Fungal species diversity in juvenile and adult leaves of *Eucalyptus globulus* from plantations affected by Mycosphaerella leaf disease

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Abstract

In recent years Mycosphaerella leaf disease (MLD) has become very common in *Eucalyptus globulus* plantations in Galicia, Northwest Spain. The etiology of MLD is complex and is associated with several species of *Mycosphaerella* and *Teratosphaeria*. A survey of the fungal mycobiota associated with juvenile and adult leaves, and with leaf litter of the same trees in MLD affected plantations was made. The goal was to identify pathogens and endophytes, to determine if the mycobiota of each leaf type differed, and if leaf litter might be a reservoir of MLD inoculum. Fungi belonging to 113 different species were isolated from leaves of juvenile and adult trees sampled at 10 locations; 81 species occurred in juvenile and 65 in adult leaves. The average number of species obtained from juvenile leaves was significantly greater ($p>0.01$) compared with adult leaves. This difference suggested that juvenile leaves are not only more susceptible to a group of pathogens, but to a wide range of fungi. Therefore, a general resistance mechanism might be lacking or be less effective in juvenile than in adult leaves. Several pathogenic species were identified in both leaf types. Leaf litter and living leaf mycobiotas were very different. However, some of the species they shared were MLD pathogens, suggesting that leaf litter could contribute inoculum of MLD.

Key words: endophytes, fungal diversity, forestry, mycobiota.

Introduction

Eucalyptus trees from Australia were first introduced into Spain in the 19th century. Presently, after *Pinus* and *Quercus*, *Eucalyptus* is the third most abundant tree genus in Spain, and *Eucalyptus globulus* is the most widely cultivated species of the genus. Commercial plantations of this species are very frequent in Galicia, in the northwest corner of the Iberian Peninsula, where about 67% of the national production volume of this species is grown (Dirección General de la Conservación de la Naturaleza, 1998). Because of the area's mild and humid climate, *E. globulus* has been a successful forestry species, with good yields and a production cycle of about 15 years. However, in recent years Mycosphaerella leaf disease (MLD), has become widespread in Galicia, and most plantations are affected by this fungal disease, particularly in young trees (Otero *et al*., 2007).

Trees affected by MLD show amphigenous necrotic leaf lesions which may coalesce, affecting a significant portion of the leaf surface. As a result premature defoliation, as well as physiological damage occurs (Pinkard and Mohammed, 2006). The disease etiology is complex because numerous species of *Mycosphaerella* and *Teratosphaeria* have been isolated from diseased leaves worldwide (Park and Keane, 1982; Carnegie *et al*., 1997; Crous, 1998; Hunter *et al*., 2004; Maxwell *et al*., 2003; Crous *et al*., 2004, 2007b, 2009a, 2009b; Jackson *et al*., 2005, 2008; Carnegie, 2007; Barber *et al*., 2008; Andjic *et al*., 2010). *Teratosphaeria nubilosa* (=*Mycosphaerella nubilosa*), and *Teratosphaeria cryptica* (=*Mycosphaerella cryptica*) are thought to be the most damaging species, but *T. molleriana*, *M. marksii*, *M. fori* (=*Pseudocercospora fori)*, and several other species have also been pinpointed as pathogens (Park and Keane, 1982; Crous and Wingfield, 1997; Hunter *et al*., 2004; 2009). In Spain *T. nubilosa* is the main causal agent of MLD in *E. globulus* (Crous *et al*., 2004; Otero *et al*., 2007). Determining whether one or more species are the causal agents of MLD is further complicated because as many as 11 different species of fungi may occur in the same ~~l~~eaf lesion (Crous *et al*., 2009d).

In addition to *Teratosphaeria* and *Mycosphaerella* species, numerous fungal taxa have also been isolated from *Eucalyptus* as pathogens, endophytes, or saprophytes (Cabral, 1985; Fisher *et al*., 1993; Crous *et al*., 1995; 2006b; 2007a; Betucci and Alonso, 1997; Simeto *et al*., 2005; Summerell *et al*., 2006; Carnegie, 2007; Slippers *et al*., 2009; Cheewangkoon et al., 2009). It is thought that some species behaving as endophytes or saprophytes in eucalypts could be pathogens from other hosts (Crous *et al*., 2009d). However, under circumstances of stress, some apparently endophytic species might become pathogenic (Slippers and Wingfield, 2007; Slippers *et al*., 2009). It could also be possible that some endophytic species could interfere with the development of

disease, as it has been demonstrated in several pathogen–endophyte interactions (Zabalgogeazcoa, 2008)

In *Eucalyptus globulus* and other species of the genus leaf development is heteroblastic, different leaf types occur during successive stages of the life of the trees (Gras *et al*., 2005). Young trees produce broad, ovate, blueish leaves, and adult trees produce narrow, lanceolate, green leaves (Figure 1). In several *Eucalyptus* species MLD incidence and severity is much greater in juvenile than in adult leaves (Pinkard and Mohammed, 2006; Hunter *et al*., 2009)., and some insect herbivores show oviposition preference for juvenile leaves (Steinbauer, 2002). Although the reason why this occurs is not well known, the increased susceptibility of juvenile leaves to MLD could be related to differences in structural or chemical defences between both types of leaves. For instance, adult leaves have thicker cuticles and lower stomatal density than juvenile leaves (James and Bell, 2001; Gras *et al*., 2005).

In this paper we report the results of a survey where the fungal assemblages associated with juvenile and adult leaves of trees from plantations affected by MLD were identified and compared. In addition, the mycobiota associated with leaf litter was compared to living leaf mycobiota and evaluated for its potential as a source of inoculum of MLD pathogens.

Materials and Methods

Plant samples

Nine commercial plantations in the province of La Coruña, in Galicia, Spain, and one in Coimbra, Portugal were sampled (Table 1). At each location, a sample consisting of one branch with several leaves was cut from an adult tree or a young tree having distinct juvenile leaves. At the 10 locations, leaves from adult and juvenile trees showed necrotic lesions typical of MLD (Figure 1). In total, 37 samples from young trees and another 37 samples from mature trees were analyzed. Young trees were at least one year old because their trunk diameters were greater than 5 cm.

The mycobiota was isolated from asymptomatic and diseased parts of leaves of each sample. Disease-free parts excised from 8 leaves of each sample were cut into square pieces of approximately 4 x 4 mm, surface disinfected with a solution of 20% domestic bleach (1% active chlorine) for 10 minutes, and rinsed with sterile water. Approximately 40 pieces were placed in two 9 cm diam Petri plates containing potato-dextrose agar with 200 mg/l of chloramphenicol. To isolate fungi from diseased tissue, living tissue from the margin of necrotic lesions was cut and processed as described above.

