Effects of water stress on the endophytic mycobiota of Quercus robur

UNIVERSITÀ DEGLI STUDI DI TORINO

Effects of water stress on the endophytic mycota of *Quercus robur*

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Oak decline is expanding throughout European and North American forests, in connection with increasing drought periods. The role of endophytic communities in oak decline is almost unknown, considering that these communities may harbour latent pathogens promoted by host weakness. This experiment was designed to assess if and in which extent endophytic mycota of adult English oak (*Quercus robur*) trtees are affected by water stress. In a typical oak plain forest of north-western Italy, two square plots were established each including 25 oak trees. In one plot a polyethylene layer was placed for rainfall flow away to occur and, consequently, to induce water stress in trees. Predawn Water Potential (PWP) and Midday Water Potential (MWP) were used as water stress indicators and measured constantly. Three seasonal samplings (spring, summer, and fall) were carried out to isolate fungal endophytes on herbaceous and woody tissues of trees from both plots. Twenty-five *taxa* were isolated, most of which were ascomycetes and anamorphic fungi. *Tubakia dryina* and *Phomopsis quercina* were the most frequently isolated fungi in annual shoots. The frequency (F) of *T. dryina* was significantly lower in water-stressed than in control trees (χ^2 test, $P<0.05$) both in summer and in autumn, when water stress was higher. The F of *P. quercina* increased over time. The F of *Monochaetia monochaeta* in annual shoots starting from the summer was significantly higher in control trees than in water-stressed trees. The correspondence analysis discriminated shoot communities of water-stressed trees from those of control trees, because of the different ratios between *T. dryina* and *P. quercina*.

Key words: climate change, endophytism, fungal ecology, oak decline

Introduction

The endophytic mycota of broad-leaved trees of temperate regions had scarcely been studied in comparison with those of pooid grasses, those of conifers or those of tropical hosts (Kumar and Hyde, 2004; Suryanarayan and Thennarasan, 2004). This is especially true for the ecological role of fungal endophytes in relation to the host, rather than for their taxonomic

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characterization. For instance, some fungi recurrently found in needles of conifers are considered as mutualistic to their host to some extent, such as *Rhabdocline parkeri* Sherwood, J.K. Stone et G.C. Carroll in Douglas fir (Carroll, 1986) or *Lophodermium conigenum* (Brunaud) Hilitzer in Scots pine (Minter and Millar, 1980a,b).

Since the end of the 1980s, the fungal communities inhabiting tissues of several deciduous trees, especially *Quercus* species, has been investigated and the main endophytic components of bark, wood or leaves are quite well known (Petrini and Fisher, 1990; Halmschlager *et al*., 1993; Collado *et al*., 1996; Anselmi *et al*., 2004). Despite a connection between these studies with the phenomenon of oak decline, information on the behaviour of fungal endophytes subjected to an increasing stress of their host is lacking. This is an impelling research issue considering that for two decades oak decline has been affecting forests throughout central and southern Europe and the USA. There is a general agreement on the hypothesis that the triggering factors, at least in the Mediterranean region, are irregular precipitations resulting in long drought periods (Cellerino and Gennaro, 2000). Drought stress is recurrent and results in trees having weaker defences against pathogens. Opportunist pathogens benefit from altered host physiological and biochemical parameters occurring after the onset of water stress (Yarwood, 1959; Houston, 1984; Boyer, 1995).

Attention should be focused on the endophytic mycota, which is always present and may comprise pathogens in a latent phase (Photita *et al*., 2004). Not all the investigators agree that endophytes should include latent pathogens (Sinclair and Cerkauskas, 1996). Several well known tree pathogens are, however, often isolated from asymptomatic tissues and, importantly, are present inside the host without needing wounds or other entry methods. Moreover, because they are in close and continuous contact with the plant, these organisms are extremely sensitive to minor changes in metabolic pathways and parameters, and are ready to take advantage of a decrease in the plants defences.

The detection and identification of fungal endophytes whose presence and/or abundance is promoted by water stress represent an essential step in the understanding of Oak decline. Some research on the mycota of declining oaks, where fungal communities of asymptomatic and declining oak trees have been compared, have already been published (Gennaro *et al*., 2003; Ragazzi *et al*., 2003). However, information on the quantitative and qualitative evolution of endophytic communities during water stress is still lacking. In this study we investigate the variation of the endophytic mycota of adult English oak (*Quercus robur* L.) trees under simulated water stress. In this manner, we were

able to assess the development over time of an endophytic mycota in relation to changes in water potential.

