Fungal community of *Eucalyptus globulus* and *Eucalyptus maidenii* stems in Uruguay

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The purpose of this research was to examine the fungal community of bark, sapwood and heartwood of *Eucalyptus globulus* ssp. *globulus* and *E. globulus* ssp. *maidenii,* two of the main species for pulp industry planted in Uruguay. Different trunk lesions are frequently found in *E. globulus* conversely to which is observed in *E. maidenii* growing in the same site. Slices from the basal portion of trunk were assessed for the fungal colonization. The main species that characterize these communities are the same but a low frequency of colonization in *E. maidenii* was remarkable. Sapwood showed the lowest and bark the highest frequency of colonization. *Aureobasidium pullulans, Botryosphaeria* spp., *Cytospora eucalypticola,* Basidiomycetes and sterile mycelia were the taxa present in almost all tissues of both hosts species. Several of these Basidiomycetes are frequently associated to *Eucalyptus* stumps. Simple correspondence analysis showed differences in community composition between bark and xylem from the two *Eucalyptus* species evidencing tissue but not host preferences. Several species present in these trunks were also found as endophytic colonizers of sprouting stumps, twigs and seedling stems of *E. globulus*. This indicates that these fungi could overcome the high stress conditions of mature trunk tissues and remain as latent colonizers.

Keywords: endophyte, bark, sapwood, heartwood, wood rotting fungi.

During the last 30 years *ca. 440 000 ha,* corresponding to 2 % of the total area of the country, have been planted with *Eucalyptus* spp. and approximately 98 % of wood produced is exported as raw material (MGAP, 2002). *Eucalyptus globulus* ssp. *globulus* Labill. *(E. globulus)* and *Eucalyptus globulus* ssp. *maidenii* (Mueller) Kirkpatrick *(E. maidenii)* are two of the main species planted in central west and south east Uruguay.

Cankers and bark cracks are frequently found in *E. globulus* trunks, but infrequently in *E. maidenii* growing in the same site. These bark lesions were assayed for fungal colonization and the main taxa associated were *Alternaria alternata, Cytospora eucalypticola, Botryosphaeria* spp., Basidiomycetes, Xylariaceae and *Sporothrix* spp. (Bettucci *et al.* 1999 a).

Similarly, these species were also present as endophytes of seedlings, sprouting stumps, twigs, flowers, capsules and seeds occurring in *Eucalyptus* in South America, and particularly in Uruguay (Bertoni & Cabral 1988, Bettucci & Alonso 1997, Bettucci *et al.* 1997, Bettucci & Saravay 1993, Bettucci *et al.* 1999*b*, Lupo *et al.* 2001).

Fungal endophytes are regarded as those present in living plant tissues (Carroll 1986, 1995), however, dead trunk tissues like heartwood could also harbour propagules of several fungal species (Boddy & Griffith 1989). Some endophytes of young stems could remain as latent colonizers in the mature tissues as new cells of bark and xylem were added by cambial activity (Boddy & Griffith 1989, Boddy 1992). Therefore, the main goal of this work was to study the composition of fungal communities of bark, sapwood and heartwood of *E. globulus* and *E. maidenii* trunks and to detect whether differences in tissue preference of each host species exist. An additional purpose was to compare the fungal community of trunks with those present as endophytes in *E. globulus* sprouting stumps and seedling stems previously studied (Bettucci & Saravay 1993, Bettucci *et al.* 1997).

Materials and methods

Sampling site

The site was located in Paysandú at the northwest of Uruguay, from 32°53'S, 31° 40'W. The climatic type according to Koeppen-Geiger classification (Strahler & Strahler 1992) is *Cfa*, temperate with all year rains. The mean annual temperature ranges from 11° to 23 °C and the annual precipitation amount ranges from 900 to 1300 mm. Low prairies were the former vegetation before tree plantations were introduced. Plantation was constituted by randomly distributed 8 year old trees of *E. globulus* and *E. maidenii*.

