A comparative study of fungal endophytes in leaves, xylem and bark of *Eucalyptus* in Australia and England

P. J. Fisher[1], O. Petrini[2] & B. C. Sutton[3]

[1]Department of Biological Sciences, Hatherly Laboratories, University of Exeter, Exeter, EX4 4PS, U. K.
[2]Mikrobiologisches Institut, ETH–Zentrum, CH–8092 Zurich, Switzerland
[3]International Mycological Institute, Bakeham Lane, Egham, Surrey TW20 9TY, U. K.

Fisher, P. J., O. Petrini & B. C. Sutton (1993). A comparative study of fungal endophytes in leaves, xylem and bark of *Eucalyptus nitens* in Australia and England. – Sydowia 45(2): 338–345.

The endophytic fungal assemblages of leaves, xylem and bark of *Eucalyptus nitens* trees taken from their natural growth range in Australia and from introduced areas in England have been studied to detect site and climate related differences in the endophyte communities. Sixty-four fungal taxa have been isolated, but only twenty account for a relative importance of more than 5% in any of the tissues examined. The Australian and British samples are clearly separated according to their geographic origin. The endophytes responsible for the separation are mainly *Cytospora eucalypticola*, very abundant in the sample units from Australia and otherwise present only in twigs from Britain that underwent drying, and *Botryosphaeria dothidea*, completely absent from the Australian samples. Australian and British isolates of *Cytospora eucalypticola* belong probably to different strains. Possible explanations for the site-related differences between Australian and British samples are presented and briefly discussed.

Keywords: correspondence analysis, ecology, endophytes, geographic differences.

Endophyte associations in aerial plant organs may range from intimate contact where the fungus inhabits the intercellular spaces and xylem vessels in the plant, to more or less superficial colonization of peripheral, often dying or dead tissues such as bark layers in plants with secondary growth. For endophytes colonising bark and phellem tissues the more appropriate term „phellophytes" has recently been proposed (Kowalski & Kehr, 1992). In the last few years, community ordination analyses have shown that endophyte communities are usually specific at the host species level (e. g., Bertoni & Cabral, 1988; Canavesi, 1987; Petrini, 1985; Petrini & Fisher, 1988; Sieber & Hugentobler, 1987; Sieber & al., 1991). Carroll & al. (1977) postulated some tissue specificity by endophytes and subsequent investigations (Bertoni & Cabral, 1988; Fisher & Petrini, 1987, 1988, 1990; Petrini & Fisher,

1988, 1990) confirmed that many endophytic fungi show a certain degree of tissue specificity.

Most ecological studies on endophytes carried out so far have dealt with hosts collected within their natural growth range. Little information, however, exists on the presence of potentially host-specific endophytic fungi in hosts growing outside their indigenous areas of distribution and no investigations have so far been made to compare the endophyte assemblages of a given host collected both inside and outside its distribution area.

Eucalyptus species are widely distributed in the southern hemisphere and have also been introduced as ornamental trees in many European countries. More than six hundred species of *Eucalyptus* occur widely throughout Australia where the genus is indigenous (Lawrence, 1951). *Eucalyptus nitens* (Deane & Maiden) Maiden can be found growing between 30° 30' and 38° 00' S latitude. Climatically this range is fairly uniform with an annual precipitation between 750 and 1250 mm. Frosts may be severe and can occur during any month of the year. *E. nitens* is therefore adapted to a cool temperate climate. *E. nitens* is an introduced species in England and in South Devon it grows at several sites. The climate of South Devon is cool temperate, with approx. 750 mm of annual rainfall and is therefore similar to that of the growth range of *E. nitens* in Australia.

In this investigation the endophytic fungal assemblages of the leaves, xylem and bark of *E. nitens* taken from its natural growth range in Australia and from introduced areas in England have been studied to detect site and climate related differences in the endophyte communities.

