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Fungal endophytes from the leaves and twigs of *Quercus ilex* L. from England, Majorca and Switzerland

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SUMMARY

Leaves of *Quercus ilex* taken from sites in England, Majorca and Switzerland have been studied to detect the influence of the geographic position of the host within and outside its native range on the composition of its endophytic fungal assemblages. Samples of stem tissue of *Q. ilex* collected from the Swiss trees were also studied to confirm tissue-specific differences. Sixty different fungal taxa were isolated, but only 28 were frequent. Of the total number of isolates from the leaves from the Swiss, British and Spanish sites 87%, 31% and 63%, respectively, were coelomycetes. Four species of *Phomopsis*, which includes *Phyllosticta ilicina* (= *Phomopsis ilicina* v. d. Aa, ined.), were the most frequent endophytes of leaves and were either absent or rare in the twig units. Two distinct kinds of sterile mycelia were common in twigs. Swiss and Spanish trees possessed fungal assemblages distinct from those present in Britain. Naturalized stands were distinguished from native stands by the presence of rather cosmopolitan and non-specific fungal taxa, rare or absent in the samples collected in the native stands. Samples derived from the native stands were colonized by more host-specific fungi. The relative frequency of two sterile mycelia in the Swiss and Spanish sites determined their separation. *Phyllosticta* (*Phomopsis*) *ilicina*, the most numerous leaf colonizer, was virtually absent from the bark and the xylem. The frequent occurrence of coelomycetes as endophytes of woody trees is briefly discussed.

Key words: *Quercus ilex* (oak), fungal ecology, fungal distribution, coelomycetes.

INTRODUCTION

Investigations on endophytic fungi of perennial trees and shrubs have demonstrated that a number of ecological factors such as the microclimate (Petrini, 1985; Canavesi, 1987; Johnson & Whitney, 1989), the age of the tissues colonized (summarized in Petrini, 1991), or anthropogenic modifications (Barklund & Rowe, 1983; Canavesi, 1987; Riesen & Close, 1987; Sieber, 1988, 1989) greatly influence endophytic assemblages. Studies of fungal endophytes of ericaceous hosts (Petrini, 1985), of conifers (Sieber-Canavesi & Sieber, 1988) and of leaves, xylem and bark of *Eucalyptus nitens* (Deane & Maiden) Maiden in Australia and England (Fisher, Petrini & Sutton, 1993) have shown that the geographic origin of the samples often determines the composition of the endophyte assemblages. Site-related differences may be caused by the density of the stands (Helander *et al.*, unpublished), but trees growing outside their natural distribution area may also tend to become colonized by indigenous fungi and not by their host-specific symbionts. Studies by Carroll, Müller & Sutton (1977), Espinosa-Garcia & Langenheim (1990), and more recently by Fisher *et al.* (1993) confirm that the endophyte assemblages of trees planted outside their original range are depauperate and consist of species different from those in native habitats.

Quercus ilex L. is an evergreen tree native to the Mediterranean region extending north in western Europe to Brittany. In Britain the species has been introduced but has become naturalized in southern England (Clapham, Tutin & Warburg, 1962). In this investigation the endophytic assemblages of the leaves of *Q. ilex* taken from sites in England, Majorca and Switzerland have been studied to detect the influence of the geographic position of the host

Table 1. *Percentage frequency of occurrence of endophytes isolated from 150 leaves taken from 3 trees at each site and 30 twig pieces from each of three trees (Swiss site only) of* Quercus ilex. *Only those fungi that were present at frequencies higher than 5% in at least one type of tissue have been included in the main table. S, Swiss site; B, British site; M, Spanish site. P1–P3, twig bark; X1–X3, twig xylem; 1–3 number of tree*