Material collection and fungal isolation

From a healthy stand 46 trees of *E. globulus* and 46 of *E. maidenii*, growing in proximity to one another, were randomly selected. Trees of each plant species were felled at 10–15 cm above the ground. From each tree, slices of 3–5 cm thickness and 18–20 cm in diameter from the basal part of the trunk were obtained. All materials were taken to the laboratory in paper bags, stored at 5 °C and processed within 24 h. From each tree slice, 20 segments of approximately 2 × 1.5 × 5 mm from inner bark, sapwood and heartwood were

dissected. *E. maidenii* bark is characteristically thicker than in *E. globulus*. Segments from each tissue were pooled and surface-sterilized by sequential immersion in 80 % ethanol for one minute, sodium hypochlorite (0.4 g active Cl/100 mL) for two minutes, washed with sterile distilled water, and then dried on sterile filter paper. To test the effectiveness of surface sterilization, segment imprints on culture medium were performed.

Segments from each tissue were randomly selected for plating. A set of 10 segments per plate, a total of 920 segments from each of the bark, sapwood and heartwood tissues of *E. globulus* and *E. maidenii*, were placed onto 9 cm Petri dishes containing 2 % MEA (Difco), pH 4.5. Plates were incubated at 24 °C for six weeks or more depending on the growth rates of fungi. Each colony that emerged from segments was transferred to fresh medium (2 % MEA, Difco; PDA, Difco and oatmeal agar) for identification. Black light was used to induce sporulation in some cultures. Identification was performed by means of conventional mycological methods following description of the cultural and micromorphological characteristics of each isolate (Sutton 1980, Ellis 1971, 1976, Dennis 1981, Gams 1983). Isolates of Basidiomycetes were identified by cultural and micromorphological characteristics and extracellular enzymes production following Stalpers (1978), Nakasone (1990) and Boidin (1997). Those cultures that failed to sporulate after 6 weeks were considered sterile mycelia.

Data analysis

The relative frequency of isolation was calculated as the number of segments colonized by a given fungus, divided by the total number of segments expressed as percentage. To evaluate to which extent fungal community was revealed by the sampling method employed, the abundance distribution and species accumulation curves for each tissue type were performed and tendencies were plotted. Moreover, the relative abundance curves were compared to lognormal theoretical model using Kolmogorov-Smirnov test (Krebs 1989). Diversity was measured for each tissue, and tree host by means of Shannon diversity index with the computer package MVSP for Windows (Kovach Computing, Anglesey, UK).

To evaluate differences in fungal composition between hosts and among tissues of each hosts simple correspondence analysis using STAT-ITCF (Service des Etudes Statistiques, Institute Technique des Céréales et des Fourrages, France) was carried out using the species, in any tissue, with a relative frequency equal or higher to 1 %. Species with relative frequency of 0.5 %, isolated from at least two tissues of both host species, were also included (Howard & Robinson 1995).

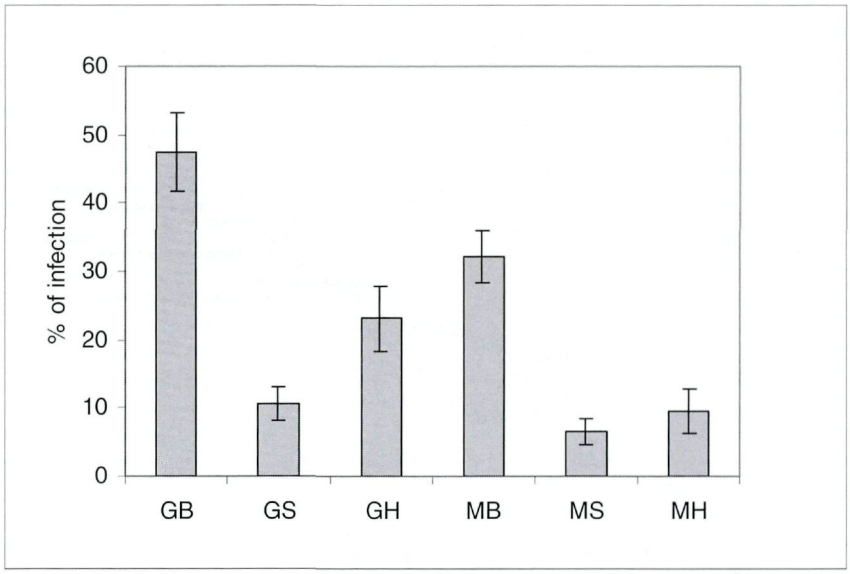

Fig. 1. Species abundance distribution from each tissue. Few species were isolated with high frequency and several were rare. The log normal distribution expected (line) for all tissues differed significantly ($P > 0.05$) from the observed data (Kolmogorov-Smirnov) except for bark of *E. globulus*.