Materials and methods

Endophytes were isolated from the leaves and branches of two trees planted about 25 years ago in the Australian National Botanic Gardens in Canberra, on a site that originally supported sclerophyllous *Eucalyptus* woodland vegetation. Further isolations were made from leaves and stems of two trees of *E. nitens* growing in the Botanic Garden on the Campus of the University of Exeter in Devon, England. The English trees were originally grown from seeds in the University glass house and planted on the present site when they were about 80 cm high. Both the Australian and British sites may be described as open grassy park lands. From each tree 50 leaves and twenty 10 cm long branch pieces (2–4 years old) were sampled at a height of approx. 2–3 m above ground. The material sampled was sent to the laboratory in paper bags and processed within 4 days. The leaves and twig pieces were thoroughly washed in running water and surface sterilized 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 sterilisation each leaf was cut into five fragments that were then placed in groups of five in Petri dishes containing 1. 5% Oxoid malt extract agar (MEA) supplemented with 250 mg/L Terramycin to suppress bacterial growth. Each branch piece was separated into bark and xylem and placed individually into sterile containers. The bark was cut into 1 cm units, referred to throughout this paper as 'segments'. From each container ten segments were randomly selected and placed in groups of five on MEA. Next the xylem pieces were cut individually into 1 cm fragments, surface sterilised again and placed on MEA. To detect differences in endophytic assemblages resulting from the use of different isolation methods, 20 additional branches taken from each tree were air dried at 18–22 C for ten weeks after the method described by Chapela & Boddy (1988) before the bark was removed and discarded. The xylem was then surface sterilised and plated onto 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.

For the statistical evaluation, the colonization frequency of the plants by a fungal species was defined as the total number of pieces of a given tissue (separate leaf or twig pieces) colonized by a given fungus.

Community ordination was performed on a reduced matrix of the raw data of the colonization frequencies that contained only those taxa with a relative importance (dominance) index (Ludwig & Reynolds, 1988) of at least 5%. The resulting matrix was analyzed by simple correspondence analysis using the package SimCA 2. 1 (Greenacre, 1986).

Results

Of the 64 fungal taxa isolated (Tab. 1), only twenty account for a relative importance of more than 5% in any of the tissues examined. The results of the ordination by simple correspondence analysis are presented in Fig. 1. The first three co–ordinates explain 76. 7% of the total inertia of the model, indicating a good fit of the model to the data. The sample units are clearly separated according to their geographic origin, with the gradient along the first axis determined mainly by *Cytospora eucalypticola*, very abundant in the sample units from Australia and otherwise present only in twigs from Britain that underwent drying, and *Botryosphaeria dothidea*, completely absent from the Australian samples. The use of different isolation methods improves the detection of distinct fungal species, as shown here, where both samples subjected to drying have yielded essentially different

Tab. 1. – Fungal isolates from *Eucalyptus nitens*. Only those fungi that accounted for a relative importance of at least 5% have been included in the detailed list and used for the statistical analysis. Figures represent the total number of isolates originating from a given tissue type. C: Canberra; E: Exeter; L: leaf; T: twig; B: bark; X: xylem; D: twigs subjected to the drying regime.

Fungal Taxon	CL	CTB	CTX	CTD	EL	ETB	ETX	ETD	Tot
Alternaria sp. 1	25	26	11	4	0	0	0	0	66
Aureobasidium sp. 1	65	0	0	0	0	0	0	0	65
Botryosphaeria dothidea (Moug.: Fr.) Ces. et de Not.	0	0	0	0	114	128	34	12	288
Cladosporium tenuissimum Cooke	37	0	0	0	0	0	0	0	37
Cytospora eucalypticola van der Westhuizen	190	133	165	86	0	0	0	12	586
Epicoccum nigrum Link	16	12	0	0	0	0	0	0	28
Hormonema sp. 1	0	0	0	0	162	2	2	0	166
Leptostroma sp.	64	0	0	0	0	0	0	0	64
Nigrospora oryzae (Berk. & Br.) Petch	0	0	0	28	0	0	0	0	28
Penicillium glabrum (Wehm.) Westl.	0	62	6	0	0	0	0	0	68
Penicillium simplicissimum (Oudem.) Thom.	0	14	37	0	0	0	0	0	51
Pestalotiopsis versicolor (Speg.) Stey.	0	0	0	48	0	0	0	0	48
Phomopsis sp.	87	0	0	0	0	0	0	0	87
Plectophomella sp.	0	0	0	0	54	0	0	0	54
Pleurophomella sp.	30	0	0	0	0	0	0	0	30
Schizophyllum commune Fr.: Fr.	0	0	0	36	0	0	0	0	36
Sordaria macrospora Auersw.	8	16	0	0	5	0	0	0	29
Trichoderma harzianum Rifai *sensu* Bissett	0	0	0	40	0	0	0	0	40
Sterile mycelium 1	27	72	147	3	0	0	0	0	249
Sterile mycelium 2	73	33	0	0	0	0	0	0	106
rare isolates	231	72	23	36	78	36	19	27	522
Total no. of isolates	853	440	389	281	413	166	55	51	2648
Total no. of tissue pieces investigated	500	200	200	200	500	200	200	200	