The plates with leaf pieces were incubated in the laboratory at room temperature and checked daily for the presence of mycelium emerging from leaf pieces. After mycelial emergence, a

small piece of mycelium was excised and transferred to a 5 cm Petri plate, and the leaf fragment was discarded from the plate.

Isolation of fungi from leaf litter

Five samples, each consisting of about 1 l of partially decomposed *Eucalyptus* leaf litter were collected from the ground surface of one *Eucalyptus* plantation (Chimparra, La Coruña). In the laboratory, a portion of about 100 ml from each sample was processed for the isolation of fungi following the process of particle filtration and plating described by Collado *et al*. (2007). After a thorough washing with 4 l of tap water, single leaf particles of 110-200 μm were deposited in 48-well tissue culture plates containing yeast malt agar. When fungi grew from a particle in a well, a mycelia fragment was transferred to a 5 cm Petri plate containing potato dextrose agar.

Isolate identification

From each plate with leaf material, a single isolate was retained when fungi of similar morphological characteristics emerged from more than one leaf piece. Isolates were first grouped into morphotypes, according to macroscopic characteristics of the mycelia. Afterwards, one or more isolates of each morphotype were identified on the basis of morphological and/or molecular characters. The molecular identification was based on the nucleotide sequence of the ITS1-5.8SrRNA-ITS2 region. The method used to amplify and sequence this region was previously described by Sánchez Márquez *et al*. (2007). The criteria for molecular identification were based on the similarity of the sequences to those of identified isolates in the public nucleotide databases. The databases were searched using the FASTA algorithm (Pearson 1990). The genus and species of the database match were accepted when the identity between our sequence and that of the database was greater than 97.0%, and only the genus was accepted when identity to a database match was from 96.9 to 95.0%. When the similarity was less than 95%, the isolates were considered unidentified. Although in some cases the taxon boundaries made with these criteria might only be approximations, in general they have been found to be acceptable estimates of fungal diversity (i.e. Arnold and Lutzoni, 2007)

Data analysis

Four types of samples were considered at each location: diseased or asymptomatic tissue from juvenile or adult leaves. The number of species identified on each individual sample was used to estimate the mean number of species found in each type of sample at each location. The significance of differences between locations, types of leaves (juvenile or adult), and types of tissue

(diseased or asymptomatic) was tested with a three way analysis of variance considering these effects.

To estimate the total number of species found in juvenile or adult leaves, the number of species present in diseased and asymptomatic tissue of each juvenile or adult tree was pooled, considering species occurring in both types of tissue only once for each tree. The significance of the difference between the mean number of species found in each type of leaf was evaluated with a t-test. This data set consisted of 74 samples, 37 from trees with juvenile leaves and another 37 of trees with adult leaves.

The similarity between the set of species (mycobiota) identified on each type of leaf and tissue was estimated using Jaccard's index of similarity (Magurran, 2004). In this case, the index was based on the number of common and different species observed between pairs of mycobiotas. The value of this index can range from 1, when all species are shared by both mycobiotas, to 0, when no species are shared by two mycobiotas.

Results

Disease incidence and severity

MLD symptoms in the form of necrotic lesions were present in young and adult trees at all 10 study locations. At all locations lesion size was considerably greater in juvenile than in adult leaves (Figure 1). In addition to the study locations, MLD was present at many other locations visited during the course of the work in 2008-2009. At all sites, juvenile tree plantations showed leaf disease, but in a few adult plantations leaf disease symptoms were not obvious.

Abundance and distribution of foliar species.

A total of 513 isolates were processed for identification. From juvenile leaves 301 isolates were obtained, 136 from asymptomatic tissue samples and 165 from diseased tissue. From adult leaves, 212 isolates were obtained, 102 from asymptomatic and 110 from diseased tissue. The isolates were classified into 113 different species; 43 of them could not be ascribed to a known taxon because they were sterile in culture, and their nucleotide sequences were less than 95% similar to any fungal taxa deposited in the EMBL nucleotide database. The 70 different species which could be ascribed to a taxonomic rank are listed in Table 3.

Of all species, 48 were observed only in juvenile leaves, 32 in adult leaves, and 33 in both types of leaves. The analyses of variance of the number of species identified in each sample

revealed statistically significant differences among locations and leaf types (Table 2). When the species found in asymptomatic and diseased tissue of each type of leaf were pooled, again more species were identified in the pooled samples of juvenile leaves (Mean: 7.46 species per sample) than in those of adult leaves (Mean: 5.00 spp./sample) (Figure 2a), and the difference between these means was statistically significant ($t = -3.329$; $p<0.01$). Therefore, fungal species diversity was greater in juvenile than in adult leaves, and juvenile leaves seemed to be susceptible to infection not only by pathogens, as evidenced by the greater severity of MLD in young trees, but in general by more fungal species than adult leaves.

More species were isolated from diseased than from asymptomatic tissue subsamples, and this occurred in juvenile and in adult leaves (Figure 2B). However, the difference between the average number of species observed in diseased (2.98 spp.) and asymptomatic (2.56 spp.) subsamples of each tree was not statistically significant (Table 2). Overall, 74 species were isolated from diseased tissue and 72 from asymptomatic tissue.

In juvenile leaves 30 of the 48 species recorded were common to asymptomatic and diseased tissue. In adult leaves 16 of the 32 species recorded occurred in both types of tissue. In juvenile as well as in adult leaves, all members of the *Mycosphaerellaceae* and *Teratosphaeriaceae* were found either in both asymptomatic and diseased tissue, or only in diseased tissue (Table 3), not a single species of these families was identified only in asymptomatic tissue.

The statistically significant location effect detected in the ANOVA (Table 2) indicated the existence of differences among the 10 study locations with respect to the number of species found in them. A correlation analysis between the number of species found in juvenile and adult leaves at each location (Table 1, Figure 3) indicated that there is a direct relationship ($p<0.01$) between the number of species observed in juvenile and adult tissue at each location.

The similarities among the composition of the mycobiotas recovered from each type of leaf and tissue, and among their combinations were estimated using Jaccard's index of similarity (Table 4). The greatest similarities occurred between the mycobiota of diseased tissue from juvenile and adult leaves, and between the mycobiotas of diseased and asymptomatic tissue of juvenile leaves. The similarity among diseased tissue from both types of leaves might be due to the presence of several species that are pathogenic or secondary invaders of diseased tissue on both leaf types. Several species with these life cycles were identified among the 28 species common to adult and juvenile diseased tissue.

The similarity among the mycobiota of diseased or asymptomatic tissue of juvenile leaves might be due to the fact that juvenile leaves are more susceptible than adult leaves to a wide range of fungal species, and some of these species may be unable to infect adult leaves, leading to a

divergence in the mycobiota of adult and juvenile leaves. Of the 81 species detected in juvenile leaves, 48 were exclusively found in this leaf type. Several of the 31 species found in both asymptomatic and diseased tissue of juvenile leaves were potential *Eucalyptus* pathogens and host-generalist endophytes (Table 3).