Materials and methods

The study site

The study was performed in the "*Bosco delle Sorti della Partecipanza*" Regional Park (Piedmont), a typical plain forest of northern Italy. This forest, located at 150 m is mainly composed of English oak, while sessile oak [*Q. petraea* (Matt.) Liebl.] and Turkish oak (*Q. cerris* L.) are sparse. The rain distribution in the area is equinoctial, with a main maximum in autumn and secondary rains in spring, and an average precipitation of about 800 mm per year. The climate is continental, but the relative humidity (R.H.) is high throughout the year and fog is frequent in the autumn and winter. In the last few years, however, long drought periods have often overcome the soil capacity for water retention. This was observed especially in some sloped areas where the water layer, which is very high in winter, greatly lowers in dry summers preventing the superficial root systems from being soaked. The texture of soil is slimy or slimy-clay, with argillic B horizon.

In the forest, a sector composed exclusively of English oaks was selected. Two square plots were established in March 2003, each of 250 m^2 and each comprising 25 trees with a mean diam. at breast height of 20 cm. Trees in one plot were subjected to artificial water stress by placing a polyethylene layer, suitably disposed and fixed to the trunks, under the crown, in order to let rainfall flow away from the plot and to avoid the so-called stem flow. The other was a control plot and it was left exposed to the atmospheric conditions without any cover.

Sampling and isolation

After bud opening (in the middle of April), after the warmest summer period (at the end of August) and during leaf fall (between the end of October and the beginning of November), five shoots of one year and three 3-years-old twigs were collected from the centre of the crown of each of the 25 trees per plot. In the same periods, five cores (1.5 cm diameter) were extracted at 1 m height from the sapwood of each tree.

The samples were stored in polyethylene bags at 5°C. Before isolation the samples were rinsed under running distilled water for 30 s, then they were surface-sterilised by immersion in a 10% hydrogen peroxide solution for 7-10

min, depending on the type of tissue. Seven minutes of immersion were adopted for the young annual shoots, and 10 minutes for the twigs and the sapwood cores. The surface sterilisation was followed by five rinsings in sterile water, in accordance with a method tested by several research units in Italy (Gennaro *et al*., 2003; Ragazzi *et al*., 2003). From each annual shoot, two fragments of 2-3 mm^3 were cut using a sterile scalpel. Along each twig four 3 mm-thick disks were cut every 3 cm; then from each disk a 2-3 mm^3 bark fragment and a 2-3 mm^3 wood fragment were obtained. From each sapwood core four bark fragments and four wood fragments were taken. All the fragments were singly placed in 5 cm diam. Petri-dishes filled with potato dextrose agar (PDA) amended with streptomycin to suppress bacterial growth (39 g/l PDA, 60 mg/l streptomycin). Dishes were incubated for five days at 23°C and 100% R.H. in the dark, and checked periodically for about 1 month to isolate all the fungal colonies growing out from the fragments. Fungal colonies were identified on the basis of their macroscopic and microscopic features.

Water stress measurements

During the vegetative season of 2003, the Predawn Water Potential (PWP) and the Midday Water Potential (MWP), the latter approximating the minimal water potential, were measured every 15 days on four representative trees selected in the central part of each plot. Measurements were taken with a pressure chamber with nitrogen flow (Skye Instruments Ltd, Llandrindod Wells, UK), according to the manufacturer's instructions.

Data interpretation and statistical analysis

The frequency of occurrence (F) was calculated for each fungus as the percentage of colonised fragments on the total number of fragments inoculated. A chi-square analysis was performed on F data (not normal distributions) with season, health state and tissue type as factors in a contingency table.

For each collecting period, the Analysis of Variance (ANOVA) was employed to compare the number of *taxa* between water-stressed and control trees.

Correspondence analysis (Sieber *et al*., 1998) on F values was conducted to assess the diversity among fungal assemblages and to identify the factors affecting such diversity.

Statistical analyses were performed using the software Statistica (StatSoft).