Rare isolates (less than 5% relative importance) are: *Alternaria tenuissima* Kunze: Fr., *Aposphaeria* sp., *Aureobasidium pullulans* (de Bary) Arnaud, *Aureobasidium* sp., *Chaetomium globosum* Kunze, *Coniella* cf. *petrakii* Sutton, *Coniella* sp., *Coniochaeta* cf. *Rosellinia xylariispora* Cooke & Ellis *sensu* Munk, *Cryptosporiopsis* sp., *Dictyopolyschema* cf. *pirozynskii* M. B. Ellis, *Exophiala* sp., *Fusarium moniliforme* Sheldon, *Gelasinospora calospora* (Mouton) C. & M. Moreau, *Gilmaniella humicola* Barron, *Harknessia hawaiiensis* Stev. & Young, *Harknessia uromycoides* (Speg.) Speg., *Hormonema* sp., *Kabatiella* sp., *Leptostroma* sp. 2, *Libertella aurantiaca* Massee, *Microsphaeropsis globulosa* (Camara) Sutton, *Microsphaeropsis olivacea* (Bonord.) Höhn., *Oidiodendron griseum* Robak., *Penicillium spinulosum* Thom, *Pestalotia matildae* Rich., *Phialophora* sp. (*Lecythophora*), *Phoma minutella* Sacc. & Penz., *Phoma* spp., *Phoma tropica* Schneider & Boerema, *Plectophomella* sp. 1, *Pleospora* sp., *Pleurophoma* sp., *Podospora tetraspora* (Winter) Cain, *Sclerophoma pythiophila* (Corda) Höhnel, *Septoria* sp., *Sporormiella intermedia* (Auersw.) Ahmed & Cain, *Sporormiella minima* (Auersw.) Ahmed & Cain, *Sporothrix* spp., *Stemphylium botryosum* Wallr., *Trichoderma koningii* Oud., *Trichoderma pseudokoningii* Rifai, *Xylaria* sp.

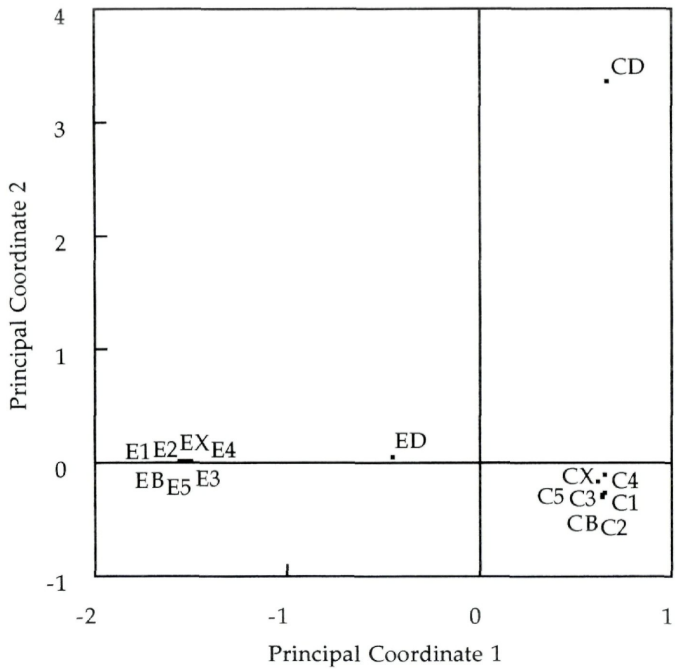

Fig. 1. – Results of the ordination by simple correspondence analysis. Only those taxa with a relative importance index of at least 5% have been used for the ordination analysis (see Tab. 1). Sample abbreviations: B, bark, C, Canberra; D, dried wood samples; E, Exeter; 1–5, leaf sample pieces 1–5; X, xylem. Total inertia explained by the first and second co-ordinate: 62%.

