A preliminary study of fungi inhabiting xylem and whole stems of *Olea europaea*

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Ascomycetes, Deuteromycetes, and Zygomycetes were isolated from six mature trees of *Olea europaea* growing on the Island of Majorca, Spain. Whole stems and xylem were colonized by quantitatively distinct assemblages of fungi. Only *Kabatina* sp. showed complete tissue specificity and was entirely confined to the xylem in all trees. Distinct fungal assemblages were also detected in trees from different sites. The colonization frequency of *Kabatina* sp. was particularly high in the samples derived from trees from the dry area. Trees growing at the humid site were characterized by low colonization levels of *Mucor racemosus*.

Keywords: Deuteromycotina, endophytes, olive trees, ecology.

Only scant information exists on the presence of symptomless fungi in the bark and xylem of woody plants. Fisher & Petrini (1988) isolated twenty-seven fungal species from apparently healthy stems and xylem of *Ulex europaeus* L. and found that the taxa were distinctly host and tissue specific. In addition, the frequency of colonization was greater or equal in whole stems than in xylem alone. In a study on endophytic fungi of twigs of *Pinus sylvestris* L. and *Fagus sylvatica* L. growing at the same site, Petrini & Fisher (1988) showed that distinct fungal assemblages colonize the two hosts. Similar results were obtained by Fisher & Petrini (1990) in a study comparing xylem and bark of *Alnus* spp. in England and Switzerland. Work on other tree species (Sieber, 1989; Sieber & al., 1991) provided additional evidence for the hypothesis that not only the leaves but also the woody organs of some plant species develop a highly specific fungal community. The present study was designed to record possible tissue specificity of fungi colonizing xylem and whole stems of *Olea europaea* L. growing at humid and dry sites on the Island of Majorca, Spain.

Materials and methods

Six mature trees of *Olea europaea* were chosen. Three trees (T1, T2, T3) were sampled from Es Salobrar de Campos, a very dry area in the South East of the Island of Majorca, Spain, with an annual rainfall of approx. 350 mm (Guijarro, 1986) and the remaining three trees (T4, T5, T6) from a rather humid area (annual rainfall: 600 mm per annum; Guijarro, 1986) on a North West facing mountain slope to the West of Valldemossa. Sampling was done at the end of January, 1991.

From each tree fifteen 10 cm long and 1 – 1.5 cm thick branches were cut. Ten of these pieces were washed in running water before surface sterilization was performed 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. Each piece was then cut into approx. 1 cm long segments that were placed in groups of five onto 1.5% Oxoid malt extract agar (MEA) supplemented with 250 mg/L Terramycin to supress bacterial growth. The remaining five pieces from each tree were stripped of their bark; surface sterilization and plating onto MEA was performed with this remaining xylem as described above. All plates were incubated at 20 ± 2 C for 5–14 d, depending on the growth rates of the fungi. Isolation was by transfer of conidia or mycelium to 2% MEA plates. Near uv light (Philips TL 40W/05) was used to induce sporulation.

For data analysis the colonization frequency of the tree tissues by a fungal species was defined as the total number of pieces of a tissue (whole stems or xylem) colonized by a given fungus.

Results and discussion

Whole stems and xylem of *O. europaea* harbour distinct fungal assemblages (Tab. 1, Fig. 1). The sample size is not large enough to allow for any definitive conclusion to be drawn but the data show clear tendencies. Only *Kabatina* sp. showed complete tissue specificity and was entirely confined to the xylem in all trees. The colonization frequency of this fungus was particularly high in the trees from the dry area (Tab. 1). *Mucor racemosus, Phoma* sp. 1, and *Sporormiella intermedia* were mainly isolated from the trees growing at the dry site whereas *Ascochytulina deflectens* preferentially colonized the trees in the humid area. There are indications that species composition and frequency of fungal communities are dependent on the geographic situation of the trees.

Trees growing at the humid site are characterized by low colonization of *M. racemosus*. The median colonization frequency is 10%, as compared with 90% for the trees growing in the dry area (Fig. 1). In

Tab. 1.– Frequency of fungal colonization (total number of pieces of a tissue colonized by a given fungus) of *O. europaea* twig pieces. For each tree 10 pieces of whole stems and five of xylem were investigated. T1, T2, T3: trees from the dry site; T4, T5, T6: trees from the humid area. S: whole stems; X: xylem.