Results

The vegetative season of 2003 was exceptionally warm and dry, resulting in very low PWP and MWP both in the water-stressed and in the control plots, associated with a strong dieback also of the trees not subjected to polythene cover. In this paper, however, we will refer to trees under cover as «stressed» and to control plot trees as «not-stressed». Significant differences in terms of both PWP and MWP between stressed and not-stressed trees resulted in September (Fig. 1).

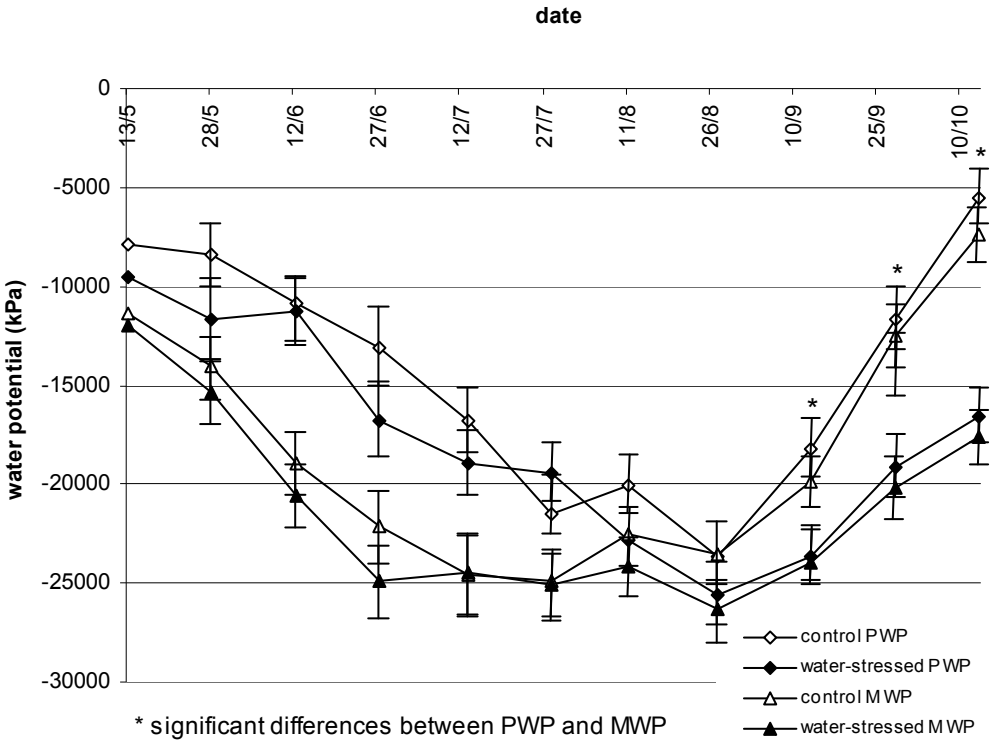

Fig. 1. Predawn Water Potential (PWP) and Midday Water Potential (MWP) of artificially water-stressed and control *Q. robur* trees measured along the whole vegetative season. Bars show standard errors.

Table 1. Frequency of occurrence (% on the total number of fragments inoculated) of fungal endophytes isolated from various tissues of water-stressed and control *Q. robur* trees in three different sampling periods.
S = annual shoots; TB = twig bark; TW = twig wood; WB = trunk bark; WW = trunk sapwood*

