A comparative study of fungal populations in healthy and symptomatic twigs and seedlings of *Eucalyptus globulus* in Uruguay

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The main goal of this work was to compare the fungal species present in endophytic assemblages of healthy twigs with those associated with twig lesions of *Eucalyptus globulus* and to compare them with fungal endophytes from seedlings. From 400 segments of healthy and symptomatic twigs of *E. globulus* 302 isolates corresponding to 49 taxa have been obtained, but only 19 taxa were isolated at frequencies higher than 2%. From 200 segments of seedlings, 328 isolates belonging to 17 taxa were isolated. *Alternaria alternata* was the main dominant species isolated from all tissues, with the exception of xylem of healthy twigs. Correspondence analysis showed differences in species composition between endophytic assemblages from stem seedlings and healthy twigs, thus suggesting that some dominant endophytic species colonizing seedlings adopt different strategies during the plant growth process. Moreover, the fungal composition of assemblages from healthy xylem differed from that of the remaining twig tissues. At the same time, differences in fungal composition between stem and leaf of seedlings were found, showing a tendency for organ specificity. Basidiomycetes with positive and negative oxidative extracellular enzyme reactions were isolated. These wood-rotting fungi probably initiate the decomposition of lignocellulosic materials before branches are detached from plants following this process in the litter.

Keywords: Fungal endophytes, wood-rotting fungi, *Cytospora chrysosperma*, *Xylaria*, ecology.

In the last 20 years *Eucalyptus* spp. have been planted as sources of fuel and pulp paper. Argentine, Chile, Brazil and Uruguay are the main producer countries in South America. Nearly 100,000 ha are forested with *Eucalyptus* spp. in Uruguay and plantations have increased annually 2.9% during the last 30 years (Ministerio de Ganadería, Agricultura y Pesca, 1991). The 98% of the wood produced is exported as raw material or for use in the paper industry (Stolovich, 1995). Uruguay is mainly covered by grassland and consequently these plantations represent an important landscape change.

Lists and descriptions of fungal species recorded from leaves, twigs and stems lesions of *Eucalyptus* spp. are available (Ferreira, 1989; Crous & al., 1989; Gibson, 1981; Sutton, 1980; Sutton & Davison, 1983; Sankaran & al., 1995). Symptomatic twigs characterized by small bark cankers have been observed in recent plantations in Uruguay. A complex of biotic and abiotic factors is considered to produce this symptom in *Eucalyptus* and other broad leaf trees (Ferreira, 1989; Bier, 1964; Sinclair & al., 1987).

Fungal endophytes have been isolated from a wide range of evergreen, deciduous and coniferous plants (Fisher & Petrini, 1990; Petrini, 1986, 1991; Petrini & Fisher, 1987, 1988; Sieber, 1989). These fungi can for a certain period probably live in some cases as neutralistic symbionts and produce symptoms only after appropriate ecological and physiological conditions occur (Bissegger & Sieber, 1994; Boddy & Griffith, 1989; Carroll, 1986, 1988, 1991; Chapela & Boddy, 1988; Shoeneweiss, 1981; Sieber & al., 1995; Wilson & Carroll, 1994). Only few investigations have been carried out on the endophytic fungi of *Eucalyptus* spp. (Bertoni & Cabral, 1988; Cabral, 1985; Fisher & al., 1993; Bettucci & Saravay, 1993; Bettucci & Alonso, 1997). The main goal of this work was to compare the species composition of endophytic fungal assemblages of healthy twigs with fungal assemblages associated with twig lesions of *E. globulus* and to compare them with fungal endophytes from seedlings.

Materials and methods

Study area

The study area was located in the south-east of Uruguay at 34° 35′ S and 55° 10′ W. Plantations were situated on a former prairie with well drained soil of low fertility. The plants were obtained from seeds of Australian origin. They were grown in polyethylene bags containing soil collected in the same site and transferred to the field in 1991. One year later, during the autumn of 1992, lesions causing bark cracks of 4–5 mm length were observed on the majority of twigs of approximately 2–5 mm diameter.