	Leaves			Twigs					
	S	B	M	P1	X1	P2	X2	P3	X3
Total number of isolates...	743	500	558	26	20	32	16	34	6
Alternaria alternata (Fr.) Keissler	7·5	4·5	4·5	0	0	0	0	0	0
Alternaria sp.	0	0	19·5	0	0	0	0	0	0
Asteromella sp.	0	7	0	0	0	0	0	0	0
Aureobasidium pullulans (de Bary) Arnaud	0	17	3·5	0	0	0	0	0	0
Cladosporium cladosporioides (Fr.) de Vries	3	9·5	2	0	0	0	0	0	0
Colletotrichum acutatum Simmonds	8·5	1·5	0	0	0	0	0	0	3·33
Coniothyrium fuckelii Sacc.	0	1·5	7	0	0	0	0	0	0
Cytospora ceratophora Sacc.	0·5	6	0	0	0	0	0	0	0
Fusarium lateritium Nees: Fr.	2·5	6	0	0	0	0	0	0	0
Geniculosporium cf. *serpens* Chesters & Greenhalgh	8·5	6·5	3	0	0	0	0	0	0
Nodulisporium anam. *Hypoxylon fragiforme* (Fr.) Kickx	1·5	6·5	14	6·7	6·67	6·67	0	16·7	6·67
Nodulisporium sp.	0·5	11·5	0	0	0	0	0	0	0
Phialophora hoffmannii (van Beyma) Schol-Schwarz	0	6	0	0	0	0	0	0	0
Phoma sp. 2	17·5	3	0	0	0	0	0	0	0
Phomopsis glandicola (Lév.) Gonz. Frag.	46·5	11·5	10·5	0	0	0	0	0	0
Phyllosticta ilicina Sacc. (= *Phomopsis ilicina* v. d. Aa ined.)	38·5	18·5	35·5	10	0	3·33	0	3·33	0
Phomopsis quercella (Sacc. & Roumeg.) Died.	23	16	4	0	0	0	0	3·33	0
Phomopsis sp. 1	28·5	0	2	10	10	0	16·7	0	3·33
Phymatotrichopsis sp.	0·5	0	8·5	0	0	0	0	0	0
Sordaria fimicola (Rob. ex Desm.) Ces. & de Not.	0	3	5·5	0	0	0	0	0	0
Sphaerographium sp.	0	1	6·5	0	0	0	0	0	0
Sporotrichum sp.	0	11	14·5	0	0	0	0	0	0
Sterile mycelium, sp. 1	2	0	17·5	1	10	46·7	10	3·33	0
Sterile mycelium, sp. 2	16	0	0	47	33·3	16·7	20	46·7	13·3
Tubakia dryina (Sacc.) Sutton	16	0·5	0	0	0	0	0	0	0
Tubakia subglobosa (Yokoyama & Tubaki) Sutton	0	3·5	8	0	0	0	0	0	0
Xylaria spp.	0	11	6	0	0	0	0	0	0

Taxa isolated with a frequency of less than 5% included: *Apiognomonia errabunda* (Rob.) Höhnel, *Ascochyta* sp., *Camarosporium* sp., *Chaetomium globosum* Kunze, *Coniochaeta* cf. *pulveracea* (Ehrh.) Munk, *Coniothyrium olivaceum* Bonorden, *Coniothyrium* sp., *Epicoccum nigrum* Link, *Geniculosporium* sp., *Gilmaniella humicola* Barron, *Microdochium caespitosum* Sutton, Pirozynski & Deighton, *Nigrospora oryzae* (Berk. & Br.) Petch, *Penicillium glabrum* (Wehmer) Westling, *Periconia* cf. *macrospinosa* Lefebvre & Johnson, *Phoma exigua* Desm., *Phoma leveillei* Boerema & G. J. Bollen, *Phoma* spp., *Phomopsis* sp. 2, *Rhinocladiella* cf. *compacta* Carrión ex de Hoog, *Sordaria humana* Fuckel, *Sporormiella intermedia* (Auersw.) Ahmed & Cain, and *Stemphylium* anam. *Pleospora herbarum* (Pers.: Fr.) Rabenh.

within and outside its native range on the composition of its endophytic assemblages. At the same time, samples of stem tissue of *Q. ilex* collected from the Swiss trees were also screened for endophytic symbionts to confirm tissue-specific differences.

