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Pathogenicity of seven species of the Botryosphaeriaceae on *Eucalyptus* clones in Venezuela

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Abstract. The Botryosphaeriaceae include several well recognised *Eucalyptus* pathogens of which various species have recently been found on *Eucalyptus* spp. in Venezuela. An initial inoculation trial was conducted using seven species (*Botryosphaeria mamane*, *B. dothidea*, *Lasiodiplodia theobromae*, *Neofusicoccum andinum*, *N. parvum*, *N. ribis* and *Pseudofusicoccum stromaticum*) on one commercially propagated clone representing a *Eucalyptus urophylla* × *E. grandis* hybrid in Venezuela. Stems were inoculated and lesion development recorded after 7 weeks. Inoculations with *B. mamane*, *B. dothidea*, *L. theobromae*, *N. andinum* and *P. stromaticum* showed little effect, but *N. parvum* and *N. ribis* caused bark swelling around the inoculation points with kino exudation. A second inoculation trial was performed on four commercial clones to evaluate variation in their tolerance to infection by *N. ribis* and *N. parvum*, which were the most pathogenic in the first trial. The clones differed significantly in their tolerance to infection by *N. parvum* and *N. ribis*, and *N. parvum* was significantly more virulent than *N. ribis* on all clones. These results illustrate the potential of using *Eucalyptus* clones to manage canker disease of *Eucalyptus* caused by species of Botryosphaeriaceae.

Additional keyword: clonal plantation forestry.

Introduction

The Botryosphaeriaceae is a cosmopolitan family of fungi, which have an exceptionally wide host range, particularly on woody plants (von Arx 1987). Many of these fungi are regarded as weak pathogens that infect stressed or wounded plants after drought, hail, wind, frost damage or insect infestation (Schoeneweiss 1984; Old 2000; Old and Davison 2000; Slippers and Wingfield 2007). Some Botryosphaeriaceae also exist as endophytes in asymptomatic tissue of hosts, including *Eucalyptus*, as latent pathogens, only expressing disease symptoms when the host defence mechanisms are reduced due to stress (Fisher *et al*. 1993; Smith *et al*. 1994, 1996; Burgess *et al*. 2005).

Infection by certain Botryosphaeriaceae has been reported to occur through wounds (von Arx and Müller 1954; Ciesla *et al*. 1996; Old 2000; Old and Davison 2000). On healthy plants, infection can also occur directly through lenticels, stomata or other openings, without causing apparent damage or disease symptoms (Smith *et al*. 1996). Species of Botryosphaeriaceae are, however, treated as opportunistic pathogens, because the diseases they cause are almost always associated with stress or wounding. Despite their pathogenic abilities, Botryosphaeriaceae are also well known as endophytes of aerial parts of woody plants, apparently existing for long periods of time in the absence of symptoms (Slippers and Wingfield 2007).

Eucalyptus species, most of which are native to Australia, represent one of the most widely planted forest species, worldwide (Poynton 1979; Turnbull 2000). Various Botryosphaeriaceae have been reported from *Eucalyptus* spp. in native environments and where they are grown as non-natives in plantations (Sankaran *et al*. 1995; Slippers *et al*. 2009), where they are associated with a wide range of disease symptoms. Taxonomic studies during the past decade have, however, revealed that identifications in many earlier reports are likely to be wrong. *Botryosphaeria dothidea* and *Neofusicoccum ribis* (=*B. ribis*) were, for example, thought to be common on *Eucalyptus*, but are now known to be generally rare on these hosts (Slippers *et al*. 2004*a*, 2004*b*). Earlier reports of these species on *Eucalyptus* is likely to represent other common species such as *Neofusicoccum parvum*, *N. australe* and *N. eucalyptorum* that were not known at the time (Slippers *et al*. 2004*a*, 2004*b*; Barber *et al*. 2005; Burgess *et al*. 2005). Slippers *et al*. (2009) lists at least 19 species of the Botryosphaeriaceae that have been confirmed from different environments using a modern taxonomic framework, backed up by molecular data. Early reports of Botryosphaeriaceae in South America should probably also be reevaluated; i.e. stick rot and canker caused by *N. ribis* reported on *E. grandis* and *E. citriodora* in Brazil (De Arruda Silveira *et al*. 2001), cankers on stems and branches

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and root disease on a *Eucalyptus* sp. caused by *N. ribis* in Argentina (Frezzi 1952) and *N. eucalypti* Sousa da Câmara causing twig lesions on *E. grandis* in Uruguay (Bettucci and Alonso 1997; Bettucci *et al*. 1999).