Material collection and fungal isolation

Samples of 20 healthy and 20 symptomatic twigs from the lower whorls of the stem were collected and samples of 20 seedlings, belonging to the same nursery, were analyzed. All materials were taken to the laboratory in paper bags, stocked at 5 C and processed within 24 h. Samples were first examined under a dissecting microscope to detect the presence of fructifications.

Surface sterilization was then performed using 4% sodium hypochlorite according to Fisher & al. (1986). Briefly, segments of approximately 2 to 5 mm length were cut off from the distal to proximal portions of the 20 healthy twigs and xylem was stripped off the bark of each segment. Likewise, segments 2–3 mm length were obtained only from the centre and edges of lesions of the 20 symptomatic twigs. From the seedlings, fifty leaves were dissected in 2×5 mm strips and 20 stems in 1–2 mm segments. All segments were surface sterilized again and then dried on sterile filter paper. A total of 400 segments from twigs and 200 from seedlings were placed separately onto 9 mm Petri dishes containing 2% malt agar and incubated at 24 C for six weeks or more depending on the growth rates of the fungi. Each colony was transferred to fresh medium to allow identification and black light was used to induce sporulation in some cultures. Those that failed to sporulate after one month were considered sterile. Isolates of basidiomycetes were identified by means of cultural characteristics and the production of extracellular oxidative enzymes (Stalpers, 1978).

Analysis of the data

The relative frequency of colonization was calculated as the percentage of isolates of a given taxon from each segment divided by the total number of segments plated out. Populations resulting from healthy and symptomatic twigs and seedlings were examined by correspondence analysis, using STAT-ITCF (Service des Etudes Statistiques, Institute Technique des Céréales et des Fourrages, France). This analysis was carried out using the species with relative frequency of 2% or greater isolated from any material (Howard & Robinson, 1995).

Results

Three hundred and two isolates belonging to 36 taxa were obtained from segments of healthy and symptomatic twigs. Seedlings were colonized by 328 isolates corresponding to 20 taxa (Tab. 1). Multiple colonization, i.e. segments colonized by more than one fungal taxon was rare. Endophytes did not fruit on twigs.

From the six basidiomycetes isolated from xylem and bark segments only three could be identified. *Bjerkandera adusta* and *Hapalopilus nidulans,* Basidiomycetes MVHC 6818 and MVHC 7056 produced laccase and peroxidase whereas *Irpex lacteus* produced peroxidase but not laccase. Basidiomycete MVHC 7055 did not produce proxidase or laccase.

Fungal assemblages from healthy and symptomatic bark appeared to be composed by few dominant taxa. *Alternaria alternata*

Tab. 1.– Endophytic fungi of *Eucalyptus globulus* twigs and seedlings. Frequency of colonization. Symbols indicate: hx: healthy xylem; hb: healthy bark; sx: symptomatic xylem; sb: symptomatic bark; tm: seedling stem; lf: seedling leaf.

Rare taxa isolated once and in only one tissue: *Acremonium* sp.; *Cerebella andropogonis* Ces.; *Curvularia clavata* Jain; *Fusicoccum eucalypti* Sousa da Camara; *Fusarium xylarioides* Steyaert; *Hapalopilus nidulans* (Pers. : Fr.) P. Karst.; *Hymenopsis typhae* Pk.; *Irpex lacteus* (Fr. : Fr.) Fr.; *Penicillium* sp.; *Phialophora radicicola* Cain; *Pleospora* sp.; *Sporormiella systenospora* Ahmed & Cain; *Xylaria* sp. MVHC 6811; *Xylaria* sp. MVHC 6846; *Xylaria* sp. MVHC 6994; *Xylaria* sp. MVHC 6842; *Xylaria* sp.; *Xylocoremium* sp.; Basidiomycete MVHC 7055; Sterile dark mycelium MVHC 6853; Sterile hyaline mycelium MVHC 6859; Sterile hyaline mycelium MVHC 6864.