MATERIALS AND METHODS

During 1993 endophytes were isolated from 1- to 2-yr-old leaves taken from three mature trees of *Q. ilex* standing in park land on the Campus of the

University of Exeter, grid reference SY 925915. From each tree, fifty leaves were cut approx. 2–3 m from the ground. The material was taken to the laboratory and processed within 24 h. The leaves were thoroughly washed in running water before surface sterilization by the immersion sequence 75 % ethanol for 1 min, 0·93–1·3 M solution of sodium hypochlorite (3–5 % available chlorine) for 3 min and 75 % ethanol for 0·5 min. After surface sterilization each leaf was cut into four fragments that were then placed serially in groups of four in Petri dishes containing 1·5 % Oxoid malt extract agar (MEA) supplemented with 250 mg l^{-1} terramycin (Pfizer) to suppress bacterial growth. The same procedure was followed for the isolation of endophytes from leaves of two trees about 50 m apart standing in park land in Cases Noves Esporles and one tree 13 km from the other two in the Valdemossa Valley, all on the Island of Majorca. Finally, Swiss leaf and stem collections were made from three trees standing in Caslano, Ct. Ticino, on the shore of the lake of Lugano. In addition to 50 leaves, three branch pieces from each tree, approx. 15 cm long, were collected and surface sterilized as described. Each piece was then separated into bark and xylem and each piece was placed in its own sterile container. The bark was cut into 1 cm units. From each container ten bark units were randomly selected and placed in groups of five on MEA. Next the xylem pieces were cut individually into 1 cm units, surface sterilized again and placed on MEA.

All plates were incubated at 20 ± 2 °C for 5–14 d, depending on the growth rates of the fungi which emerged. Isolation was by transfer of mycelium, conidia or ascospores to 2 % MEA plates. Near UV-light (Philips TL 40W/05) was used to induce sporulation.

Only those fungi that were present at frequencies higher than 5 % in at least one type of tissue were used for the ordination analysis. Data were transformed in percentage frequencies, defined as the number of isolates derived from a given leaf or twig unit sample divided by the total number of units plated out for each sample, to account for differences in size between leaf and twig samples. Simple correspondence analysis was performed on the reduced matrix of the data using the package SimCA 2.1 (Greenacre, 1986). Twig samples were treated as supplementary variables in the correspondence analysis, since the experimental design in this part of the study was inherently different from the one used for the isolations from the leaves.

RESULTS

Of approx. 60 different fungal taxa isolated, only 28 were present at frequencies of colonization higher than 5 % in at least one type of tissue (Table 1). Of the total number of isolates from the leaves from the Swiss, British and Spanish sites 87 %, 31 % and 63 %, respectively, were coelomycetes. Four species of *Phomopsis* were the most frequently isolated endophytes of leaves and were either absent or rare in the twig units. Two distinct species of sterile mycelia were common in twigs and present only to a lesser extent in some leaf samples (Table 1, Fig. 2).

The correspondence analysis (Fig. 1) showed that the Swiss and Spanish trees possessed fungal assemblages distinct from those in Britain. The first axis of the correspondence analysis was determined by the gradient naturalized stand–native stands and the gradient was produced by the presence of rather cosmopolitan and non-specific fungal taxa such as *Aureobasidium pullulans*, *Asteromella* sp., *Cladosporium cladosporioides*, *Cytospora ceratospora*, *Fusarium lateritium*, *Phialophora hoffmannii* and *Xylaria* sp. in the British trees. These fungi, on the other hand, were rare or absent in the samples collected in the native stands. The samples derived from the native stands were colonized by more host-specific fungi such as *Phomopsis glandicola*, *P. quercella*, *Phyllosticta* (*Phomopsis*) *ilicina*, *Tubakia dryina*, *T. subglobosa* and the two sterile mycelia sp. 1 and sp. 2 (Fig. 2). The relative frequency of these taxa in the two sites (Fig. 2) determined their separation on the second axis of the correspondence analysis (Fig. 1).