	SPRING										SUMMER										AUTUMN									
	water-stressed					control					water-stressed					control					water-stressed					control				
fungal species	S	TB	TW	WB	WW	S	TB	TW	WB	WW	S	TB	TW	WB	WW	S	TB	TW	WB	WW	S	TB	TW	WB	WW	S	TB	TW	WB	WW
Acremonium strictum W. Gams	0,0	0,0	2,7	0,0	3,0	0,0	0,0	0,9	0,0	4,5	0,0	0,0	0,5	0,0	0,9	0,0	0,0	2,5	0,0	0,0	0,0	0,0	1,1	0,0	0,0	0,0	0,0	0,0	0,0	5,1
Cladosporium cladosporioides (Fr.) de Vries	5,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Colletotrichum acutatum J.H. Simmonds	0,0	0,0	0,0	0,0	0,0	0,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Coniothyrium fuckelii Sacc.	7,2b	2,2a	0,7a	0,0a	0,0a	5,0ab	2,9a	0,0a	0,0a	0,0a	4,2a	0,0a	0,0a	0,9a	0,0a	8,8b	1,1a	0,0a	0,0a	0,0a	3,3a	3,3a	0,0a	0,7a	0,0a	5,0ab	2,7a	1,5a	0,0a	0,0a
Dematiaceous sterile mycelium 1	0,0	0,0	0,0	2,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Dematiaceous sterile mycelium 2	0,0	0,0	0,0	0,0	0,0	0,0	1,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,9	0,0
Dendrodochium sp.	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,2	0,0	0,0	0,0	0,0	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,2	0,0	0,0	0,0	0,0
Diplodina sp.	0,0	0,0	3,3	0,0	5,0	0,0	0,0	0,7	0,0	0,0	0,0	0,0	0,0	0,0	2,5	0,0	0,0	5,5	0,0	1,6	0,0	0,0	0,0	0,0	4,3	0,0	0,0	0,7	0,0	1,7
Epicoccum nigrum Link	0,0	0,0	0,0	0,0	0,0	1,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Eutypella sp.	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,0	0,0	0,0	0,0	0,0
Fusarium lateritium Nees:Fr.	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,3	0,0	0,0	0,0	0,0	0,0	0,0
Hyaline sterile mycelium	0,0	0,0	1,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,7	0,0	0,0
Monochaetia monochaeta (Desm.) Allesch. in Rabenh	5,6b	0,0a	0,0a	0,0a	0,0a	7,1b	1,2a	0,0a	0,0a	0,0a	4,0ab	3,1a	0,0a	0,0a	0,0a	10,2b	2,5a	0,0a	0,0a	0,0a	0,5a	0,0a	0,0a	0,0a	0,0a	5,1b	0,9a	0,0a	0,0a	0,0a
Monocillium sp.	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Mucor hiemalis Wehmer	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Nigrospora sphaerica (Sacc.) E.W. Mason	0,0	0,0	0,0	0,0	0,0	0,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,1	0,0	0,0	0,0	0,0
Nodulisporium sp.	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Oedocephalum sp.	0,0a	0,7a	1,6a	0,0a	6,7b	0,0a	0,0a	4,1ab	0,0a	3,0a	0,0a	0,0a	0,7a	0,0a	1,5a	0,0a	1,1a	5,0ab	0,0a	0,9a	0,0a	0,5a	0,0a	0,0a	2,6a	0,0a	0,0a	3,7a	0,0a	5,2ab
Phoma sp. 1	5,6ab	3,3a	0,0a	0,0a	0,0a	7,7ab	0,0a	0,0a	0,0a	0,0a	5,9ab	0,0a	0,0a	0,0a	0,0a	10,1b	2,6a	0,0a	0,0a	0,0a	1,1a	0,0a	0,0a	0,0a	0,0a	6,7ab	0,0a	0,0a	0,0a	0,0a
Phoma sp. 2	2,0a	2,5a	0,0a	1,7a	0,0a	1,3a	4,9a	0,7a	0,7a	0,0a	0,7	5,2ab	0,0a	0,9a	0,5a	1,1a	3,7a	0,0a	2,2a	0,0a	0,0a	9,9ab	0,0a	0,5a	0,0a	0,9a	7,1ab	0,0a	3,3a	0,7a
Phomopsis quercina (Sacc.) Höhn.	12,0b	2,3a	0,0a	0,0a	0,0a	5,1ab	3,7a	0,0a	0,0a	0,0a	11,2b	1,3a	0,0a	0,0a	0,0a	1,7a	1,9a	0,0a	0,7a	0,0a	24,3c	2,5a	0,0a	1,7a	0,0a	3,3a	3,4a	0,0a	0,5a	0,0a
Ramichloridium anceps (Sacc. et Ell.) de Hoog	0,0	0,0	0,0	0,0	0,0	0,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Scytalidium lignicola Pesante	0,0a	0,0a	5,0a	0,0a	1,1a	0,0a	0,7a	3,3a	0,0a	3,5a	0,5a	0,0	6,7ab	0,0a	0,0a	0,0a	3,7a	2,1a	3,9a	0,0a	1,3a	2,5a	5,0a	5,4a	0,0a	0,0a	7,4ab	7,7ab	2,7a	0,7a
Trichoderma viride Pers.: Fr.	0,0	5,0	0,0	0,0	0,0	0,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Tubakia dryina (Sacc.) Sutton	3,2a	1,8a	0,0a	0,0a	0,0a	9,2ab	0,8a	0,0a	0,0a	0,0a	4,7a	1,1a	0,0a	0,0a	0,0a	14,9b	0,0a	0,0a	0,0a	0,0a	0,7a	0,7a	0,0a	0,0a	0,0a	15,6b	0,0a	0,0a	0,0a	0,0a