The 70 species that could be assigned to a known taxon were classified into 26 families (Table 3). With the exception of the basidiomycetes *Bullera unica* and *Hypholoma fasciculare*, all other taxa belonged to the Ascomycota. The taxonomic families represented by more species were *Teratosphaeriaceae* (9 species), *Pleosporaceae* (7), *Mycosphaerellaceae* (6), and *Xylariaceae* (5). These four families included 38 % of the identified taxa.

In terms of species abundance, the most important taxa were *Hormonema* sp. (isolated from 49 samples), *Valsa fabianae* (35 samples), *Readeriella dimorphospora* (27), *Neofusicoccum eucalyptorum* (27), *Teratosphaeria molleriana* (19), *Mycosphaerella fori* (=*Pseudocercospora fori*) (19), *Alternaria* spp. (15), *Cladosporium* spp. (12), and *Penicillium* spp. (10).

Leaf litter mycobiota.

From the 5 leaf litter samples, 281 isolates were obtained, and a set of 149 were classified into 77 different species. Twenty seven species could be ascribed to a known taxon (Table 5), and the remaining 50 were classified as unknown fungi because they were vegetative mycelia and no close match was found in nucleotide databases. The most abundant taxa in leaf litter were *Phaeomoniella* spp. (14 isolates), *Cladosporium* spp. (6), Unknown species 5279 and 5281 (5 each), *Phlogicylindrium eucalyptorum* (5), *Ceratobasidium* sp. (4), and *Teratosphaeria molleriana* (4).

When the leaf litter mycobiota was compared to that from tree leaves, 10 species were common to both (Table 4), four of these common species were pathogens and lesion associated species (*Teratosphaeria molleriana*, *T. parva*, *T. nubilosa*, and *Mycosphaerella marksii*). The similarity between the mycobiota of living leaves and leaf litter was very low (Jaccard: 0.054) compared with the values observed among the mycobiota of living leaves, which averaged 0.287 (Table 4). This result indicates that in general the leaf litter mycobiota differed from that of living leaves, but nevertheless, some potential pathogens might have an alternative saprophytic habit and survive in litter.

Discussion

Our observations reaffirm that leaf disease in *Eucalyptus globulus* is widespread in Galicia, as observed by Otero *et al*. (2007), who found the disease in 95% of 157 Galician plantations that they studied. The fact that in our study significantly many more fungal species occurred in juvenile leaves than in adult ones indicates that in general juvenile leaves are more prone to infection by

fungi, pathogens as well as endophytes. The reduced incidence and severity of MLD in adult leaves does not seem to be the result of increased resistance to some particular pathogen species, adult leaves seem to be more resistant to infections by multiple fungal species. Therefore, a wide-spectrum defence mechanism present in adult leaves seems to be absent or less efficient in juvenile leaves.

Anatomical differences between juvenile and adult leaves might explain their differential susceptibility to fungal infections. Adult leaves of *E. globulus* and other species have thicker cuticles, and lower stomatal density than juvenile leaves (James and Bell, 2001; Gras *et al*., 2005). Comparative studies of juvenile leaves in susceptible and resistant genotypes of *E. globulus* have shown that anatomical characteristics such as palisade mesophyll density, cuticle thickness, and wax coverage of stomata, are associated to resistance (Smith, 2006; Smith *et al*., 2006). Juvenile leaves of resistant trees of *E. nitens* have thicker cuticles and a higher density of palisade cells than those of susceptible trees. Palisade cell density is related to the speed of formation of the necrophyllactic periderm, a barrier layer of lignified and suberized cells containing defensive chemicals around the point of fungal attack or injury (Smith *et al*., 2006, 2007). Necrophyllactic periderm develops from palisade cell differentiation, and the fact that juvenile *E. nitens* leaves have palisade mesophyll in both leaf surfaces, while *E. globulus* has a single adaxial layer appears to be related to a faster rate of periderm development and increased MLD resistance in *E. nitens* (Smith *et al*., 2007). Adult leaves of several *Eucalyptus* species have more layers of palisade mesophyll, thicker cuticles, and lower stomatal density than adult leaves (James and Bell, 2001, Gras et al. 2005). Therefore, some characters associated to MLD resistance in juvenile leaves of resistant genotypes are present in adult leaves. These anatomical differences with adult leaves could make juvenile leaves an easier substratum for penetration by fungi.

Other mechanisms of general resistance differing between adult and juvenile leaves like waxes or metabolites present inside leaves or in the cuticle surface could also limit the access of some fungal species to adult tissue (Li et al., 1997; Gras et al., 2005; Steinbauer *et al*., 2009).

When the similarities among the mycobiotas of all possible types of leaf and tissues were estimated, the greatest similarity occurred between diseased tissues of juvenile and adult leaves. This relatively high similarity (0.363) could be due to the presence of pathogens able to infect both types of tissue. As expected if this were true, many of the 28 species common to both types of diseased tissue were pathogens or lesion-associated taxa like *Neofusicoccum eucalyptorum, Teratosphaeria dimorpha, Mycosphaerella* spp., *M. fori, M. parva, Readeriella dimorphospora, R. eucalypti, R. callista, R. tasmanica*, *Sydowia eucalypti*, *Teratosphaeria molleriana, T. nubilosa,* and *Valsa fabianae* (Adams *et al*., 2005; Barber *et al*., 2008; Crous *et al*., 1995, 2006a, 2009a, 2009b,

2009c; Hunter *et al*., 2004, 2009; Park and Keane, 1982; Pérez *et al*., 2008; Slippers and Wingfield, 2007; Smith *et al*., 2001; Summerell *et al*., 2006). Other species found in both types of tissue were common endophytes occurring in diverse plant taxa, like *Alternaria* spp., *Cladosporium* spp., *Epicoccum* spp., *Penicillium* spp., *Pleospora herbarum,* and *Stemphyllium solani* (Sánchez Márquez *et al*., 2007).

The strong correlation between the number of species found between juvenile and adult leaves at each location, and the similarity between species found in healthy and diseased tissue in juvenile leaves indicate transfers of inoculum. In addition to the species found in both types of diseased leaves, other pathogens identified in this survey were *Alysidiella parasitica* (Summerell *et al*., 2006), *Botryosphaeria dothidea* (Smith *et al*., 1994), *Teratosphaeria africana, Mycosphaerella marksii, M. walkeri,* and *M. madeirae* (Crous 1998; Crous *et al*., 2009d). One conclusion based on the relatively large diversity and abundance of potentially pathogenic species found in this study is that the sanitary outlook for *Eucalyptus* globulus in this region is far from optimal.