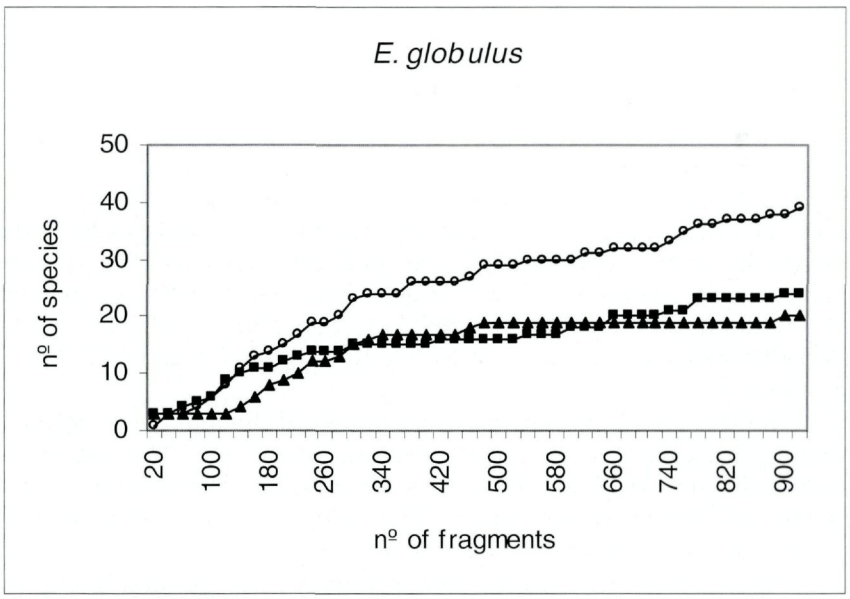

Fig. 2. Number of tissue segments colonized in *E. globulus* (G) and *E. maidenii* (M). B: bark, S: sapwood, H: heartwood. Bars indicates standard deviations.

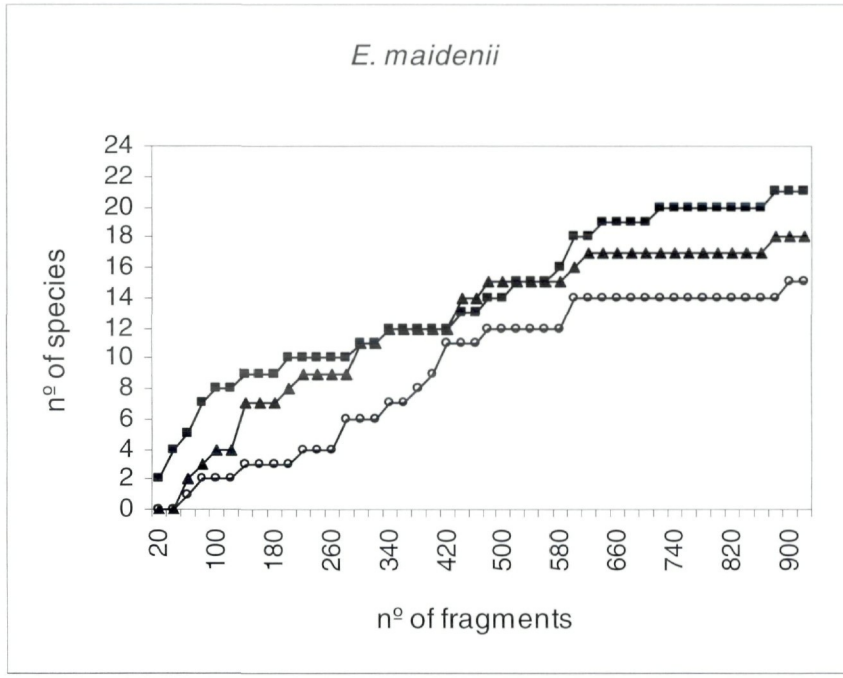

Fig. 3. Species accumulation curves tendency showing the number of species found from each 20 additional segments plated out. Symbols indicate: bark: . . .; sapwood: —; heartwood: -.-.-

Results

From 5520 segments, 1196 isolates belonging to 94 taxa were obtained from bark, sapwood and heartwood with the number of taxa ranging from 18 (sapwood of *E. maidenii*) to 40 (bark of *E. globulus*) (Tab. 1). Communities in all tissues of the two hosts did not fit with lognormal distribution ($P > 0.05$), except *E. globulus* bark (Fig. 1). The percent of segments colonized ranged from nearly 6.6 % (sapwood of *E. maidenii*) to 47.6 % (bark of *E. globulus*) (Fig. 2), with overall average colonization of 27.1 % for *E. globulus* and 16.2 % for *E. maidenii*.