P. ilicina, the most frequent leaf colonizer, was virtually absent from the bark and the xylem. Only

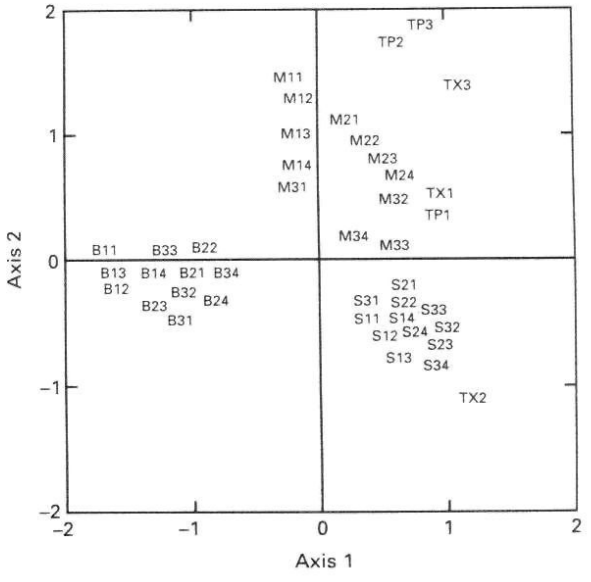

Figure 1. Results of the ordination by simple correspondence analysis. Only those fungi that were present at frequencies higher than 5 % in at least one type of tissue of *Quercus ilex* have been included in analysis (see Table 1). B, British leaves; M, Spanish leaves; S, Swiss leaves; T, twigs; P, bark; X, xylem. The first number (1–3) in the leaf sample codes refers to the tree number, the second (1–4) to the leaf piece: 1, petiole; 2, leaf base; 3, leaf middle; 4, leaf tip. Total inertia explained by the first four co-ordinates: 68 %.

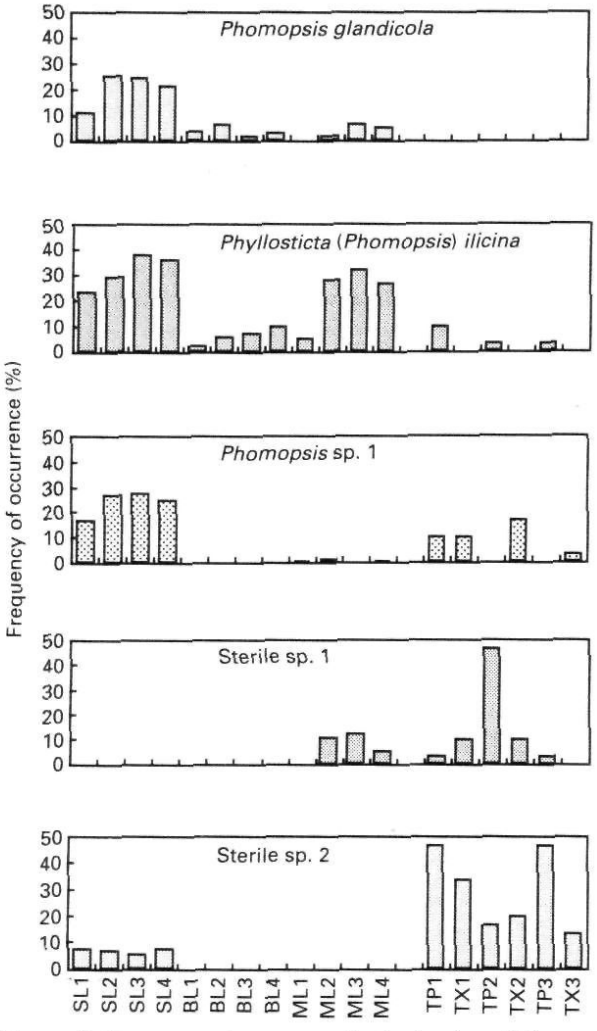

Figure 2. Percentage frequency of colonization of *Quercus ilex* by the five most frequent endophytic fungi. Symbols as in Fig. 1. Numbers 1–4 (L) encode the leaf portions: 1, petiole; 2, leaf base; 3, leaf middle; 4, leaf tip. Numbers 1–3 (T) refer to the tree numbers.

the anamorph of *Hypoxylon fragiforme* and the two sterile mycelia were present in large amounts in these tissues (Fig. 2).