The recent description of several new genera in the Botryosphaeriaceae (Crous *et al*. 2006) impacts heavily on the taxonomy of fungi on *Eucalyptus*. Crous *et al*. (2006) used a larger sample and sequence dataset than most previous studies and distinguished 10 lineages within the Botryosphaeriaceae. Many of these were recognised as genera, corresponding largely to anamorph conidial characters. Species of four of these genera occur on *Eucalyptus*, namely *Botryosphaeria* (anamorph *Fusicoccum*), *Neofusicoccum* and *Pseudofusicoccum* (both formerly in *Fusicoccum*), and *Lasiodiplodia*. Of these, *Neofusicoccum* is the most common and diverse genus on *Eucalyptus* in most areas of the host distribution, while *Lasiodiplodia* tends to be the dominant genus in tropical environments.

Despite the importance of the Botryosphaeriaceae as canker pathogens on commercially grown eucalypts, the variation in susceptibility among clones has not been widely explored as a means to manage disease problems. Recent studies have led to the identification of seven species of the Botryosphaeriaceae from *Eucalyptus* in Venezuela (Mohali *et al*. 2006, 2007). The aim of this study was to test the pathogenicity of these species in field inoculations on a commercially exploited *Eucalyptus* clone. A second aim was to determine whether a suite of commercially propagated clones differ in their susceptibility to infection by the most pathogenic Botryosphaeriaceae occurring on *Eucalyptus* in Venezuela.

Methods

Fungal isolates

Isolates of the Botryosphaeriaceae used in this study (Table 1) were collected in previous studies (Mohali *et al*. 2006, 2007). These isolates originated from stems and branches of *Eucalyptus urophylla* × *E. grandis* hybrids and *Acacia mangium*, growing in plantations in Acarigua, Portuguesa State, and from *Eucalyptus* sp. growing in the Cordillera Los Andes in Mérida State, Venezuela (Table 1). The Botryosphaeriaceae were collected from asymptomatic plant tissue, as well as from trees exhibiting discolouration of the wood or dieback, and from entirely dead trees.

Isolates were grown on 2% malt extract agar (MEA; DIFCO, Detroit, MI) at 25°C and stored on this medium at 4°C. All isolates used in this study are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa.

Pathogenicity tests

Inoculation experiments were conducted in January 2004. Inoculations were made on 2-year-old trees of ~7 m in height and 25–35 cm in diameter, in plantations of *E. urophylla* × *E. grandis* hybrids in Acarigua, Portuguesa state. For each tree, a piece of bark was removed with a cork borer (1.5 cm diameter) to expose the cambium. Bark discs were replaced by agar discs of the same size bearing the test species of Botryosphaeriaceae. The wounds were covered with the original bark discs and sealed with masking tape to prevent desiccation. Lesion lengths that developed in the cambium were measured 7 weeks after inoculation.

Seven species of the Botryosphaeriaceae were used in the trial, six of which had been isolated from trees in the same area where the study was conducted. Two isolates of each of the seven Botryosphaeriaceae were used in the inoculation trial (Table 1).

The test fungi were grown on 2% MEA in Petri dishes at 25°C for 14 days before inoculation. A total of 280 trees (20 trees per isolate/40 per taxon) were inoculated onto the stems of a single *E. urophylla* × *E. grandis* hybrid clone (256). Twenty trees of the same clone were inoculated with sterile MEA plugs to serve as controls.

A second inoculation trial was conducted using only a single isolate each of *N. ribis* (CMW13409) and *N. parvum*

Table1. Isolates of different Botryosphaeriaceae from Venezuela used in the inoculation trials
All isolates were collected by S. Mohali in 2003

Isolate No.[A]	Species	Host	Origin
CMW13355	*Neofusicoccum parvum*	*Eucalyptus urophylla* × *E. grandis*	Acarigua, Portuguesa state
CMW13419	*N. parvum*	*E. urophylla* × *E. grandis*	Acarigua, Portuguesa state
CMW13362	*N. ribis*	*E. urophylla* × *E. grandis*	Acarigua, Portuguesa state
CMW13409	*N. ribis*	*E. urophylla* × *E. grandis*	Acarigua, Portuguesa state
CMW13373	*Botryosphaeria dothidea*	*E. urophylla* × *E. grandis*	Acarigua, Portuguesa state
CMW13381	*B. dothidea*	*E. urophylla* × *E. grandis*	Acarigua, Portuguesa state
CMW13370	*B. mamane*	*E. urophylla* × *E. grandis*	Acarigua, Portuguesa state
CMW13397	*B. mamane*	*E. urophylla* × *E. grandis*	Acarigua, Portuguesa state
CMW13487	*Lasiodiplodia theobromae*	*E. urophylla* × *E. grandis*	Acarigua, Portuguesa state
CMW13488	*L. theobromae*	*E. urophylla* × *E. grandis*	Acarigua, Portuguesa state
CMW13366	*Pseudofusicoccum stromaticum*	*E. urophylla* × *E. grandis*	Acarigua, Portuguesa state
CMW13426	*P. stromaticum*	*Acacia mangium*	Acarigua, Portuguesa state
CMW13444	*Neofusicoccum andinum*	*Eucalyptus* sp.	Mérida, Mérida state
CMW13455	*N. andinum*	*Eucalyptus* sp.	Mérida, Mérida state

[A]Isolates come from the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa.