The species accumulation curve evidenced the number of species found with each 20 additional segments plated out. The tendency curves did not reach saturation, as indicated by the absence of an asymptote in any tissue except for *E. globulus* sapwood in which asymptote was achieved from 800 segments (Fig. 3).

From all taxa isolated, 51 were exclusively distributed in one of the three tissues of *E. globulus*. In *E. maidenii,* 18 taxa were isolated exclusively in one tissue. In both hosts these tissue specific taxa were

isolated with a low relative frequency. Conversely, the taxa isolated with the highest relative frequency, at least in one tissue, were those common to the two hosts and present in nearly all tissues. In sapwood and heartwood the number of isolates as well as the number of taxa was low. In both hosts, the fungal diversity of sapwood and heartwood was similarly low (Tab. 2). Conversely, in bark the number of isolates and taxa was relatively high.

The bark community of two hosts was dominated by *Botryosphaeria* spp., *Cytospora eucalypticola* Van der Westh., *Pestalotiopsis guepinii* (Desm.) Steyaert and sterile mycelia. *Botryosphaeria* spp. were the taxa isolated with the highest relative frequency (Tab. 1). *Phialophora* spp., Basidiomycetes, *Aureobasidium pullulans* (de Bary) G. Arnaud and sterile mycelia, were the most common taxa in xylem of both hosts. Sterile hyaline and dark mycelia corresponded to 25 and 14 different morphotaxa respectively, distributed in all tissues of the two *Eucalyptus* species. The remaining species were rare (< 1 %).

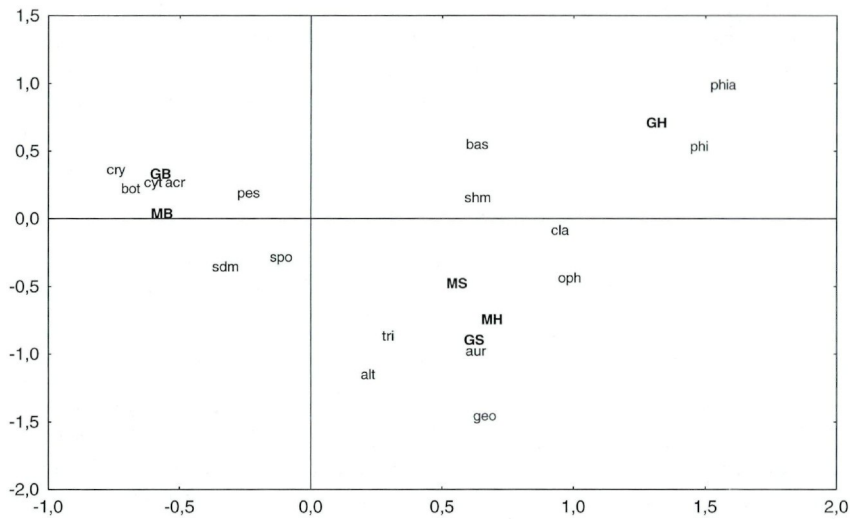

Fig. 4. Simple correspondence analysis. Ordination of hosts tissues according to the endophytic composition on the two first axes. Total inertia explained by the first two co-ordinates axes: 76,32 %. Variables are the relative frequencies of isolation of species with frequency equal or higher than 1 % and those with 0.5 % but isolated from more than one tissue of the two host species. Symbols for hosts tissues: G: *E. globulus;* M: *E. maidenii:* B: bark; S: sapwood; H: heartwood; symbols for the species are: acr: *Acremonium* spp.; alt: *Alternaria alternata;* aur: *Aureobasidium pullulans;* bas: Basidiomycete (32 taxa); bot: *Botryosphaeria* spp.; cla: *Cladosporium cladosporioides;* cry: *Cryptosporiopsis eucalypti;* cyt; *Cytospora eucalypticola;* geo: *Geotrichum* spp.; oph: *Ophiostoma piliferum;* pes: *Pestalotiopsis guepinii;* phia: *Phialemonium* spp.; phi: *Phialophora* spp.; spo: *Sporothrix* spp.; sdm: Sterile dark mycelium; shm: Sterile hyaline mycelium; tri: *Trichoderma* spp.