* numbers with the same letters are not significantly different following chi-square analysis (P<0.05). *Taxa* with no letters are intended as without any significant difference in their frequencies.

Table 2. Total number and mean number of fungal *taxa* isolated from various tissues of water-stressed and control *Q. robur* trees in three different sampling periods. S = annual shoots; TB = twig bark; TW = twig wood; WB = trunk bark; WW = trunk sapwood.

		Total number of species		**Mean number of species**	
		Water-stressed	**Control**	**Water-stressed**	**Control**
Spring	S	8	11	5.56	6.96**
	TB	7	7	4.36	4.28NS
	TW	6	5	3.72	2.84**
	WB	2	2	0.48	0.44NS
	WW	4	3	1.48	1.36NS
Summer	S	8	11	4.96	6.36**
	TB	4	8	2.08	4.68**
	TW	3	4	1.16	1.44NS
	WB	2	5	0.52	2.80**
	WW	4	2	1.68	0.48**
Autumn	S	6	9	3.72	5.92**
	TB	6	5	2.76	2.76NS
	TW	2	4	0.48	2.52**
	WB	5	4	2.56	2.24NS
	WW	2	5	0.44	2.20**

** and NS indicate significant at $P \leq 0.01$ and not significant ($P > 0.05$)

Twenty-five fungal *taxa* were isolated; three of these were isolated exclusively from stressed trees and eight exclusively from control trees (Table 1). Two *taxa* persisted as dematiaceous sterile mycelia. The zygomycetes included one *taxon*, whereas the remainding fungi belonged to ascomycetous or anamorphic species.

With the exception of twig-wood (TW) samples collected in the spring isolated from control trees was either significantly higher than that of taxa isolated from stressed trees or no significant differences occurred (Table 2).

Tubakia dryina and *Phomopsis quercina* were the most frequent taxa isolated from the herbaceous tissues of annual shoots (Table 1). They showedopposite trends with respect to the water stress level of the hosts. *Tubakia dryina* was significantly less frequent in stressed trees than in control trees both in the summer sampling and in the autumn sampling ($P<0.05$), whereas *Phomopsis quercina* was significantly more frequent in stressed trees. This difference was highly significant in the autumn ($P<0.01$), when the frequency of *P. quercina* had a remarkable increase. An inverse ratio between *Tubakia dryina* and *Phomopsis quercina* isolated from the annual shoots is especially evident in the autumn. While in the samples collected from the control plot the former species was over 15% F and the latter under 5% F, in those from the water-stressed plot *P. quercina* reached almost 25% F and *Tubakia dryina* was reduced to near zero.

Although less strongly characterised, *Monochaetia monochaeta* showed a trend comparable with that of *Tubakia dryina*, since a difference of its frequencies between stressed and non-stressed trees became significant starting from the summer sampling and was constant until the autumn (higher about 5% in control trees, $P<0.05$). A decrease in the frequency of this species occurred during the advanced vegetative season, however the decrease was not significant.

Among the fungi isolated from the bark tissues of twigs, a species of *Phoma* (referred as *Phoma* sp. 2 in Table 1) should be mentioned, as frequencies varyied from 2.5% to almost 10% towards the autumn, but did not differentiate significantly. Only *Acremonium strictum* and *Oedocephalum* sp. were constant in isolation frequencies from sapwood tissues.

Correspondence analysis showed some distinct groups of communities (Fig. 2). At the positive limit of the first axis the annual shoot samples are grouped, i.e. those characterised by the highest number of species isolated, whereas at the opposite limit the wood samples are confined. This central axis is substantially shaped by quantitative elements. The second axis gives further indications of the distribution patterns. The shoot samples collected from the control trees are all positioned at higher scale values than those collected from stressed trees and this fact may be related to a qualitative difference in the mycota composition. The same is true for the relative positions of the wood samples obtained from the twigs and from the sapwood, mainly grouped at lower and higher scale values, respectively.