In this study, many species that have been described in eucalypts from Australia, New Zealand, or South Africa were found in Spanish plantations. This suggests that a significant part of the mycobiota identified in this study is *Eucalyptus*-specific. The specificity of many components of the mycobiota is supported by the fact that the *Eucalyptus* mycobiota shows little similarity to that of the endophytes of grasses *Ammophila arenaria* and *Elymus farctus* (Table 5), surveyed in Galician populations contiguous to *Eucalyptus* plantations (Sánchez Marquez *et al*., 2008). Out of 10 taxa which are shared by *Eucalyptus* and the mycobiota of these two grasses, nine were host-generalist endophytes (*Alternaria* spp., *Arthrinum* spp., *Aureobasidium pullulans*, *Cladosporium* spp., *Cordyceps bassiana, Epicoccum* spp., *Penicillium* spp., *Stemphillium solani,* and *Torrubiella confragosa*). Interestingly, one isolate of *Valsa fabianae,* an *Eucalyptus* pathogen was obtained from a plant of *Elymus farctus*. Although *Eucalyptus* mycobiota has been thought to be family-specific, many species reported from hosts in the *Myrtaceae* have been isolated from hosts of other families (Crous *et al*., 2008, 2009d, Cheewangkoon *et al*., 2009).

A *Hormonema* species was the most abundant taxon found in *Eucalyptus* trees; it was found in all types of leaves and tissue types, and at nine of the 10 locations studied. Although other fungi with black yeast-like *Hormonema* synanamorph stages, e.g. *Sydowia eucalypti* (anamorph *Selenophoma eucalypti*) and *Selenophoma australiensis,* have been described from living *Eucalyptus* leaves (Cheewangkoon *et al.,* 2009), the strains isolated in this study were not conspecific with our isolate of *S. eucalypti,* a species reported from Portugal (Cheewangkoon *et al.,* 2009) (about 87% pairwise identity), nor did they appear closely related to *H. carpetanum,* a species common in central Spain (Bills *et al*. 2004).

The mycobiota observed in leaf litter suggests that its species composition differs significantly from that of living leaves. Leaf litter fungi might be more specialized for a saprobic habitat. It is remarkable that four of the ten fungal taxa common to living leaves and leaf litter were leaf pathogens (*Teratosphaeria nubilosa, T. molleriana, T. parva* and *Mycosphaerella marksii*). Therefore, these species might have some saprobic capability, and leaf litter may play a role in their life cycles as an inoculum reservoir.

In conclusion, this study shows that the species richness of fungal assemblages from juvenile leaves is greater than that of adult leaves. This indicates that juvenile leaves are more easily infected than adult leaves not just by pathogens, but by a wide range of fungal species. Therefore, the fact that MLD symptoms are more severe in juvenile than in adult trees might not be due to specific reactions between juvenile leaves and some particular pathogen species, but to less developed mechanisms of general defence against fungal invasion in juvenile than in adult leaves. Knowledge of differences in nonspecific structural (e.g. cuticle properties, stomatal density) or biochemical defence mechanisms (e.g. responses to pathogen associated molecular patterns, waxes and other epicuticular chemicals) between juvenile and adult leaves could be very useful to apply in processes of selection for more resistant tree genotypes. Further study of the interactions among endophytes and pathogens in *Eucalyptus* phylloplanes may reveal antagonistic interactions which might be applicable for disease control strategies in nursery trees (Arnold *et al*., 2003). The study also shows that although leaf litter mycobiota is different from that of living leaves, it contains some pathogenic species, and leaf litter could have a role as an inoculum reservoir. In addition, numerous fungal species, including pathogens, associated to *Eucalyptus* trees in other parts of the world are present in Spanish plantations.

References

Adams, G.C., Wingfield, M.J., Common, R., Roux, J. (2005) Phylogenetic relationships and morphology of *Cytospora* species and related teleomorphs (*Ascomycota*, *Diaporthales*, *Valsaceae*) from *Eucalyptus*. *Studies in Mycology* **52**, 1-147.

Andjic, V., Whyte, G., Hardy, G., Burgess, T. (2010) New *Teratosphaeria* species occurring on eucalypts in Australia. *Fungal Diversity* **43**, 27-38.

Arnold, A.E., Lutzoni, F. (2007) Diversity an host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? *Ecology* **88**, 541-549.

Arnold, A.E., Mejia, L.C., Kyllo, D., Rojas, E.I., Maynard, Z., Robbins, N., Herre, E.A. (2003) Fungal endophytes limit pathogen damage in a tropical tree. Proceedings of the National Academy of Sciences USA **26**, 15649-15654.

Barber, P.A., Carnegie, A.J., Burgess, T.I., Keane, P,J. (2008) Leaf diseases caused by *Mycosphaerella* species in *Eucalyptus globulus* plantations and nearby native forest in the green Triangle region of southern Australia. *Australasian Plant Pathology* **37**, 472-481.

Betucci, L., Alonso, R. (1997) A comparative study of fungal populations in healthy and symptomatic twigs of *Eucalyptus grandis* in Uruguay. *Mycological Research* **101**, 1060-1064.

Bills, G.F., Collado, J., Rubial, C., Peláez, F., Platas, G. (2004). *Hormonema carpetanum sp. nov*., a new lineage of dothideaceous black yeasts from Spain. *Studies in Mycology* **50**, 149–157.

Cabral, D. (1985). Phyllosphere of *Eucalyptus viminalis*: Dynamics of fungal populations *Transactions of the British Mycological Society* **85**, 501-511.

Carnegie, A.J. (2007) Forest health condition in New South Wales, Australia, 1996-2005. I. Fungi recorded from eucalypt plantations during forest health surveys. *Australasian Plant Pathology* **36**, 213-224.

Carnegie, A.J., Keane, P.J., Podger, F.D. (1997) The impact of three species of *Mycosphaerella* newly recorded on *Eucalyptus* in Western Australia. *Australasian Plant Pathology* **26**, 71-77.

Cheewangkoon, R., Groenewald, J.Z., Summerell, B.A., Hyde, K.D., To-anun, C., Crous P.W. (2009) Myrtaceae, a cache of fungal biodiversity. *Persoonia* **23**, 55-85.

Collado, J., Platas, G., Paulus, B., Bills, G.F. (2007) High throughput culturing of fungi from plant litter by a dilution to extinction technique. *FEMS Microbiology Ecology* **60**, 521-533

Crous, P.W. (1998) *Mycosphaerella* spp. and their anamorphs associated with leaf spot diseases of *Eucalyptus*. St. Paul, USA: APS Press.

