	0.07	Q049	-	-	0.72
Group 1	0.01	0.02	0.02	Q067	-	-	0.23
<i>Quercirhiza incrustata</i> *	2.99	-	-	Q048	-	-	0.20
Q102 Cortinariaceae*	1.45	-	-	Q021	-	-	0.17
Q101	0.67	-	-	Q044	-	-	0.16
Q004	0.36	-	-	Q023	-	-	0.15
<i>Lactarius chrysorrheus</i> *	4.27	1.91	-	Q050	-	-	0.15
Q096 <i>Lactarius</i> sp.*	0.30	3.78	-	Q058	-	-	0.15
<i>Quercirhiza araneosa</i> *	1.25	0.44	-	Q019	-	-	0.14
<i>Quercirhiza bicolor</i> *	1.18	0.32	-	Q043	-	-	0.14
Q099	0.81	0.04	-	Q025	-	-	0.11
Q040	0.47	0.31	-	Q038	-	-	0.11
Q037	0.05	0.48	-	Q041	-	-	0.10
Q078 <i>Tuber</i> sp.	0.25	0.06	-	Group_4	-	-	0.87

with an asterisk in Table 2) occurred with different relative frequencies in the three decline classes. Overall, Wilk's Lambda found highly significant differences between classes (Wilk's $\lambda=0.20$; $P<0.0001$). In addition, the means of every pair of classes compared with Mahalanobis squared distances always showed significant differences ($P<0.0001$). Two discriminant functions were calculated and CAN 1 and CAN 2 were employed to plot the separation among the three classes in Fig. 2. Within the variation described by multivariate analysis, CAN 1 was responsible for 65.9% of between-class variation and separated class 3 from classes 1 and 2. CAN 2, accounted for 34.1% of between-class variation and separated class 1 from class 2. Thirteen morphotypes were highly correlated with the discriminant functions (Table 3). Among them, Q76 was positively correlated with CAN 1, Q96 was negatively correlated with CAN 2, and Q74 was positively correlated with both CAN 1 and CAN 2.

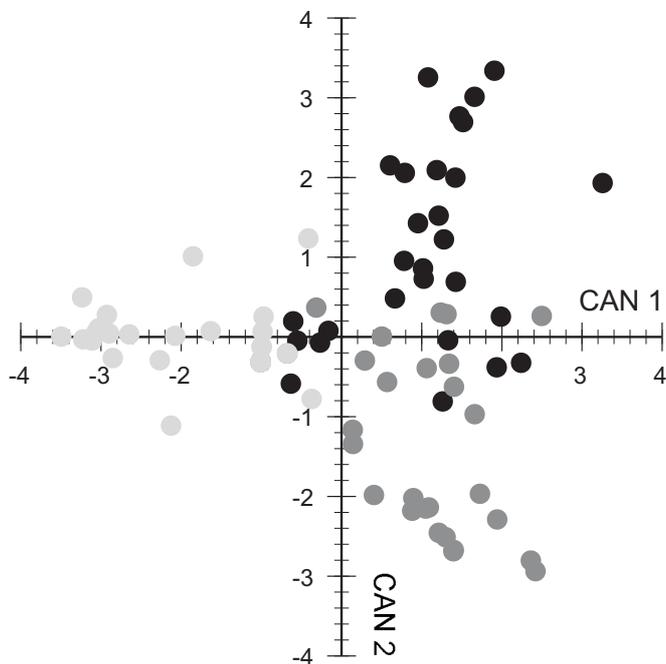


Figure 2. Canonical discriminant analysis of the 17 morphotypes identified by an asterisk in Tab. 2 and Mahalanobis squared distances ($P<0.0001$). Black, grey and white dots indicate class 1, 2 and 3, respectively.