endophytic taxa than the fresh xylem. This is illustrated by the separate position of the samples in Fig. 1. Tab. 1 shows that the main colonizers found in Australian samples, with few exceptions, are completely absent from British *Eucalyptus nitens*. *Cytospora eucalypticola*, dominant in all samples from Australia, could be detected only in dried British xylem samples, although British isolates differed in cultural characteristics. Australian and British isolates may thus be considered different strains. *Aureobasidium* sp. 1, *Leptostroma* sp., *Pestalotiopsis versicolor*, *Phomopsis sp.*, *Pleurophomella sp.*, *Schizophyllum commune*, and two prominent sterile mycelia are present exclusively in Australian samples, while *Botryosphaeria dothidea*,

Hormonema sp. 1 and *Plectophomella* sp. are completely absent from them. *Pestalotiopsis versicolor, S. commune* and *Trichoderma harzianum* have only been isolated from dried twigs. Looking at the total number of isolates obtained from each tissue type it is clear that virtually in each case more then twice the number of isolates were obtained from the Australian tissues than from the British ones (Tab. 1).

Discussion

With the exception of *Cytospora eucalypticola*, the main endophytic colonizers of *Eucalyptus* tissues in Australia are not present to any extent in the British samples. Although only 2 trees each (1100 tissue pieces) were investigated from both the Australian and British sites, the much higher incidence of fungal colonization in the Australian material is striking. One possible explanation may be that the indigenous fungal and tree floras in the Australian samples are physiologically better adapted to each other than the British fungal flora is to the trees introduced from Australia. On the other hand, since horizontal transmission of fungal endophytes is probably the rule for woody plants, these differences could well be only the result of the absence of inoculum, so that trees planted outside their natural distribution area would tend to become colonized by indigenous fungi. The almost complete absence of a host–specific endophyte such as *Cytospora eucalypticola* from the British samples supports this hypothesis. Carroll & al. (1977) have studied the endophyte assemblages of both Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] and coastal redwood [*Sequoia sempervirens* (D. Don) Endl.] collected outside their natural range. Carroll & al. (1977) made specific comparisons of the fungi on native vs. introduced Douglas fir trees. Similar comparisons for coastal redwood can be inferred by comparing the fungi recovered from trees in France by Carroll & al. (1977) and the lists published by Espinosa–Garcia & Langenheim (1990). The results by Carroll & al. (1977) and Espinosa–Garcia & Langenheim (1990) confirm that the endophyte assemblages on trees planted outside their original range are depauperate and consist of species different from those in native habitats.

The presence of a closely related strain of *Cytospora eucalypticola* in the British samples might suggest time and climate related changes in this species which may have originally been introduced from Australia to Britain in *E. nitens* seeds or seedlings. Comparative studies of both Australian and British isolates at the biochemical level, making use of isozyme or DNA analysis techniques, may explain the relationship between Australian and British strain of *C. eucalypticola*.

Drying the branches for ten weeks before isolation of endophytes has resulted in the recovery of only a few fungal taxa that could not be isolated using the routine isolation method on fresh material. Chapela & Boddy (1988) isolated endophytes from beech twigs using a variety of methods and described endophyte assemblages different from those reported by Kowalski & Kehr (1992) and Petrini & Fisher (1988). In this connection Boddy & Griffith (1989) postulated that water content may be a major determinant in the development of endophytes in beech (*Fagus sylvatica* L.) sapwood. Thus the drying regime may supress fast-growing fungal taxa sensitive to drought, allowing viable, stress-tolerant and slow-growing species to grow from the dried tissues.