Crous, P,W., Groenewald, J.Z., Mansilla, J.P., Hunter, G.C., Wingfield, M.J. (2004) Phylogenetic reassessment of *Mycosphaerella* spp. and their anamorphs occurring in *Eucalyptus*. *Studies in Mycology* **50**, 195-214.

Crous, P.W., Groenewald, J.Z., Summerell, B.A., Wingfield, B.D., Wingfield, M.J. (2009a) Co-occuring species of *Teratosphaeria* on *Eucalyptus*. *Persoonia* **22**, 38-48.

Crous, P.W., Lennox, C.L., Sutton, B.C. (1995) *Selenophoma eucalypti* and *Stigmina robbenensis* spp. nov. from *Eucalyptus* leaves on Robben Island. *Mycological Research* **99**,648-652.

Crous, P.W., Mohammed, C., Glen, M., Verkley G.J.M., Groenewald, J.Z. (2007a) *Eucalyptus* microfungi known from culture. 3. *Eucasphaeria* and *Sympoventuria* genera nova, and new species of *Furcaspora*, *Harknessia*, *Heteroconium* and *Phacidiella*. *Fungal Diversity* **25**, 19-36.

Crous, P.W., Slippers, B., Wingfield, M.J., Rheeder, J., Marassas W.F.O., Philips, A.J.L., Alves, A., Burgess, T., Barber, P., Groenewald, J.Z. (2006a) Phylogenetic lineages in the *Botryosphaeriaceae*. *Studies in Mycology* **55**, 235-253.

Crous, P.W., Summerell, B.A., Carnegie, A.J., Mohammed, C., Himaman, W., Groenewald, J.Z. (2007b) Foliicolous *Mycosphaerella* spp. and their anamorphs on *Corymbia* and *Eucalyptus*. *Fungal Diversity* **26**, 143-185.

Crous, P.W., Summerell, B.A., Mostert, L., Groenewald, J.Z. (2008) Host specificity and speciation of *Mycosphaerella* and *Teratosphaeria* species associated with leaf spots of *Proteaceae*. *Persoonia* ***20****, 59-86.*

Crous, P.W., Summerell, B.A., Carnegie, A .J., Wingfield, M.J., Groenewald, J.Z. (2009b) Novel species of *Mycosphaerellaceae* and *Teratosphaeriaceae*. *Persoonia* **23**, 99-118.

Crous, P.W., Summerell, B.A., Carnegie, A .J., Wingfield, M.J., Hunter, G.C., Burgess, T.I., Andjic, V., Barber, P.A., Groenewald, J.Z. (2009c) Unraveling *Mycosphaerella*: do you believe in genera? *Persoonia* **23**, 99-118.

Crous, P.W., Verkley, G.J.M., Groenewald, J.Z. (2006b) *Eucalyptus* microfungi known from culture. 1. *Cladoriella and Fulvoflamma* genera nova, with notes on some other poorly known taxa. *Studies in Mycology* **55**, 53-63.

Crous, P.W., Wingfield, M.J. (1997) *Colletogloeopsis*, a new coelomycete genus to accommodate anamorphs of two species of *Mycosphaerella* on *Eucalyptus*. *Canadian Journal of Botany* **75**, 667-674.

Crous, P.W., Wingfield, M.J., Groenewald, J.Z. (2009d). Niche sharing reflects a poorly understood biodiversity phenomenon. *Persoonia* **22**, 83-94.

Dirección General de la Conservación de la Naturaleza. (1998) Segundo inventario forestal nacional. Madrid: Ministerio de Medio Ambiente.

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

Gras, E.K., Read, J., Mach, C.T., Sanson G.D., Clissold, F.D. (2005) Herbivore damage, resource richness and putative defences in juvenile *versus* adult *Eucalyptus* leaves. *Australian Journal of Botany* **53**, 33-44.

Hunter, G.C., Crous, P.W., Carnegie, A.J., Wingfield, M.J. (2009) *Teratosphaeria nubilosa*, a serious leaf disease pathogen of *Eucalyptus* spp. in native and introduced areas. *Molecular Plant Pathology* **10**, 1-14.

Hunter, G.C., Roux, J., Wingfield, B.D., Crous, P.W., Wingfield, M.J. (2004). *Mycosphaerella* species causing leaf disease in South African *Eucalyptus* plantations. *Mycological Research* **108**, 672-681.

Jackson, S.L., Maxwell A., Burgess T.I., Hardy, G.E. Dell, B. (2008) Incidence and new records of *Mycosphaerella* species within a *Eucalyptus globulus* plantation in western Australia. *Forest Ecology and Management* **255**, 3931-3937.

Jackson, S.L., Maxwell, A., Dell, B., Hardy, G.E.S. (2005) New disease records of Mycosphaerella leaf disease from eucalypts in Western Australia.. *Australasian Plant Pathology* **34**, 423-424.

James, S.A., Bell, D.T. (2001) Leaf morphological and anatomical characteristics of heteroblastic *Eucalyptus globulus* ssp. *globulus* (*Myrtaceae*). *Australian Journal of Botany* **49**, 259-269.

Li, H., Madden, J.L., Potts, B.M. (1997) Variation in leaf waxes of the Tasmanian *Eucalyptus* species.I. Subgenus S*ymphyomyrtus*. *Biochemical Systematics and Ecology* **25**, 631-657.

Magurran, A.E. (2004) Measuring Biological Diversity. Oxford, UK: Blackwell Publishing.

Maxwell, A., Dell, B., Neumeister-Kemp, H., Hardy, G.E.S. (2003) *Mycosphaerella* species associated with Eucalyptus in southwestern Australia - new species, new records and a key. *Mycological Research* **107**, 351-359.

Otero, L., Aguín, O., Sainz, M.J., Mansilla, P. (2007) Incidencia y severidad de la enfermedad foliar causada por *Mycosphaerella spp*. en eucaliptales de Galicia. *Boletín del CIDEU* **4**, 3-8.

Park, R.F., Keane, P.J. (1982) Leaf diseases of *Eucalyptus* associated with *Mycosphaerella* species. *Transactions of the British Mycological Society* **79**,101-105.

Pearson, W.R. (1990) Rapid and Sentive Sequence Comparison with PASTP and FASTA. *Methods in Enzymology* **183**, 63-98.

Pérez, C.A., Altier, N., Simeto, S., Wingfied, M.J., Slippers, B., Blanchette, R.A. (2008) *Botryosphaeriacae* from *Eucalyptus* and native *Myrtaceae* in Uruguay. *Agrociencia* **12**,19-30.

Pinkard, E.A., Mohammed C.L. (2006) Photosynthesis of *Eucalyptus globulus* with Mycosphaerella leaf disease. *New Phytologist* **170**, 119-127.