Table 3. Pearson correlation coefficients between the discriminant functions and the morphotypes employed in the multivariate analysis. Values followed by asterisk are significant with $P<0.05$

Morphotype	CAN 1	CAN 2
Q1	-0.45*	0.05
Q3	0.20	0.05
Q9	0.28*	0.15
Q20	0.21*	-0.11
Q24	-0.63*	0.01
Q27	0.41*	0.02
Q28	0.10	0.26*
Q29	0.15	-0.40*
Q30	0.26*	0.20
Q33	0.12	0.34*
Q39	-0.11	0.15
Q74	0.23*	0.44*
Q76	0.24*	-0.51*
Q89	0.20	-0.10
Q92	0.16	0.06
Q96	0.31*	-0.49*
Q102	0.15	0.30*

Discussion

Decline symptoms were more severe when trees were farther away from surface water or groundwater, and when the saline concentration of the water was higher (A.R.P.A., Emilia-Romagna Region, 2002 unpublished data), suggesting that, as reported by other authors (Schütt and Cowling, 1985; Manion and Lachance, 1992), drought and water salinity are important environmental factors predisposing the less resistant genotypes to decline (Thomas and Büttner, 1992; Nagarajan and Natarajan, 1999; Shi *et al.*, 2002).

At the experimental plot these features were highly evident and homogeneous, since here asymptomatic, lightly declining and strongly declining coeval trees from the same gametic origin all grew close together.

An examination of the rootlets irrespective of the decline severity of the tree revealed that rootlet ramification level was positively correlated with non-vital tips, negatively with vital tips, and non-significantly with ectomycorrhizal tips. This suggested that, regardless of ectomycorrhization, and similarly to what happens above-ground with bud

necrosis, when a rootlet tip dies the rootlet produces new tips proximally, increasing the ramification and decreasing the soil volume explored (Sarrig *et al.*, 1993). Furthermore, irrespective of the ramification, rootlet length was correlated negatively with non-vital tips, positively with ectomycorrhized tips, and non-significantly with vital and unmycorrhized tips, corroborating Smith and Read (1977) who found a positive effect of ectomycorrhization on rootlet development.

When we examined the data by decline class and shifted from asymptomatic to declining trees, both the number of non-vital tips and the extent of ramification gradually doubled. Therefore, as previously reported on *Quercus robur* by Causin *et al.* (1996), the extent of ramification of rootlets having the same total length and the incidence of non-vital tips in this forest were decline-discriminant features, but the degree of ectomycorrhization was not.

From a qualitative point of view, the study corroborated Kovacs *et al.* (2000), who reported that the relative frequency of some morphotypes was related to decline severity, since multivariate analysis of the consortium shaped by the 17 most frequent morphotypes showed that their relative frequency differed strongly between asymptomatic, weakly declining and strongly declining trees, so that the severity of a decline can also be characterised through below-ground features, by determining the recovery frequency of the more frequent ectomycorrhizae independently of the least common ones.

Furthermore, about a third of the morphotypes detected occurred in all three decline classes, but the number and frequency of morphotypes assigned exclusively to one class increased with aggravating decline (tripling from asymptomatic to strongly declining trees), but decreased in frequency. Unfortunately, little is known about the dynamics and the ecological role of the ectomycorrhizal community in forest ecosystems, and still lesser about that in declining trees (Linderman, 1988; Perrin and Estivalet, 1989; Horton and Bruns, 2001). The results of the study on trees at different stages of decline and growing in close proximity to each other in the same environment, suggested that the ectomycorrhizal community changes as an indirect result of selective pressure exerted by environmental stresses on the tree and

its rootlets. These rootlets, depending on individual susceptibility, gradually decline, maintaining their full ectomycorrhization but losing their ability to select the most efficient fungal symbionts so that other, less efficient, symbionts are enabled to take their place, in accordance with a well-known principle of colonisation, that both the composition of a fungal population, and the relative frequency of the members within it, gradually change when the medium is changed (Pugh, 1980; Grayston and Campbell, 1996; Lilleskov and Bruns, 2001; Shi *et al.*, 2002). In this hypothesis, while the morphotypes common to both asymptomatic and declining trees may have had a wider adaptive range, the few ectomycorrhizae occurring exclusively on asymptomatic trees would be gradually replaced by a greater number of ectomycorrhizae growing exclusively on severely declining trees. Such ectomycorrhizae would be essential to maintain the stability of the modified ecosystem processes but would probably be less efficient than the previous ones (Zhou and Sharik, 1997; Loreau *et al.*, 2001). Examples of ectomycorrhizal fungi that do not act in a mutualistic manner throughout their entire life cycle (colonizing the tip as weak saprophytes or parasites, or growing saprophytically away from the tip, or forming resting stages) are well known (Agerer and Waller, 1993; Johnson *et al.*, 1997; Hibbett *et al.*, 2000), and the saprophytic or resting ability of some fungi colonising the most strongly declining trees, rapidly growing as mycelium or producing sclerotia on artificial media, or even generating a developed but immature sporocarp (*Xerocomus* Q024), was revealed by our laboratory observations (unpublished data).