From 32 Basidiomycetes taxa, 17 were identified to genus level and 4 to species according to the cultural and micromorphological characteristics and to production of extracellular oxidative enzymes. These species were *Lentinus tigrinus* (Bull.) Singer, *Bjerkandera adusta* (Willd:) P. Karst., *Peniophora molesta* Boidin, Lanquetin et Gilles and *Phanerochaete magnoliae* (Berk. & Curt.) Bursdall. Only *L. tigrinus* was present in all tissues of both hosts. All the 32 Basidiomycetes produced extracellular oxidative enzymes characteristic of white rotting fungi.

The simple correspondence analysis showed that the two first coordinate axes explained 76.32 % of the total inertia, indicating a good fit of the data to the model (Fig. 4). Axis one accounted for 55.2 % of the total inertia and separated bark fungal assemblages of both *Eucalyptus* species from those of wood. *Botryosphaeria* spp. contributed 22.2 % and *C. eucalypticola* 10 % to the inertia of this axis associated mainly to bark of the two plant species. *Phialophora* spp. contributed 43.5 % to the inertia of this axis and mainly characterized *E. globulus* heartwood.

Axis two accounted for 21.2 % of the total inertia and explained some differences between heartwood of *E. globulus* and the other woody tissues of both hosts. *Aureobasidium pullulans* and *Geotrichum candidum* Link mainly associated with woody tissues of both hosts contributed 50.6 % of this axis. Basidiomycetes and *Phialophora* spp. contributed 17.9 % and characterized *E. globulus* heartwood.

Tab. 1. – Endophytic fungi of *Eucalyptus globulus* and *E. maidenii* trunk tissues. Frequency of colonization (%). B: bark; S: sapwood; H: heartwood.

	E. globulus			*E. maidenii*		
	B	S	H	B	S	H
Acremonium sp. FI 764					0.43	
Acremonium strictum W. Gams	2.17					
Alternaria alternata (Fr.) Keissl.	0.22	0.76		0.33		0.11
Aspergillus niger Tiegh.	0.11			0.33	0.22	0.33
Aureobasidium pullulans (de Bary) G. Arnaud	0.11	1.52	0.98	1.52	1.63	2.72
Basidiomycete	1.41	0.11	2.28	0.54	0.11	0.43
Bjerkandera adusta (Willd.) P. Karst.	0.22					
Botryosphaeria spp.	18.15	0.11	0.11	14.89	0.43	0.54
Cladosporium cladosporioides (Fresen.) G.A. de Vries	0.11		0.43		0.65	
Cryptosporiopsis eucalypti Sankaran & B. Sutton	0.98			0.43		
Curvularia pallescens Boedijn		0.11		0.11	0.22	
Cytospora eucalypticola Van der Wahrs.	13.15	0.76	0.43	5.54	0.43	