Sánchez Márquez, S., Bills, G.F., Zabalgogeazcoa, I. (2007) The endophytic mycobiota of *Dactylis glomerata. Fungal Diversity* **27**, 171-195.

Sánchez Márquez, S., Bills, G.F., Zabalgogeazcoa, I. (2008) Diversity and structure of the fungal endophytic assemblages from two sympatric coastal grasses. *Fungal Diversity* **33**, 87-100.

Simeto, S, Alonso R, Tiscornia S, Betucci L. (2005) Fungal community of *Eucalyptus globulus* and *Eucalyptus maidenii* stems in Uruguay. *Sydowia* **57**, 246-258.

Slippers, B., Burgess, T., Pavlic, D., Ahumada, R., Maleme, H., Mohali, S., Rodas, C., Wingfield, M.J. (2009) A diverse assemblage of *Botryosphaeriaceae* infect *Eucalyptus* in native and non-native environments. *Southern Forests* **71**, 101-110

Slippers, B., Wingfield, M.J. (2007) *Botryosphaeriaceae* as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biology Reviews* **21**, 90–106.

Smith, A.H. (2006) The development of strategies for the management and research of foliar pathogens on eucalypt plantations: using *Mycosphaerella* as a case study. University of Tasmania, Ph.D. Thesis.

Smith, H., Crous, P.W., Wingfield, M.J., Coutinho, T..A., Wingfield, B.D. (2001). *Botryosphaeria eucalyptorum* sp. nov., a new species in the *B. dothidea*-complex on *Eucalyptus* in South Africa. *Mycologia* **93**, 277–285.

Smith, A.H., Gill, W.M., Pinkard, E.A., Mohammed C.L. (2007) Anatomical and histochemical defence responses induced in juvenile leaves of *Eucalyptus globulus* and *Eucalyptus nitens* by *Mycosphaerella* infection. Forest Pathology **37**, 361-373.

Smith, H., Kemp, G.H.J., Wingfield, M.J. (1994) Canker and die-back of *Eucalyptus* in South Africa caused by *Botryosphaeria dothidea*. *Plant Pathology* **43**, 1031-1034.

Smith, A.H., Pinkard, E.A., Hunter, G.C., Wingfield M.J., Mohammed C.L. (2006) Anatomical variation and defence responses of juvenile *Eucalyptus nitens* leaves to Mycosphaerella leaf disease. Australasian Plant Pathology **35**, 725-731.

Steinbauer, M.J. (2002) Oviposition preference and neonate performance of *Mnesampela privata* in relation to heterophylly in *Eucalyptus dunnii* and *E. globulus*. *Agricultural and Forest Entomology* **4**, 245-253.

Steinbauer, M.J., Davies, N.W., Gaertner, C., Derridj, S. (2009) Epicuticular waxes and plant primary metabolites on the surfaces of juvenile *Eucalyptus globulus* and *E. nitens* leaves. *Australian Journal of Botany* **57**, 474-485.

Summerell, B.A., Groenewald, J.Z., Carnegie, A., Summerbell, R.C., Crous, P.W. (2006) *Eucalyptus* microfungi known from culture. 2. *Alysidiella*, *Fusculina* and *Phlogicylindrium* genera nova, with notes on some other poorly known taxa. *Fungal Diversity* **23**, 323-350.

Zabalgogeazcoa, I. (2008). Fungal endophytes and their interactions with plant pathogens. *Spanish Journal of Agricultural Research* **6**, 138-146.

Table 1. Locations of the tree plantations studied, number and type of trees sampled at each site, and number of fungal species identified in trees of each type at each location.

Location	Number of trees sampled		Number of species identified		
	juvenile	adult	juvenile	adult	total
1. As Pontes	5	5	17	15	24
2. Barqueira	5	5	26	17	32
3. Chimparra	3	3	10	6	11
4. Confurco	2	2	22	11	26
5. Corredeira	3	3	4	5	8
6. Espiñaredo	5	5	23	16	32
7. Infesta	5	5	27	20	40
8. San Antonio do Corbeiro	2	2	12	9	20
9. Vilaboa	2	2	18	11	23
10. Vale da Quebrada	5	5	26	15	37
TOTAL	**37**	**37**	**81**	**65**	**113**

Table 2. F values of the ANOVA of the mean number of species found in diseased or asymptomatic tissue samples obtained from juvenile or adult leaves.

Source of variation	df	F
Location (Lo)	9	7.73**
Leaf type (Le)	1	23.79**
Tissue type (Ti)	1	2.82 ^{NS}
Lo x Le	9	1.45 ^{NS}
Lo x Ti	9	1.67 ^{NS}
Le x Ti	1	0.16 ^{NS}
Lo x Le x Ti	9	0.46 ^{NS}
Error	108	

**: significant at P<0.05

NS: not significant

Table 3. Fungal species identified in asymptomatic (As) or diseased (Dis) tissue samples of juvenile and adult leaves of *Eucalyptus globulus* and their abundance in terms of the number of samples positive for the presence of a given taxon. The identifications were based in the percentage of similarity of each isolate to its closest match at the EMBL nucleotide database. One sample of asymptomatic tissue and another of diseased tissue were obtained from each tree, 148 samples in total.