	Code	hx	hb	sx	sb	tm	lf
Alternaria alternata (Fr.) Keissler	alt	5	51	22	62	89	98
Arthrinium phaeospermum (Corda) Ellis	art	1	2	2			
Bjerkandera adusta (Willd.: Fr.) P. Karst.	bjd			11	3		
Botrytis cinerea (Pers.: Pers.) Persoon	bot					14	
Cladosporium cladosporioides (Fresen.) de Vries	cla	1			1	11	6
Cytospora chrysosperma Pers.: Fr.	cyt			3	7	32	
Drechslera hawaiiensis (Bugnicourt) Subram. & Jain ex M. B Ellis	drc			2		2	
Epicoccum purpurascens Ehrenb.: Schlecht.	epi		3	3	8	3	2
Eupenicillium brefeldianum (E. Dodge) Stolk & Scott	eub					3	
Fairmaniella leprosa (Fairm.) Petrak & Syb.	fai	1			3		
Fusarium oxysporum Schlecht.	fus					11	
Geniculosporium sp. MVHC 6840	gen				2		
Hainesia lythri (Desm.) Hohn.	hai					8	2
Harknessia hawaiiensis Stev. & Young	har					3	
Nigrospora sacchari (Speg.) Mason	nsa		1		3		
Nigrospora sphaerica (Sacc.) Mason	nph	3	11	2	8	1	2
Penicillium purpurogenum Stoll	ppu					3	
Pestalotiopsis guepinii (Desm.) Stey.	pes	1	1		2		
Phoma multirostrata (Mathur & al.) Dorenbosch & Boerema	phm					2	4
Phoma sorghina (Sacc.) Boer. Doern. & van Kest.	phs	1	9	9	5	16	6
Phoma tropica Schneider & Boerema	pht					3	
Phomopsis arnoldiae Sutton	pha		3		3		
Trichoderma harzianum Rifai	tri						8
Ulocladium chartarum (Preuss) Simmons	ulo		3		4		
Xylaria sp. MVHC 6826	xya	1			4		
Xylocoremium sp. MVHC 6812	xyc		1		2		
Basidiomycete MVHC 6818	bas1	5					
Basidiomycete MVHC 7056	bas2	7					
Sterile hyaline mycelium MVHC 7173	shm					5	
Rare taxa		9	2	5	6	2	
Total isolates 630		35	86	58	123	194	142
Total segments 600		100	100	100	100	100	100

was the only dominant species. *Phoma sorghina*, *Nigrospora sphaerica*, *Epicoccum purpurascens*, *Phomopsis arnoldiae* and *Cytospora chrysosperma* were also common, though less frequent species. *Xylaria* spp. were mainly isolated from symptomatic bark. Basidiomycetes, on the other hand, were frequently isolated from the xylem of healthy twigs. Conversely, symptomatic xylem shared nearly the same most frequent species with bark.

A. alternata was commonly obtained from stem and leaves of seedlings. *Botrytis cinerea* and *Cytospora chrysosperma* were mostly present in leaves and stems, respectively, and *Cladosporium cladosporioides*, *Hainesia lythri* and *Phoma sorghina* were frequently isolated from stems.