Discussion

The lack of rain in 2003 may have affected the objectives of the present research. As demonstrated by PWP and MWP values, the trees growing in the control plot were also affected by water stress in summer and this may have reduced a potential divergence between the mycota of control and water-stressed trees. Despite this, we were able to detect significant differences in the frequencies of *Tubakia dryina*, *Monochaetia monochaeta* and *Phomopsis quercina*. The first two species were more frequently associated with control oak trees, and this finding is in agreement with the results of previous studies (Gennaro *et al*., 2003). A certain recovery of hydration during the autumn was probably sufficient to restore a suitable habitat for these fungi in the control trees, especially for *Tubakia dryina*, whose frequency in these hosts became comparable to that of *Phomopsis quercina* in the water-stressed trees.

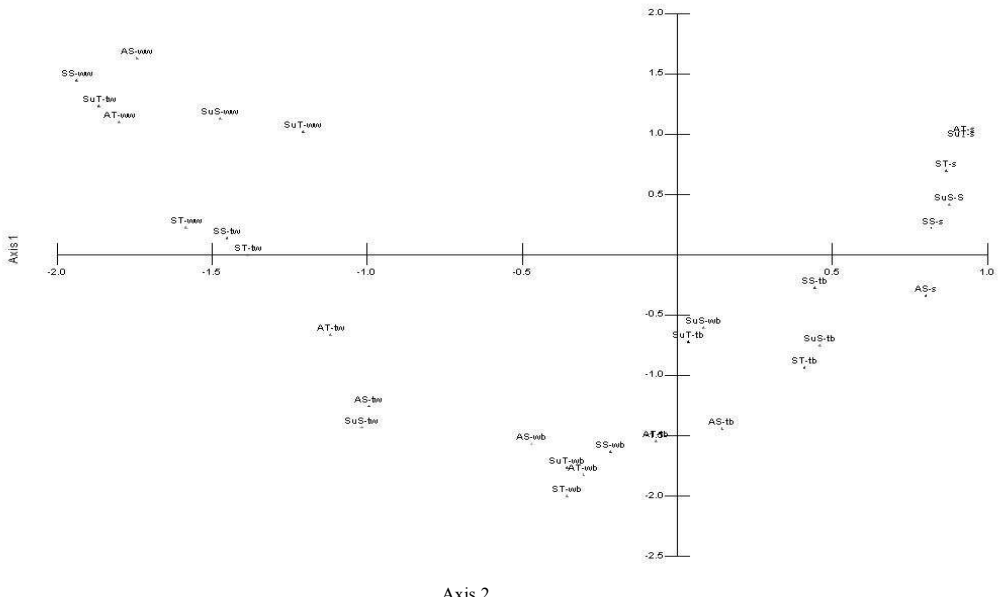

Fig. 2. Ordination by correspondence analysis of frequencies of endophytic fungi from different tissues of artificially water-stressed and control *Q. robur* trees in three different samplings. Total inertia explained by the first two principal axes: 70.6%. First letters: S = spring, Su = summer, A = autumn; second letter: S = water-stressed, T = control; small letters: s = annual shoots, tb = twig bark, tw = twig wood, wb = trunk bark, ww = trunk sapwood.

Tubakia. dryina is known to be one of the main fungal endophytes of asymptomatic English oaks in northern Italy (Gennaro *et al*., 2003). Several records of this fungus as a leaf pathogen, agent of necrotic spots, have been reported both from North America and Europe (Munkvold and Neely, 1990), especially in Red oak (*Q. rubra* L.), but occasionally on Turkish oak (Belisario, 1990). Nevertheless, none of these reports were associated with a decline syndrome and resulting attacks were typical of a weak pathogen. In the present research, *T. dryina* was isolated as a specific endophyte of a European oak, and the taxonomic relationship of these isolates with those pathogenic isolates from introduced Red oak should be investigated through molecular and physiological approaches. Molecular approaches have been used to assess the endophytic diversity of *Alternaria alternata* associated with pine (e.g. Guo *et al*., 2004), and endophytic *Colletotrichum* species associated with various hosts (Photita *et al*., 2005).