	E. globulus			*E. maidenii*		
	B	S	H	B	S	H
Epicoccum purpurascens Ehrenb.			0.65			
Eupenicillium brefeldianum (B. O. Dodge) Stolk & D. B. Scott				0.22		
Fusarium sp. FI 813, FI 819		0.11	0.11			
Geotrichum candidum Link	0.22	1.30	0.11			
Graphium spp.	0.33		0.22			0.11
Hymenochaetaceae FI 1155	0.54					
Hymenochaetaceae FI 748	0.33	0.11	0.11	0.11		
Hyphoderma sp. FI 1150			0.22			
Lentinus tigrinus (Bull.) Singer	0.22	0.43	0.43	0.11	0.33	0.11
Leptodontium elatius (F. Mangenot) de Hoog						0.33
Leptodothiorella sp. FI 874						0.33
Moniliella suaveolens var. *nigra* (Lindner) Arx		0.22				
Mucor sp. FI 690		0.33				
Nectria sp. FI 2430	0.22					
Neoplaconema cymbiforme Z. Q. Yuan & C. Mohammed	0.43					
Nigrospora sphaerica (Sacc.) E. W. Mason	0.11			0.11		
Ophiostoma piliferum (Fr.) Syd. & P. Syd.	0.11		0.33			0.54
Paecilomyces farinosus (Holmsk.) A. H. S. Br. & G. Sm.	0.11	0.22				
Peniophora molesta Boidin, Lanq. & Gilles	0.11					
Peniophora sp. FI 1148	0.54					
Peniophora sp. FI 700, FI 691			0.54			
Pestalotiopsis guepinii (Desm.) Steyaert	3.48	0.54	0.87	1.74		0.33
Phanerochaete magnoliae (Berk. & Curtis) Burds.			0.44		0.11	
Phanerochaete sp. FI 1003	0.54					
Phanerochaete sp. FI 721, FI 729			0.33			
Phellinus sp. FI 1156			0.33			
Phellinus sp. FI 978			0.22			0.11
Phialemonium sp. FI 921			0.54		0.11	
Phialophora fastigiata (Lagerb. & Melin) Conant		0.11	3.70		0.76	1.09
Phialophora richardsiae (Nannf.) Conant		0.98	6.52			0.54
Phialophora spp.		0.33	0.33			
Phlebia sp. FI 1141			0.33			
Septoria sp. FI 914				0.54		

	E. globulus			*E. maidenii*		
	B	S	H	B	S	H
Sporothrix eucalypti M. J. Wingf., Crous & W. J. Swart	0.65			0.43	0.11	0.11
Sporothrix inflata de Hoog			0.11			
Sporothrix shenckii Hektoen & C.F. Perkins		0.22				0.22
Sporothrix sp. FI 710	0.11			0.11		
Sterile dark mycelium	1.41	0.43		3.48	0.33	0.98
Sterile hialine mycelium	0.54	0.76	1.52	0.76		0.11
Trechispora sp. FI 1001			0.33			
Trichoderma harzianum Rifai	0.33					
Trichoderma sp. FI 959	0.11	0.43	0.11	0.54	0.43	0.65
Total number of isolates 1196	438	97	213	298	61	89
Total number of taxa 94	40	27	38	24	18	21
Total number of segments 5520	920	920	920	920	920	920

Rare taxa: *Acremonium kiliense* Grütz; *Acremonium stromaticum* W. Gams & R.H. Stover; *Arthrinium sphaerospermum* Fuckel; *Aspergillus ochraceus* G. Wilh.; *Botryotrichum piluliferum* Sacc. & Marchal; *Cylindrocarpon* sp. FI 788; *Drechslera hawaiensiis* M.B. Ellis; *Fusarium nygamai* L.W. Burgess & Trimboli; *Fusarium solani* (Mart.) Sacc.; *Hainesia lythri* (Desm.) Höhn.; *Microspaheropsis olivacea* (Bonord.) Höhn.; *Mucor hachijyoensis* Ts. Watan; *Mucor hiemalis* Wehmer; *Phialemonium obovatum* W. Gams & McGinnis; *Phoma fimeti* Brunaud; *Phoma sorghina* (Sacc.) Boerema, Dorenb. & Kesteren; *Rhinocladiella atrovirens* Nannf.; *Rhizomucor* sp. FI 736; *Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not.; *Sporothrix inflata* de Hoog; *Sporotrichum aureum* Link; *Torula herbarum f. quaternella* Sacc.; *Trichoderma koningii* Oudem.; *Xylaria* sp. FI 1147; *Xylaria* sp. FI 939

Tab. 2. – Measures of diversity for endophytic communities of tissues, organs and plant hosts. Symbols indicate: H': Shannon'n diversity index; J: evenness; S: total number of species.

	H'	J	S
GB	2.115	0.573	40.0
GS	2.896	0.879	27.0
GH	2.805	0.771	38.0
MB	1.911	0.609	24.0
MS	2.529	0.875	18.0
MH	2.532	0.832	21.0

Discussion

The fungal communities of trunks were constituted by several taxa with a low frequency of colonization mainly in woody tissues. The low index of diversity of all tissues analyzed confirm that the endophytic communities of trees planted outside their original

location are depauperate in composition (Espinosa-García & Langenheim 1990, Fisher *et al.* 1993).