The correspondence analysis performed on the frequency of 28 taxa showed that the three first axes accounted for 80% of the total inertia. The principal coordinate axes 1–2 (56.8%) separated four distinct clusters of closely related species (Fig. 1). The first axis (32% of total inertia) separated twigs from seedlings, and the second axis

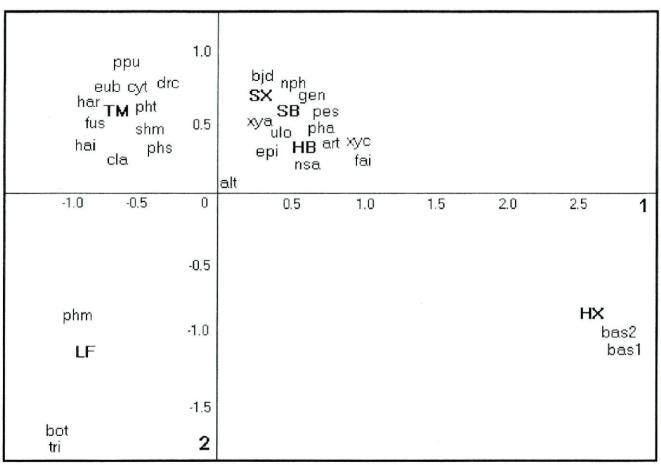

Fig. 1.– Correspondence analysis. Ordination of seedlings and healthy and symptomatic twigs on the two first axes. Variables are the relative frequencies of isolation. The first axis (1) separates twig tissues from seedlings. The second axis (2) shows differences between xylem, characterized by basidiomycetes, and healthy bark and symptomatic tissues characterized by a set of commonly isolated species. It also shows differences between seedling leaves and stems. – Symbols used indicate: HX, healthy xylem; HB, healthy bark; SX, symptomatic xylem; SB symptomatic bark; LF leaves; TM stems. Symbols for the species are indicated in Tab. 1.

(24.8%), allowed to distinguish between healthy xylem and the other twig tissues. At the same time, it showed differences between seedling leaves and stems. Basidiomycetes were most frequent in the xylem, and several taxa such as *Geniculosporium* sp., *N. sphaerica*, *B. adusta*, and Xylariaceae were associated with bark of healthy twigs and symptomatic tissues. *B. cinerea* and *Phoma tropica* were common in leaves, whereas *C. chrysosperma* and *Phoma multirostrata* colonized seedling stems.

Discussion

The number of taxa obtained from each material and the frequency of isolates were very low, confirming previous findings that suggested that the endophytic communities of trees planted outside their original location are depauperate in composition (Espinosa-García & Langenheim, 1990; Fisher & al., 1993). Moreover, the low frequency of these taxa could indicate that the indigenous fungal flora and *E. globulus* are not yet physiologically well adapted to each other in Uruguay.

Correspondence analysis demonstrated differential colonization of twigs and seedlings by endophytic fungi. It also showed that the assemblage of species isolated from symptomatic xylem was more related to that of symptomatic bark than to that of healthy xylem, suggesting that different types of injured tissues were colonized by similar taxa. Basidiomycetes were dominant in xylem of healthy twigs, confirming earlier observations on sprouting stumps of *E. globulus* (Bettucci & Saravay, 1993). These fungi are probably adapted to live under high water potential. The assemblages from xylem and bark of symptomatic twigs shared several species such as *A. alternata, Phoma sorghina, E. purpurascens* and *C. chrysosperma*. *Cytospora* spp. are known to be weak pathogens of several species of *Eucalyptus* in Argentine (Sarasola & Sarasola, 1959), Australia (Davison & Tay, 1983; Old & al., 1986; Old & al., 1991), South-Africa (Crous & al., 1989) and Spain (Fernández de Ana, 1982). *Cytospora* was also found to be an endophytic colonizer in healthy twigs of *Eucalyptus nitens* (Fisher & al., 1993) and in sprouting stumps of *E. globulus* in Uruguay (Bettucci & Saravay, 1993).

The endophytic community of seedling leaves was characterized by *B. cinerea*. This is a very common species in *Eucalyptus* seedlings and constitute a severe problem for seedlings growing under very humid conditions (Ferreira, 1989; Crous & al., 1989). According to Sieber (1989), the difference between seedling stems and leaves suggests some tendency to organ specificity. As in this study *C. chrysosperma* was frequently present in seedling stems and rarely in symptomatic twigs, one could speculate that this potential pathogen

remain latent until favorable conditions for expansion occur, such as wounds or growth cracks that favour its pathogenicity. The fungus is then able to colonize exposed xylem and bark (Shoeneweiss, 1983).