Monochaetia monochaeta was also been frequently associated with control trees, corroborating the results of a previous research performed in the same stand (Gennaro *et al*., 2003). In concomitant studies carried out in north-

eastern and central Italy, however, a species of *Monochaetia* was isolated from shoots of various oak species independently of the health state of the host (Ragazzi *et al*., 2003). *Monochaetia* species are well-known producers of compounds with anti-fungal activity (Li *et al*., 2001) and also *M. monochaeta* may be pathogenic in post-harvest syndromes on tropical fruits (Pino and Sanabria de Albarracin, 1995). The behaviour of this fungus in English oak is therefore uncertain and needs to be clarified.

Species of *Phomopsis* are often referred to as having weak pathogenic behaviour on *Quercus* (Hartmann *et al*., 1999; Luque *et al*., 2000; Ragazzi *et al*., 2001), and the higher isolation frequencies of *P. quercina* in water-stressed trees, in this study, are in agreement with this character. Nevertheless, a possible involvement of this fungus in the oak decline, as a secondary pathogen, need to be assessed.

Water stress seems to have induced a progressive qualitative shift of the endophytic mycota of English oak, with *P. quercina* being more frequent than *T. dryina*. This fact is also mirrored in the correspondence analysis, where the shoot fungal communities of control and water-stressed hosts resulted in distinct positions along one axis, probably because of the different ratios between the two main fungi, i.e. *T. dryina* and *P. quercina*. In an analogous way, the different positions along the same axis of the communities inhabiting twig wood and trunk sapwood, respectively, are probably linked to the higher frequencies of *Scytalidium lignicola* in the former tissue.

The fungal communities of annual shoots yielded a higher number of taxa in comparison when compared to that of the other investigated tissues. This is agreement with previous findings (Gennaro *et al*., 2003). The mean number of taxa isolated from annual shoots was significantly lower in water-stressed than in non stressed trees. This was true in all sampling periods, even when stressed and control trees could not be differentiated in terms of water potential (i.e. spring and summer). The mycota associated with herbaceous tissues may be more sensitive than mycota associated with woody tissues to fluctuations in water content.

The limited colonisation of woody tissues by fungal endophytes in this study, notable in the correspondence analysis, is not a new finding. Kowalski and Kehr (1992) reported that only 9% of *Q. robur* samples were colonised by fungi in their woody parts and do not appear to be affected by water stress.

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References

Anselmi, N., Cellerino, G.P., Franceschini, A., Granata, G., Luisi, N., Marras, F., Mazzaglia, A., Mutto Accordi, S. and Ragazzi, A. (2004). Geographic distribution of fungal endophytes of *Quercus* sp. in Italy. In: *Endophytism in Forest Trees* (eds. A. Ragazzi, S. Moricca and I. Dellavalle). Italian Academy of Forest Sciences, Italy: 73-89.

Belisario, A. (1990). *Tubakia dryina* (Sacc.) Sutton segnalato per la prima volta su *Quercus rubra* L. in Italia. Informatore Fitopatologico 40: 54-56.

Boyer, J.S. (1995). Biochemical and biophysical aspects of water deficits and the predisposition to disease. Annual Review of Phytopathology 33: 251-274.

Carroll, G.C. (1986). The biology of endophytism in plants with particular reference to woody perennials. In: *Microbiology of the Phyllosphere* (eds. N.J. Fokkema and J. van den Heuvel). Cambridge University Press, UK: 205-222.

Cellerino, G.P. and Gennaro, M. (2000). Drought as predisposing factor in oak decline. In: *Decline of Oak Species in Italy – Problems and Perspectives* (eds. A. Ragazzi and I. Dellavalle). University of Firenze – Research National Council – Italian Academy of Forest Sciences, Italy: 157-175.

Collado, J., Platas, G. and Peláez, F. (1996). Fungal endophytes in leaves, twigs and bark of *Quercus ilex* from Central Spain. Nova Hedwigia 63: 347-360.

Gennaro, M., Gonthier, P. and Nicolotti, G. (2003). Fungal endophytic communities in healthy and declining *Quercus robur* L. and *Q. cerris* L. trees in northern Italy. Journal of Phytopathology 151: 529-534.