Sequence accession	Sequence-based Identification	Family	% similarity	Number of isolates				Total
				Juvenile		Adult		
				As	Ds	As	Ds	
	In both leaf and tissue types							
FR667958	*Cladosporium* spp.	*Davidiellaceae*	100.0	4	5	1	2	12
FR667959	*Epicoccum* spp.	*Pleosporaceae*	100.0	4	6	1	3	14
FR668023	*Hormonema* spp.	*Dothioraceae*	98.7	6	16	14	13	49
FR667960	*Mycosphaerella fori*	*Mycosphaerellaceae*	99.1	5	8	4	2	19
FR667961	*Mycosphaerella* spp.	*Mycosphaerellaceae*	98.7	13	13	7	6	39
FR667956	*Neofusicoccum eucalyptorum*	*Botryosphaeriaceae*	100.0	8	5	7	7	27
FR667962	*Penicillium* spp.	*Trichocomaceae*	99.8	4	3	1	2	10
FR667957	*Readeriella dimorphospora*	*Teratosphaeriaceae*	99.2	1	9	4	13	27
FR667963	*Stemphylium solani*	*Pleosporaceae*	100.0	5	1	1	1	8
FR667978	*Sydowia eucalypti*	*Dothioraceae*	99.5	1	5	3	2	11
FR667964	*Teratosphaeria molleriana*	*Teratosphaeriaceae*	99.8	10	2	3	4	19
FR667965	*Valsa fabianae*	*Valsaceae*	100.0	3	12	5	15	35
-	Unknown fungus 4429		84.1	5	4	18	5	32
-	Unknown fungus 4840		87.5	1	3	1	2	7
	In both leaf types							
FR667966	*Alternaria* spp.	*Pleosporaceae*	99.5	8	6	0	1	15
FR667968	*Cladosporium herbarum*	*Davidiellaceae*	100.0	4	2	0	2	8
FR667969	*Glomerella cingulata*	*Glomerellaceae*	100.0	2	0	1	0	3
FR667970	*Lanzia griseliniae*	*Rutstroemiaceae*	97.3	0	1	0	1	2
FR667971	*Nemania difusa*	*Xylariaceae*	99.6	1	0	1	0	2
FR667972	*Phialemonium curvatum*	*Cephalothecaceae*	99.8	1	0	0	3	4
FR667973	*Phaeomoniella* spp.	*Herpotrichiellaceae*	97.1	0	4	2	2	8
FR667974	*Pleospora herbarum*	*Pleosporaceae*	100.0	0	2	0	1	3
FR667975	*Readeriella callista*	*Teratosphaeriaceae*	100.0	1	3	0	1	5
FR667976	*Readeriella eucalypti*	*Teratosphaeriaceae*	100.0	0	1	0	1	2
FR667977	*Readeriella tasmanica*	*Teratosphaeriaceae*	100.0	0	1	0	1	2
FR667967	*Teratosphaeria dimorpha*	*Teratosphaeriaceae*	98.8	2	3	0	1	6
FR667979	*Teratosphaeria nubilosa*	*Teratosphaeriaceae*	100.0	6	2	0	1	9
FR667980	*Teratosphaeria parva*	*Teratosphaeriaceae*	99.4	0	3	0	1	4
FR667981	*Torrendiella* spp.	*Sclerotiniaceae*	96.1	3	4	1	0	8
FR667982	*Torrubiella confragosa*	*Cordycipitaceae*	99.8	1	0	2	0	3
FR667983	*Xylaria* spp.	*Xylariaceae*	97.8	0	1	1	3	5
FR667984	*Sordariomycete* sp.	incertae sedis	100.0	1	1	1	0	3
-	Unknown fungus 4640		79.2	0	2	0	2	4
	Only in juvenile leaves							
FR667985	*Alysidiella parasitica*	incertae sedis	99.1	2	0	0	0	2
FR667986	*Arthrinium* sp.	*Apiosporaceae*	95.2	2	1	0	0	3
-	*Aspergillus tubingensis*	*Trichocomaceae*	n.s.*	1	0	0	0	1
FR667987	*Aspergillus versicolor*	*Trichocomaceae*	99.8	0	1	0	0	1
FR667988	*Aureobasidium pullulans*	*Dothioraceae*	100.0	0	3	0	0	3
FR667989	*Botryosphaeria dothidea*	*Botryosphaeriaceae*	100.0	0	1	0	0	1
FR667990	*Botryotinia fuckeliana*	*Sclerotiniaceae*	99.8	0	1	0	0	1
FR667991	*Bullera unica*	*Tremellaceae*	99.6	1	0	0	0	1
FR667992	*Colletotrichum*	*Glomerellaceae*	100	1	2	0	0	3

	gloeosporioides							
FR667993	*Coniothyrium fuckelii*	*Leptosphaeriaceae*	99.3	0	1	0	0	1
	Cryptosporiopsis	*Dermataceae*	97.8					
FR667994	*melanigena*			1	0	0	0	1
FR667995	*Diaporthe viticola*	*Diaporthaceae*	100	2	2	0	0	4
FR667996	*Dicyma pulvinata*	*Xylariaceae*	100	0	2	0	0	2
FR667997	*Gnomonia ischnostyla*	*Gnomoniaceae*	99.8	0	1	0	0	1
FR667998	*Guignardia mangiferae*	*Botryosphaeriaceae*	99.6	1	0	0	0	1
FR667999	*Harknessia capensis*	incertae sedis	99.1	1	1	0	0	2
FR668000	*Hypholoma fasciculare*	*Strophariaceae*	98.1	1	0	0	0	1
FR668001	*Hypoxylon howeanum*	*Xylariaceae*	100	2	1	0	0	3
	Annulohypoxylon	*Xylariaceae*	98.5					
FR668002	*multiforme*			1	0	0	0	1
FR668003	*Leotiomycete* sp.	*Herpotrichiellaceae*	98.9	0	1	0	0	1
FR668004	*Massarina corticola*	*Massarinaceae*	100	0	1	0	0	1
FR668005	*Mollisia cinerea*	*Dermataceae*	98.6	0	2	0	0	2
FR668006	*Mycosphaerella madeirae*	*Mycosphaerellaceae*	100	1	1	0	0	2
FR668007	*Mycosphaerella marksii*	*Mycosphaerellaceae*	100	0	1	0	0	1
	Mycosphaerella	*Mycosphaerellaceae*	99.8					
FR668008	*punctiformis*			3	1	0	0	4
FR668009	*Mycosphaerella walkeri*	*Mycosphaerellaceae*	100	1	1	0	0	2
	Pestalotiopsis	*Amphisphaeriaceae*	99.6					
FR668010	*maculiformans*			0	1	0	0	1
FR668011	*Pestalotiopsis uvicola*	*Amphisphaeriaceae*	100	0	1	0	0	1
FR668012	*Phoma macrostoma*	*Pleosporaceae*	99.6	0	1	0	0	1
FR668013	*Pleospora* sp.	*Pleosporaceae*	99.7	2	0	0	0	2
FR668014	*Teratosphaeria africana*	*Teratosphaeriaceae*	99.8	4	1	0	0	5
FR668015	*Torrendiella eucalypti*	*Sclerotiniaceae*	97.7	1	0	0	0	1
-	16 unknown species**			8	8	0	0	16
	Only in adult leaves							
-	*Cordyceps bassiana*	*Cordycipitaceae*	n.s.*	0	0	1	0	1
FR668017	*Cordyceps memorabilis*	*Cordycipitaceae*	99.8	0	0	1	0	1
FR668018	*Cytospora sp.*	*Valsaceae*	95.9	0	0	0	2	2
FR668019	*Leptosphaerulina chartarum*	*Pleosporaceae*	99.8	0	0	1	0	1
FR668016	*Nigrospora* sp.	incertae sedis	99.6	0	0	0	1	1
FR668020	*Paraconiothyrium variabile*	*Montagnulaceae*	99.6	0	0	1	0	1
FR668021	*Pezicula* sp.	*Dermataceae*	100	0	0	1	0	1
FR668022	*Preussia* sp.	*Sporormiaceae*	98.7	0	0	2	0	2
-	Unknown fungus 4107		79.6	0	0	2	0	2
-	23 unknown species**			0	0	14	9	23

Notes: * n.s.: not sequenced; **: one isolate of each species

Table 4. Jaccard's indexes of similarity calculated for pairwise comparisons of the mycobiota identified in each type of tissue. Additionally, the *Eucalyptus* leaf mycobiota was compared to those of *Ammophila arenaria* and *Elymus farctus*, two coastal grasses from populations adjacent to *Eucalyptus* plantations (Sánchez Márquez *et al*., 2008).