To extend our knowledge of the successional structure of the ectomycorrhizal community on declining holm-oak, and to verify the cause-and-effect relationships between ectomycorrhizal population dynamics and tree decline severity, further investigations are essential. For this purpose, detected morphotypes are being characterised by morphological and molecular means, and the functional interactions between these morphotypes and asymptomatic holm-oaks subjected to increasing levels of both water and saline stress are being studied by measuring morphological and physiological below-ground parameters such as the root biomass.

Acknowledgements

The authors wish to thank F. Curzola, A. Fiora, A. Fioravanti, E. Gallo, L. Lorenzon, M. Sales and E. Zanetti for their help in field and laboratory observations, G. Naccarato and G. Nobili for kind co-operation during the field studies, and J. Garbaye for critical revision of the manuscript.

Literature cited

- Agerer R., (ed.), 1987–2002. *Colour Atlas of Ectomycorrhizae*. Einhorn-Verlag, Schwäbisch Gmünd, D.
- Agerer R., 1991. Characterization of ectomycorrhiza. In: *Methods in Microbiology: Techniques for the Study of Mycorrhiza* (J.R. Norris, D.J. Read, A.K. Varma, ed.), Academic Press Inc., San Diego, CA, USA, 25–73.
- Agerer R., (ed.), 1996–2002. *Descriptions of Ectomycorrhizae*. Einhorn-Verlag, Schwäbisch Gmünd, D.
- Agerer R. and G. Rambold, 1998. *DEEMY, a DELTA-based System for Characterization and DEtermination of Ectomycorrhizae, v.1.1*. Institute for Systematic Botany, Section Mycologie, University of München, D.
- Agerer R. and K. Waller, 1993. Mycorrhizae of *Entoloma saepium*: parasitism or symbiosis? *Mycorrhiza* 3, 145–154.
- Anselmi N., G.P. Cellerino, A. Franceschini, G. Granata, N. Luisi, F. Marras, A. Mazzaglia, S. Mutto Accordi, A. Ragazzi and A. Vannini, 2002. Studi sull'endofitismo di patogeni fungini di debolezza implicati nel deperimento delle querce in Italia. In: *Proceedings of SIPaV/AIPP Congress "L'endofitismo di Funghi e Batteri Patogeni in Piante Arboree e Arbustive"* (Franceschini A., Marras F., ed.), Maggio 19–21, 2002, Sassari - Tempio Pausania, Italy, 43–59.
- Baar J. and F.W. De Vries, 1995. Effects of manipulation of litter and humus layers on ectomycorrhizal colonization potential in Scots pine stands of different age. *Mycorrhiza* 5, 267–272.
- Blaschke H., 1994. Decline symptoms on roots of *Quercus robur*. *European Journal of Forest Pathology* 24, 386–398.
- Blaschke H., T. Jung, E. Paoletti and F. Bussotti, 1995. First reports of *Phytophthora* on roots of declining *Quercus ilex* in central Italy. *Giornale Botanico Italiano* 129(2), 132.
- Bruhn J.N., J.J. Wetteroff, J.D. Mihail, J.M. Kabrick and J.B. Pickens, 2000. Distribution of *Armillaria* species in upland Ozark Mountain forests with respect to site, overstory species composition and oak decline. *European Journal of Forest Pathology* 30, 43–60.
- Cairney J.W.G. and S.M. Chambers, 1999. *Ectomycorrhizal Fungi: Key Genera in Profile*. Springer-Verlag, Berlin, D, 369 pp.