Two rare but interesting species, *Irpex lacteus* and *Sporormiella systenospora* were isolated as endophytes from healthy xylem. The former is a frequent colonizer of burnt woody tissues of *Baccharis* spp. (Borges da Silveira, 1990) and the latter has been isolated as endophyte of *Ulex* sp. (Carroll, 1991). Both species grow under post-fire conditions and could be very important for mineral immobilization. In fact, *Eucalyptus* plantations are very frequently burnt by wildfire during the warm and dry summer in Uruguay.

Basidiomycetes and Xylariaceae probably initiate the decomposition of lignocellulosic materials before branches are detached from plants and afterward in the litter (Basham & Anderson, 1977; Chapela & Boddy, 1988).

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References

Basham, J. T. & W. H. Anderson (1977). Defect development in second-growth sugar maple in Ontario. I. Microfloral infection relationships associated with dead branches. – Can. J. Bot. 55: 934-976.

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

Bettucci, L. & R. Alonso (1997). A comparative study of fungal populations in healthy and symptomatic twigs of *Eucalyptus grandis* in Uruguay. – Mycol. Res., in press.

—— & M. Saravay (1993). Endophytic fungi of *Eucalyptus globulus*: a preliminary study. – Mycol. Res. 97: 679–682.

Bier, J. E. (1964). The relation of some bark factors to canker susceptibility. – Phytopathol. 54: 250–278.

Bissegger, M. & T. Sieber (1994). Assemblages of endophytic fungi in coppice shoots of *Castanea sativa*. – Mycologia 86: 648–655.

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

Borges da Silveira, R. M. (1990). Aphyllophorales Poliporoides (Basidiomycetes) do Parque Nacional de Aparados da Serra, RS. – MSc. Thesis. Universidade Federal do Rio Grande do Sul. Porto Alegre. Brazil.

Cabral, D. (1985). Phyllosphere of *Eucalyptus viminalis*. Dynamics of fungal populations. – Trans. Brit. Mycol. Soc. 85: 501–511.