The main species that characterized these communities are the same in *E. globulus* and *E. maidenii,* but the frequency of colonization in *E. maidenii* was remarkably low. The composition of fungal communities that colonized both host trunks were similar to that found in twigs, seedling stems and sprouting stumps of *E. globulus* (Bettucci & Saravay 1993, Bettucci *et al.* 1997, Bettucci *et al.* 1999*b*) whereas the frequency of colonization was higher in young tissues than in trunks. A set of species considered almost exclusively endophytes (Bills & Polishook 1992) were dominant components of the community present in these trunks. *P. guepinii* was commonly found in *Eucalyptus* spp. in Uruguay (Bettucci & Saravay 1993, Bettucci & Alonso 1997, Bettucci *et al.* 1997, Bettucci *et al.* 1999*b*) and is a common endophyte in temperate (Fisher *et al.* 1993, Barengo *et al.* 2000, Bills & Polishook 1992) and tropical (Bayman *et al.* 1998) tree species.

Among fungi present only in woody tissues the species of *Phialophora* were the most important. These fungi have revealed the ability to colonize and persist in several species of standing tree or in dead wood under strikingly different ecological conditions (Bettucci 1984, 1985, Menkis *et al.* 2004). Species of *Phialophora* and *A. pullulans* produce blue stain in untreated coniferous sapwood but are infrequently found in hardwood (Butcher 1970) and hence they could not represent a risk for *Eucalyptus* timber or chips.

Sporothrix eucalypti M.J. Wingf., Crous & W.J. Swart was mentioned as die back producer and it is considered pathogen of leaves and buds of young *Eucalyptus* plants (Wingfield 1993). Its presence in trunks analyzed here, could impact on sprouting stump. *Cryptosporiopsis eucalypti* Sankaran & B. Sutton a pathogen that produce leaf spot and shoot blight (Old *et al.* 2002) was frequently present in Uruguay as foliar endophyte (Bettucci 2003).

Several isolates of Basidiomycetes were present in bark and heartwood of *E. globulus* but few in *E. maideni.* The higher water content of functional sapwood from living trees constitute a high stress condition for fungal growth that could explain the lower number of isolates of wood rotting Basidiomycetes and other taxa in this tissue (Boddy 1992). In young *E. globulus* stems wood rotting Basidiomycetes were also present as endophytes with higher relative frequency but lower specific richness than in trunks analyzed here (Bettucci & Saravay 1993).

Six months after trees were felled, fruit bodies of *L. tigrinus, B. adusta, P. molesta,* and *P. magnoliae* were found associated with stumps from which slices were taken. The conditions to which stumps were exposed, such as higher oxygen availability and drying,

favoured the fruit bodies development from latent propagules present in wood. Consequently, these fungi became involved in the decomposition process of stump lignocellulosic materials.

Botryosphaeria spp. and *Cytospora* spp. are also common endophytes mainly in bark of *Eucalyptus* spp., as well as in native Myrtaceae in Uruguay (Bettucci & Alonso 1997, Bettucci *et al.* 2004; Alonso, 2004) and in other countries (Smith *et al.*, 1996; Fisher *et al.* 1993). *Botryosphaeria* was also considered as an *Eucalyptus* pathogen producing cankers and bark cracking (Smith *et al.* 1994, mith *et al.* 1996) and as weak pathogen in plants growing under unfavorable environmental conditions (Houston, 1993, Old *et al.* 1986). The bark lesions more frequently present in *E globulus* are improbable due to *Botryosphaeria,* since experimental inoculations evidenced the inability of *Botryosphaeria* spp. to penetrate healthy tissues and to produce lesions (Alonso 2004). Apparently, as with other endophytes, virulence of *Botryosphaeria* spp. on *Eucalyptus* will depend on the plant development and on environmental conditions under which it is growing (Schulz 2005).

Trunk lesions in *E. globulus* trunks could serve as infection courts for basidiospores of *I. jamaicensis* associated to trunk heart rot in Uruguay (Martínez 2005). The low incidence of bark lesion in *E. maidenii*, could be explained by the thicker bark and the absence of heart rot to the higher xylem density in relation with *E. globulus*. These structural characteristics could constitute an important barrier to fungal establishment (Blanchette & Biggs 1992) as well as resistance to unfavourable environmental conditions.

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