	T1S	T1X	T2S	T2X	T3S	T3X	T4S	T4X	T5S	T5X	T6S	T6X
Alternaria tenuissima (Kunze ex Pers.) Wiltshire	5	2	4	1	6	0	10	2	5	2	5	0
Arthrinium phaeospermum (Corda) M.B. Ellis	0	0	0	0	0	0	0	1	1	0	2	4
Ascochytulina deflectens (Karst.) Petrak	3	0	1	0	0	0	2	1	7	0	5	0
Aureobasidium pullulans (De Bary) Arnaud	3	0	3	0	0	0	1	0	6	2	5	0
Cladosporium tenuissimum Cooke	1	0	1	1	0	0	3	0	3	0	3	0
Cytospora sp.	0	0	0	0	0	0	4	1	2	0	1	0
Kabatina sp.	0	5	0	4	0	2	0	4	0	2	0	2
Mucor plumbeus Bon.	0	0	6	0	9	0	9	2	1	0	1	0
Mucor racemosus Fres.	9	0	10	0	9	0	1	0	0	0	2	0
Nigrospora oryzae (Berk. & Br.) Petch	2	0	0	0	0	0	1	0	1	0	4	0
Penicillium spp.	2	0	5	0	1	1	2	0	2	0	2	0
Phoma sp. 1	8	0	0	0	1	1	0	0	0	0	0	0
Ramularia sp.	2	0	1	0	0	0	1	0	2	0	6	0
Sordaria macrospora Auersw.	4	1	1	1	7	0	8	1	0	0	1	2
Sporormiella intermedia (Auersw.) Ahmed & Cain	5	2	2	1	1	1	0	0	0	0	0	0
Sterile mycelia	7	3	4	3	3	3	4	0	9	0	9	0

Rare isolates: *Alternaria* sp., *Epicoccum purpurascens* Ehrenb. ex Schlecht, *Hormonema* spp., *Microsphaeropsis* sp., *Mortierella vinacea* Dixon-Stewart, *Nodulisporium* sp., *Pestalotiopsis guepinii* (Desm.) Stey., *Phialophora* sp., *Phoma* spp., *Phomopsis* sp., *Pleospora herbarum* (Pers.: Fr.) Rabenhorst, *Pleurophoma* sp., *Rhizoctonia* sp., *Trichoderma polysporum* (Link ex Pers.) Rifai.

previous studies of fungi of woody perennials Mucorales were recorded only from the roots of *Pinus sylvestris* (Fisher & al., 1990) and were virtually absent from the aerial parts of the trees. Since in this study all isolates come from apparently healthy twigs, and the spores and mycelia of these fungi are not likely to survive surface sterilization in such large numbers (Tab. 1), the emergence of mycelium with sporangia from just below the bark may be indicative of the onset of decay. Further investigations are required to ascertain whether and in which form these taxa may spend the early part of their life cycle in the tissues without causing any deterioration of the plant tissues.

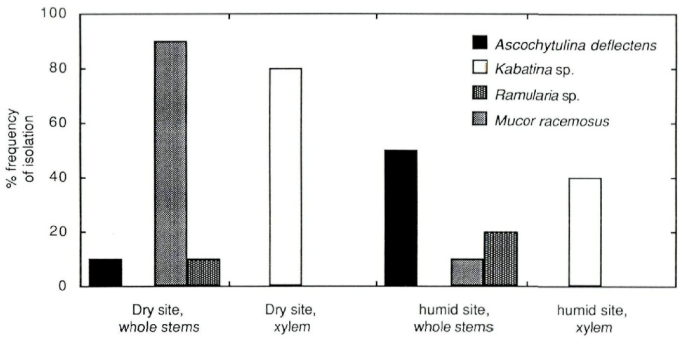

Fig. 1. – Bar chart of the colonization frequencies (median values of percentages) of some prominent colonizers of whole stems and xylem of olive trees.

For the fungal species recorded from both whole stem and xylem, colonization of both bark and xylem can be postulated by hyphal penetration from the bark into the xylem. The surface-sterilization procedures have been accurately tested for effectiveness against surface-dwelling epiphytes and saprophytes from bark (Fisher and Petrini, 1988; 1990). As the periderm of woody plants represents a structurally heterogeneous substrate, it is more difficult to differentiate truly internal from external fungi. Of all fungal taxa isolated at least *Kabatina* sp. can be regarded as a true endophyte. The mode of infection of xylem specific fungi such as *Kabatina* sp. remains unclear but the presence of this slow-growing fungus in the bark cannot be excluded, since other fast-growing species could have concealed its presence. The use of other isolation methods which involve alternate moisture regimes (Chapela & Boddy, 1988) and extensive histology studies are needed to further understand the mode of infection of xylotropic fungi.

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