Guo, L.D., Xu, L., Zheng, W.H. and Hyde, K.D. (2004). Genetic variation of *Alternaria alternata*, an endophytic fungus isolated from *Pinus tabulaeformis* as determined by random amplified microsatelites (RAMS). Fungal Diversity 16: 53-65.

Halmschlager, von E., Butin, H. and Donaubauer, E. (1993). Endophytische Pilze in Blättern und Zweigen von *Quercus petraea*. European Journal of Forest Pathology 23: 51-63.

Hartmann, G., Habermann, M., Kruger, F. and Niemeyer, H. (1999). Waldschutzsituation 1998/99 in Niedersachsen und Schleswig-Holstein. Forst und Holz 54: 219-226.

Houston, D.R. (1984). Stress related to diseases. Arboricultural Journal 8: 137-149.

Kowalski, T. and Kehr, R.D. (1992). Endophytic fungal colonization of branch bases in several forest tree species. Sydowia 43: 137-168.

Kumar, D.S.S. and Hyde, K.D. (2004). Biodiversity and tissue-recurrence of endophytic fungi from *Tripterygium wilfordii*. *Fungal Diversity* 17: 69-90.

Li, J.Y., Harper, J.K., Grant, D.M., Tombe, B.O., Bashyal, B., Hess, W.M. and Strobel G.A. (2001). Ambuic acid, a highly functionalized cyclohexenone with antifungal activity from *Pestalotiopsis* spp. and *Monochaetia* sp. Phytochemistry 56: 463-468.

Luque, J., Parlade, J. and Pera, J. (2000). Pathogenicity of fungi isolated from *Quercus suber* in Catalonia (NE Spain). Forest Pathology 30: 247-263.

Minter, D.W. and Millar, C.S. (1980a). Ecology and biology of three *Lophodermium* species on secondary needles of *Pinus sylvestris*. European Journal of Forest Pathology 10: 169-181.

Munkvold, G.P. and Neely, D. (1990). Pathogenicity of *Tubakia dryina*. Plant Disease 74: 518-522.

Petrini, O. and Fisher, P.J. (1990). Occurrence of fungal endophytes in twigs of *Salix fragilis* and *Quercus robur*. Mycological Research 94: 1077-1080.

Photita, W., Lumyong, S., Lumyong, P., McKenzie, E.H.C. and Hyde, K.D. (2004). Are some endophytes of *Musa acuminata* latent pathogens? Fungal Diversity 16: 131-140.

Photita, W., Taylor, P.W.J., Ford, R., Lumyong, P., McKenzie, E.H.C. Hyde, K.D. and Lumyong, S. (2005). Morphological and molecular characterization of *Colletotrichum* species from herbaceous plants in Thailand. Fungal Diversity 18: 117-133

Pino, P. and Sanabria de Albarracin, N. (1995). Diagnostico de enfermedades fungicas postcosecha en mango (*Mangifera indica* L.) para exportacion. Ernstia 5: 73-79.

Ragazzi, A., Moricca, S., Capretti, P., Dellavalle, I., Mancini, F. and Turco, E. (2001). Endophytic fungi in *Quercus cerris*: isolation frequency in relation to phenological phase, tree health and the organ affected. Phytopathologia Mediterranea 40: 165-171.

Ragazzi, A., Moricca, S., Capretti, P., Della valle, I. and Turco, E. (2003). Differences in composition of endophytic mycobiota in twigs and leaves of healthy and declining *Quercus* species in Italy. Forest Pathology 33: 31-38.

Sieber, T.N., Petrini, O. and Greenacre, M.J. (1998). Correspondence analysis as a tool in fungal taxonomy. Systematic and Applied Microbiology 21: 433-441.

Sinclair, J.B. and Cerkauskas, R.F. (1996). Latent infection vs. endophytic colonization by fungi. In: *Endophytic Fungi in Grasses and Woody Plants – Systematics, Ecology and Evolution* (eds. S.C. Redlin and L.M. Carris). APS Press, USA: 3-29.

Suryanarayanan, T.S. and Thennarasan, S. (2004). Temporal variation in endophyte assemblages of *Plumeria rubra* leaves. Fungal Diversity 15: 197-204.

Yarwood, C.E. (1959). Predisposition. In: *Plant Pathology, Vol. I* (eds. J.G. Horsfall and A.E. Dimond). Academic Press, USA: 521-562.

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