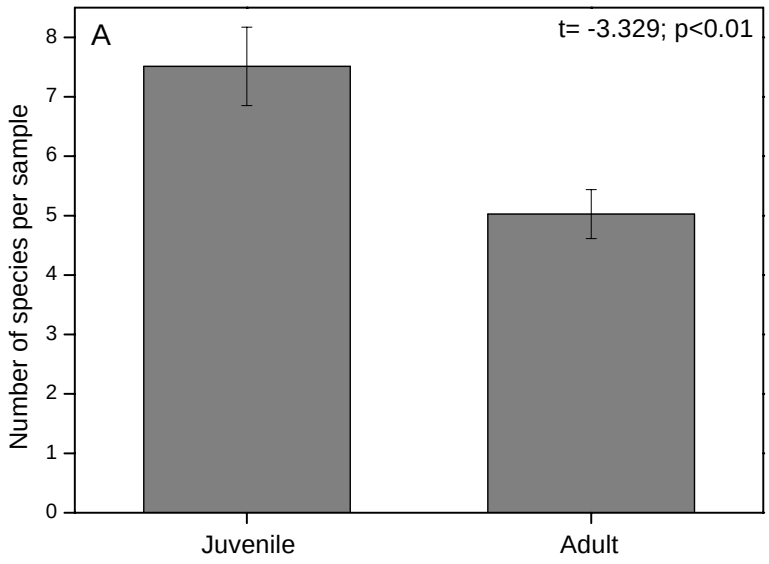
Mycobiotas compared		**Number of species on each mycobiota**		**Number of shared species**	**Similarity index**
1	**2**	**1**	**2**		
Juvenile leaves	Adult leaves	83	66	34	0.298
Asymptomatic tissue	Diseased tissue	75	73	32	0.278
Juvenile asymptomatic	Juvenile diseased	52	64	28	0.329
Adult asymptomatic	Adult diseased	41	41	17	0.265
Juvenile asymptomatic	Adult asymptomatic	52	41	20	0.271
Juvenile diseased	Adult diseased	64	41	28	0.363
Juvenile asymptomatic	Adult diseased	52	41	20	0.285
Juvenile diseased	Adult asymptomatic	64	41	18	0.209
Leaf total	Leaf litter	115	77	10	0.054
Leaf total	*Ammophila arenaria*	115	75	10	0.055
Leaf total	*Elymus farctus*	115	54	9	0.055
Ammophila arenaria	*Elymus farctus*	75	54	27	0.264

Table 5. Species and number of isolates identified in leaf litter samples

Species	Family	Number of isolates
Bionectria ochroleuca	*Nectriaceae*	1
Ceratobasidium sp.	*Ceratobasidiaceae*	4
Cladosporium spp.	*Davidiellaceae*	6
Epicoccum nigrum	*Pleosporaceae*	1
Fusicladium amoenum	*Venturiaceae*	1
Hormonema sp.	*Dothioraceae*	1
Lachnum virgineum	*Hyaloscyphaceae*	1
Lachnum sp.	*Hyaloscyphaceae*	2
Leptosphaerulina chartarum	incertae sedis	1
Mollisia fusca	*Dermateaceae*	1
Mycosphaerella fori	*Mycosphaerellaceae*	2
Mycosphaerella marksii	*Mycosphaerellaceae*	1
Nectria mariannaeae	*Nectriaceae*	3
Paraconiothyrium variabile	*Montagnulaceae*	1
Penicillium spp.	*Trichocomaceae*	2
Phaeomoniella spp.	*Herpotrichiellaceae*	14
Phlogicylindrium eucalyptorum	*Amphisphaeriaceae*	5
Pilidium acerinum	*incertae sedis*	1
Plectosphaerella cucumerina	*Plectosphaerellaceae*	1
Stilbella sp.	*incertae sedis*	1
Sympoventuria capensis	*Venturiaceae*	2
Teratosphaeria molleriana	*Teratosphaeriaceae*	4
Teratosphaeria nubilosa	*Teratosphaeriaceae*	1
Teratosphaeria parva	*Teratosphaeriaceae*	1
Trichoderma brevicompactum	*Hypocreaceae*	1
Trichoderma taxi	*Hypocreaceae*	1
Umbelopsis sp.	*Umbelopsidaceae*	1
50 Unknown species		87

Figure 1. Mycosphaerella leaf disease in juvenile (left) and adult (right) leaves of *Eucalyptus globulus*. Usually lesions are larger in juvenile than in adult leaves.

Figure 2. **A**. Average number of species (± standard error) found in leaf samples from juvenile and adult trees. The number of species found in diseased and asymptomatic tissue of 37 juvenile and 37 adult trees was compared. **B**. Average number of fungal species found in subsamples of asymptomatic (a) or diseased (d) tissue obtained from leaves of juvenile (j) or adult (a) trees.



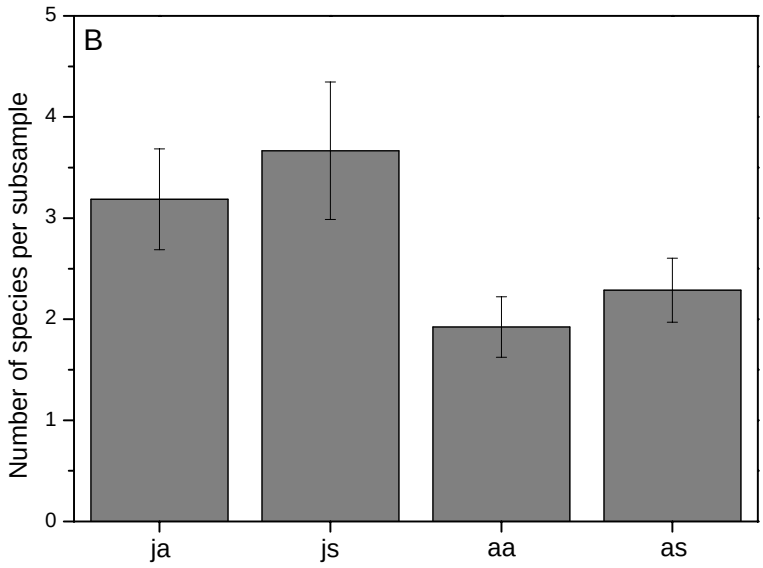


Figure 3. Relationship between the number of fungal species found in adult and juvenile trees at each location.