DISCUSSION

The endophytic assemblages of the *Q. ilex* trees were composed of a number of cosmopolitan species such as *Alternaria* spp., *Aureobasidium pullulans*, *Cladosporium cladosporioides*, of fungi such as the xylariaceous anamorphs *Geniculosporium* spp. and *Nodulisporium* spp. known to live endophytically in a large number of hosts but to fruit only on a few of them, and of fungal taxa that can be defined as host-specific. The latter included a large number of coelomycetous taxa such as *Phomopsis glandicola*, *P. quercella*, *Phyllosticta* (*Phomopsis*) *ilicina*, and *Tubakia dryina* and *T. subglobosa*.

Results of correspondence analysis showed that the position of the samples on the first axis reflects their geographic origin, with trees growing within their natural range hosting an endophytic flora more host-specific than that present in trees planted outside their natural range. For instance, *Aureobasidium pullulans*, a common opportunistic endophytic colonizer of many plant species was prominent in the British leaves, scarce in Majorca and absent from the Swiss leaves. Fisher *et al.* (1993) noted that the endophytic assemblages of *Eucalyptus nitens* in Australia were different from those isolated from British samples of the same tree species. These authors suggested that the endophyte assemblages outside the host's natural range were depauperate and consisted of species different from those in native habitats. The set-up of the study used by Fisher *et al.* (1993) did, however, not allow any firm conclusion to be drawn. The present study was specifically designed to detect such site-specific differences and confirms the hypothesis of Fisher *et al.* (1993), as well as earlier results of Carroll *et al.* (1977) who studied the endophyte assemblages of Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] and coastal redwood [*Sequoia sempervirens* (D. Don) Endl.]. The second axis of the correspondence analysis separates the Swiss and Spanish samples, both characterized by rather host-specific endophyte species. This probably reflects the climatic conditions present at both sites. The richest fungal flora and the highest incidence of some fungal species was found in leaves from the trees on the shore on the lake of Lugano. This may be the result of higher humidity caused by the close proximity to the lake shore, although no humidity measurements are available for the various sites to support this hypothesis. Within each site, gradients can be seen that approximately reflect the topographic position of the leaf fragments. In general, leaf tips were more heavily colonized than the bases, although the difference is not statistically significant. No qualitative differences in species composition could be detected. Tissue specificity by endophytes could, however, be demonstrated by the rare occurrence of leaf endophytes in the wood. The position of the Swiss twig samples along the second axis of the correspondence analysis was closer to the Spanish leaf samples than to the Swiss ones. This apparent anomaly is caused by the comparatively few species and the small number of isolates present in the woody tissues.

In this, as in previous studies of wood-inhabiting endophytes (summarized in Petrini, 1986), coelomycetes form a dominant group. In general, coelomycetous anamorphs are widespread colonizers of plant tissues, in which they can be present either as pathogens or endophytes (e.g. Sutton, 1980, Petrini, 1986). An example where a coelomycete has evolved competitive ability which favours its host is *Phomopsis oblonga* (Desm.), an abundant bark-inhabiting saprotroph of *Ulmus glabra* (Huds.) in northern and western Britain where it can limit the breeding of the

beetle vectors of Dutch elm disease and may also physically prevent *Ceratocystis ulmi* (Buis.) Moreau, from invading colonized tissues by utilizing most of the easily assimilated substrates (Webber & Hedger, 1986). The possible role of coelomycete endophytes in general, and particularly of such high concentrations as have been found in leaves in this study, is as yet poorly understood and will require further investigation.

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