In this study, only limited tissue-specificity has been detected. *Aureobasidium* sp., *Hormonema* sp. 1, *Leptostroma* sp., *Plectophomella* sp., *Pleurophomella* sp. and *Phomopsis* sp. are apparently confined to the leaf tissues, while other endophytes appear to colonize all tissues without discrimination. These results are in contrast to those obtained by Bertoni & Cabral (1988) with *Eucalyptus viminalis*, where marked tissue preferences have been observed. The endophyte assemblage reported by Bertoni & Cabral (1988) is, however, distinct from the one detected here, and host-related as well as geographical differences may account for the discrepancy. On the other hand, it may be not entirely appropriate to draw a conclusion about the absence of host-specific endophytes based on the reduced sample of *E. nitens* population studied. It cannot be excluded that host-specific endophytes could be isolated from samples taken from natural stands within the growth range of *E. nitens*.

Acknowledgments

We are grateful to Mr A. Slee (CSIRO Division of Plant Industry, Australian National Herbarium, Canberra, Australia) for collecting the Australian material. Thanks are also due to Dr. G. C. Carroll (Eugene, Oregon, USA) for useful comments and suggestions on the manuscript.

References

Bertoni, M. D. & D. Cabral (1988). Phyllosphere of *Eucalyptus viminalis*. II: distribution of endophytes. – Nova Hedwigia 46: 491–502.

Boddy, L. & G. S. Griffith (1989). Role of endophytes and latent invasion in the development of decay communities in sapwood of angiospermous trees. – Sydowia 41: 41–73.

Canavesi, F. (1987). Beziehungen zwischen endophytischen Pilzen von *Abies alba* Mill. und den Pilzen der Nadelstreue. – Dissertation ETH No. 8325, Swiss Federal Institute of Technology, Zürich, Switzerland.

Carroll, F. E., E. Müller & B. C. Sutton (1977). Preliminary studies on the incidence of needle endophytes in some European conifers. – Sydowia 29: 87–103.

Chapela, I. H. & L. Boddy (1988). Fungal colonization of attached beech branches I. Early stages of development of fungal communities. – New Phytologist 110: 39–45.

Espinosa–Garcia, F. J. & J. H. Langenheim (1990). The leaf fungal community of a coastal redwood population – diversity and spatial patterns. – New Phytol. 116: 89–97.

Fisher, P. J. & O. Petrini (1987). Location of fungal endophytes in tissues of *Suaeda fruticosa*: a preliminary study. – Trans. Br. mycol. Soc. 89: 246–249.

—— & —— (1988). Tissue specificity by fungi endophytic in *Ulex europaeus*. – Sydowia 40: 46–50.

—— & —— (1990). A comparative study of fungal endophytes in xylem and bark of *Alnus* species in England and Switzerland. – Mycol. Res. 94: 313–319.

Greenacre, M. J. (1986). SimCA: a program to perform simple correspondence analysis. – Amer. Stat. 51: 230–231.

Kowalski, T. & R. D. Kehr (1992). Endophytic fungal colonization of branch bases in several forest tree species. – Sydowia 44: 137–168.

Lawrence , G. H. M. (1951). Taxonomy of Vascular Plants. – Macmillan, New York: 633 – 634.

Ludwig, J. A. & J. F. Reynolds, (1988). Statistical Ecology. A primer on methods and computing. – John Wiley & Sons, New York, 337 pp.

Petrini, O. (1985). Wirtsspezifität endophytischer Pilze bei einheimischen Ericaceae. – Bot. Helv. 95: 213–238.

—— & P. J. Fisher (1988). A comparative study of fungal endophytes in xylem and whole stems of *Pinus sylvestris* and *Fagus sylvatica*. – Trans. Br. mycol. Soc. 91: 233–238.

—— & —— (1990). Occurrence of fungal endophytes in twigs of *Salix fragilis* and *Quercus robur*. – Mycol. Res. 94: 1077–1080.

Sieber, T. N. & C. Hugentobler (1987). Endophytische Pilze in Blättern und Ästen gesunder und geschädigter Buchen (*Fagus sylvatica* L.). – Eur. J. For. Pathol. 17: 411–425.

——, F. Sieber–Canavesi & C. E. Dorworth (1991). Endophytic fungi of red alder (*Alnus rubra*) leaves and twigs in British Columbia. – Canad. J. Bot. 69: 407–411.

(Manuscript accepted 7th June 1993)