- Camussi A., F. Moeller, E. Ottaviano and M. Sari Gorla, 1986. *Metodi Statistici per la Sperimentazione Biologica*. Zanichelli, Bologna, Italy, 500 pp.
- Causin R., L. Montecchio and S. Mutto Accordi, 1996. Probability of ectomycorrhizal infection in a declining stand of common oak. *Annales des Sciences Forestieres* 53, 743–752.
- Economou A., E. Beccu, G. Canu, S. Cocco, F. Bussotti, E. Cenni, A. Cozzi, M. Ferretti, M. Andrada de Conceição and G. Sanchez Peña, 1994. *Alberi della Regione Mediterranea, Guida per la Valutazione delle Chiome*. CEC-UN/ECE, Brussels, Geneva, CH.
- Fisher P.J., O. Petrini, L.E. Petrini and B.C. Sutton, 1994. Fungal endophytes from the leaves and twigs of *Quercus ilex* L. from England Majorca and Switzerland. *New Phytologist* 127, 133–137.
- Gardes M. and T.D. Bruns, 1993. ITS primers with enhanced specificity for basidiomycetes – applications to the identification of mycorrhiza and rusts. *Molecular Ecology* 2, 113–118.
- Goodman D.M., D.M. Durall, J.A. Trofymow and S.M. Berch, 1996. *Concise Descriptions of Some North American Ectomycorrhizae*. Canada Forest Service, Victoria, CA, USA.
- Grayston S.J. and C.D. Campbell, 1996. Functional biodiversity of microbial communities in the rhizosphere of hybrid larch (*Larix eurolepis*) and Sitka spruce (*Picea sitchensis*). *Tree Physiology* 16, 1031–1038.
- Guillaumin J.J., C.H. Bernard, C. Delatour and M. Belgrand, 1985. Contribution à l'étude du dépérissement du chêne: pathologie racinaire en forêt de Tronçais. *Annales des Sciences Forestieres* 42, 1–22.
- Halmschlager E., 1998. The possible role of *Armillaria* spp. and *Phytophthora* spp. in the Oak Decline Complex. In: *Proceedings of the Division 7 IUFRO Working Party, Disease/Environment Interactions in Forest Decline* (Cech T., Hartmann G., Tomiczek C., ed.), March 16–21, 1998, Vienna, Austria, 49–56.
- Hibbett D.S., L.B. Gilbert and M.J. Donoghue, 2000. Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. *Nature* 407, 506–508.
- Horton T.R. and T.D. Bruns, 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Molecular Ecology* 10, 1855–1871.
- Johnson N.C., J.H. Graham and F.A. Smith, 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist* 135, 575–585.
- Jung T., H. Blaschke and W.F. Osswald, 2000. Involvement of *Phytophthora* species in central and western European oak decline and the influence of site factors on the disease. *Plant Pathology* 49, 706–718.
- Kovacs G., M. Pausch and A. Urban, 2000. Diversity of ectomycorrhizal morphotypes and oak decline. *Phyton - Annales Rei Botanicae* 40, 109–116.
- Lilleskov E.A. and T.D. Bruns, 2001. Nitrogen and ectomycorrhizal fungal communities: what we know, what we need to know. *New Phytologist* 149, 154–158.
- Linderman R.G., 1988. Mycorrhizal interactions with the rhizosphere microflora: the mycorrhizosphere effect. *Phytopathology* 78, 366–371.
- Loreau M., S. Naem, P. Inchausti, J. Bengtsson, J.P. Grime, A. Hector, D.U. Hooper, M.A. Huston, D. Raffaelli, B. Schmid, D. Tilman and D.A. Wardle, 2001. Biodiversi-