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

—— (1988). Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. – Ecology 69: 2–9.
—— (1991). Beyond pest deterrence- Alternative strategies and hidden costs of endophytic mutualism in vascular plants. – In: J. Andrews & S. Hirano (eds.) Microbial ecology of leaves. Springer-Verlag, USA: 358–375.
Chapela, I. & L. Boddy (1988). Fungal colonization of attached beech branches. II. Spatial and temporal organization of communities arising from latent invaders in bark and functional sapwood, under different moisture regimes. – New Phytol. 110: 47–57.
Crous, W. P., P. S. Knox-Davies, & M. J. Wingfield (1989). A list of *Eucalyptus* leaf fungi and their potential importance to South African Forestry. – Suid-Africaanse Bosboutydsfrik 149: 17–29.
Davison, E. & C. Tay (1983). Twig, branch, and upper trunk cankers of *Eucalyptus marginata*. – Plant Dis. 67: 1285–1287.
Espinosa-Garcia, F. J. & J. H. Langenheim (1990). The endophytic fungal community in leaves of a coastal red-wood population diversity and spatial patterns. – New Phytol. 116: 89–97.
Fernández De Ana, F. J. (1982). La *Cytospora* spp. parásito del *Eucalyptus gigantea* en Galicia. – Anales del INIA-Servicio Forestal 5: 195–198.
Ferreira, F. A. (1989). Patologia Forestal; principais doenças no Brasil.– Sociedade de Investigaçoes Forestais, Brasil-Viçosa: 570 p.
Fisher, J. P., A. E. Anson & O. Petrini (1986). Fungal endophytes in *Ulex europaeus* and *Ulex gallii*. – Trans. Brit. Mycol. Soc. 86: 153–156.
—— & O. Petrini (1990). A comparative study of fungal endophytes in xylem and bark of *Alnus* species in England and Switzerland. – Mycol. Res. 94: 313–319.
——, —— & B. C. Sutton (1993). A comparative study of fungal endophytes in leaves, xylem and bark of *Eucalyptus* in Australia and England. – Sydowia 45: 338–345.
Gibson, I. A. S. (1981). A canker disease of *Eucalyptus* new to Africa.– Food and Agriculture Organization. Food and Agricultural Digest Forest Genetic Resources Information 10: 23–24.
Howard, P. J. A. & Ch. Robinson (1995). The use of correspondence analysis in studies of successions of soil organisms. – Pedobiologia 39: 518–527.
Ministerio de Ganadería Agricultura y Pesca. Dirección Forestal. 1991. Proyecto de desarrollo Forestal. – BIRF UR 3131 PNUD/FAO–URU/90/005.
Old, K., D. Murray, G. Kile, J. Simpson & K. Malafant (1986). The pathology of fungi isolated from eucalypt cankers in South Eastern Australia. – Australia For. Res. 16: 37–50.
—— Z. Yuan & T. Kobayashi (1991). A *Valsa* teleomorph for *Cytospora eucalyptticola*.– Mycol. Res. 5: 1253–1256.
Petrini, O. (1986). Taxonomy of endophytic fungi of aerial plant tissue. – In: Fokkema N. & J. van den Heuvel (eds). Microbiology of the Phyllosphere. Cambridge University Press, Cambridge: 175–187.
—— (1991). Fungal endophytes of tree leaves. – In: J. Andrews & S. Hirano (eds.). Microbial ecology of leaves. Springer-Verlag, USA: 179–197.
—— & P. J. Fisher (1987). Fungal endophytes in *Salicornia perennis*. – Trans. Brit. Mycol. Soc. 87: 647–651.
—— & —— (1988). A comparative study of fungal endophytes in xylem and whole stem of *Pinus sylvestris* and *Fagus sylvatica*. – Trans. Brit. Mycol. Soc. 91: 233–238.
Sankaran, B., C. Sutton & M. Balasundaran (1995). *Cryptosporiopsis eucalypti* sp. nov., causing leaf spots of eucalypts in Australia, India and U.S.A. – Mycol. Res. 99: 827–830.
Sarasola, A. A. & M. A. Sarasola (1959). Enfermedades del eucalito en la Argentina. – IDIA 139: 1–11.

Shoeneweiss, D. (1981). The role of environmental stress in diseases of woody plants. – Plant Dis. 65: 308–314.
—— (1983). Drought predisposition to *Cytospora* canker in blue spruce. – Plant Dis. 67: 308–314.
Sieber, T. N. (1989). Endophytic fungi in twigs of healthy and diseased Norway spruce and white fir. – Mycol. Res. 92: 322–326.
——, T. Kowalski & O. Holdenrieder (1995). Fungal assemblages in stem and twig lesions of *Quercus robur* in Switzerland. – Mycol. Res. 99: 534–538.
Sinclair W., Lyon, H. H. & W. T. Johnson (1987). Diseases of trees and shrubs. – Cornell University Press, Ithaca, Can. & London, U.K.
Stalpers, J. A. (1978). Identification of wood-rotting Aphyllophorales in pure culture. – Studies Mycol. 16: 1–243.
Stolovich L. (1995). Forestación ¿un negocio para quien?. – ITeM-CIEDUR. Uruguay.
Sutton, B. C. (1980). The Coelomycetes. – C.A.B., Surrey, 696 pp.
—— & E. M. Davison (1983). Three stromatic Coelomycetes from western Australia. – Trans. Brit. Mycol. Soc. 81: 291–301.
Wilson D. & G. C. Carroll (1994). Infection studies of *Discula quercina*, an endophyte of *Quercus garryana*. – Mycologia 86: 635–647.

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