- ty and ecosystem functioning: current knowledge and future challenges. *Science* 294, 804–808.
- Manion D. and D. Lachance, 1992. *Forest Decline Concepts*. APS Press, St. Paul, Minnesota, MN, USA, 249 pp.
- Marçais B., O. Cael and C. Delatour, 2000. Relationship between presence of basidiomes, above-ground symptoms and root infection by *Collybia fusipes* in oaks. *European Journal of Forest Pathology* 30, 7–17.
- Ministero Agricoltura e Foreste (ed.), 1984. *Gran Bosco della Mesola. Piano di Gestione Naturalistica per il Decennio 1980–1989*. Officine Grafiche Calderini, Bologna, I, 189 pp.
- Montecchio L., S. Rossi and R. Causin, 2001a. “*Quercirhiza incrustata*” + *Quercus ilex* L. *Descriptions of Ectomycorrhizae* 5, 73–78.
- Montecchio L., S. Rossi, A. Grendene and R. Causin, 2001b. *Cortinarius ionochlorus* R. Maire + *Quercus ilex* L. *Descriptions of Ectomycorrhizae* 5, 35–40.
- Montecchio L., S. Rossi, A. Grendene and R. Causin, 2002. *Amphinema byssoides* (Pers.: Fr.) J. Erikss. + *Quercus ilex* L. *Descriptions of Ectomycorrhizae* 6, 1–6.
- Montecchio L., S. Rossi, A. Grendene and R. Causin, 2004. “*Quercirhiza cistidiophora*” + *Quercus ilex* L. *Descriptions of Ectomycorrhizae* 7 (in press).
- Montecchio L., S. Rossi, A. Grendene, R. Causin and S. Mutto Accordi, 1999a. “*Quercirhiza araneosa*” + *Quercus ilex* L. *Descriptions of Ectomycorrhizae* 4, 91–96.
- Montecchio L., S. Rossi, A. Grendene, R. Causin and S. Mutto Accordi, 1999b. “*Quercirhiza bicolor*” + *Quercus ilex* L. *Descriptions of Ectomycorrhizae* 4, 97–102.
- Nagarajan G. and K. Natarajan, 1999. The use of Box-Behnken design of experiments to study in vitro salt tolerance by *Pisolithus tinctorius*. *World Journal of Microbiology and Biotechnology* 15, 197–203.
- Panaiotis C., C. Carcaillet and M.M. Hamedi, 1997. Determination of the natural mortality age of an holm oak (*Quercus ilex* L.) stand in Corsica (Mediterranean Island). *Acta Oecologica* 18, 519–530.
- Perrin R. and Estivalet, 1989. Mycorrhizal association and forest decline. *Agriculture, Ecosystems & Environment* 28, 381–387.
- Pugh G.J.F., 1980. Strategies in fungal ecology. *Transactions of the British Mycological Society* 75, 1–14.
- Sarig S., Roberson E.B., Firestone M.K., 1993. Microbial activity soil structure: response to saline water irrigation. *Soil Biology and Biochemistry* 25, 693–697.
- SAS Institute Inc., 1990. *SAS/STAT User's Guide*. Version 6, 4th Ed. Vol. 1 and 2, Cary, NC, USA.
- Schütt P., E.B. Cowling, 1985. Waldsterben, a general decline of forests in central Europe: symptoms, development and possible causes. *Tree Disease* 69, 548–558.
- Shi L.B., M. Guttenberger, I. Kottke and R. Hampp, 2002. The effect of drought on mycorrhizas of beech (*Fagus sylvatica* L.): changes in community structure, and the content of carbohydrates and nitrogen storage bodies of the fungi. *Mycorrhiza* 12, 303–311.
- Smith S.E. and D.J. Read, 1977. *Mycorrhizal symbiosis*. Academic Press Inc., San Diego, CA, USA, 605 pp.
- Tedersoo L., U. Kõljalg, N. Hallenberg and K.H. Larsson, 2003. Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. *New Phytologist* 159, 153–165.
- Thomas F.M. and G. Büttner, 1992. Excess nitrogen, drought and winter frost as possible predisposing factors of oak decline in northern Germany. In: *Proceedings of the International Congress Recent Advances in Studies on Oak Decline* (Luisi N., Lerario P., Vanini A., eds.), September 13–18, 1992, Selva di Fasano, Brindisi, Italy, 285–291.
- White T.J., T.D. Bruns, S. Lee and J. Taylor, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Diagnostics and Forensics. In: *PCR protocols - A guide to Methods and Applications* (Innis M., Gelfand D., Sninsky J., White T, ed.) Academic Press Inc., San Diego, CA, USA, 315–322.
- Zhou M. and T.L. Sharik, 1997. Ectomycorrhizal associations of northern red oak (*Quercus rubra*) seedlings along an environmental gradient. *Canadian Journal of Forest Research* 27, 1705–1713.

Accepted for publication: March 30, 2004