(CMW13355), which were the most pathogenic species in the trial used to assess pathogenicity of the different species of the Botryosphaeriaceae. This inoculation was made on four commercially propagated clones (113, 138, 213, 276) representing *E. urophylla* × *grandis* hybrid*s*. An equal number of trees (20 trees per isolate) were inoculated with sterile MEA plugs to serve as controls. Inoculations were made using the identical procedures to those used in the first inoculation trial. The lengths of the lesions were measured 7 weeks after inoculation with the fungi.

Statistical analyses

ANOVA were computed using the Statistical Package for the Social Sciences or 'SPSS' computer program (Nei *et al*. 2005) for the lesion length in every treatment, and using Tukey's procedure for the comparison of means ($P = 0.05$).

Results

Inoculation of a single E. urophylla × E. grandis *clone with seven species of Botryosphaeriaceae*

There were significant differences ($P > 0.0001$, d.f. = 6, $F = 57, 84$) in the lesion lengths induced by the different species, as well, between isolates by species of the Botryosphaeriaceae inoculated onto clone 256 (Fig. 1). It was thus possible to separate the isolates into three groups according to their level of virulence (a, b, c; $P = 0.05$; Fig. 1). The control inoculations with MEA plugs produced no lesions in the outer bark and the wounds had begun to heal and produce callus after 7 weeks (Fig. 2*a*).

Isolates of *Botryosphaeria mamane* and *Lasiodiplodia theobromae* did not produce lesions and were not statistically different from the controls (Fig. 1). *B. dothidea* produced very small lesions that also did not differ significantly from the control (Fig. 1). Sites of inoculation with these three Botryosphaeriaceae had started to produce callus tissue around the wounds by the time the trial was terminated.

Neofusicoccum andinum and *Pseudofusicoccum stromaticum* produced small lesions, but those were significantly larger than those for *B. mamane* and *L. theobromae* (Fig. 1). The lesion length between isolates of *N. andinum* and *P. stromaticum* differed significantly for both species. The points of inoculation with *N. andinum* and *P. stromaticum* had started to heal and produce callus by the end of the trial.

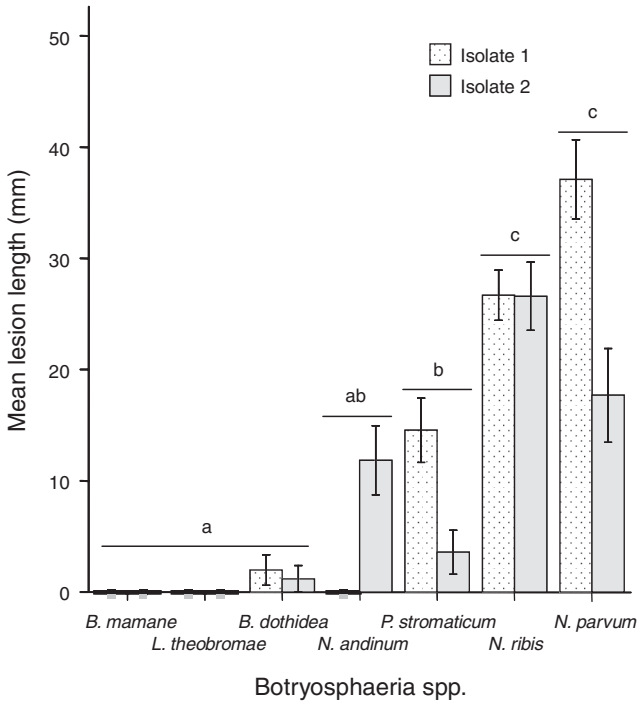

Fig. 1. Mean lesion length values (mm) of different Botryosphaeriaceae inoculated on *Eucalyptus urophylla* × *E. grandis* hybrid (clone 256) in Venezuela. Lesions were measured 7 weeks after inoculation. Isolates with the same letter above the bar do not differ significantly ($P = 0.05$).

N. ribis and *N. parvum* produced significantly larger lesions than all the other Botryosphaeriaceae included in this study (Figs 1 and 2*b*). Both species produced bark swelling around the inoculation points and in some cases the bark was cracked. Black kino exudation (Fig. 2*c*, white arrow) was observed when the outer bark was removed from the points of inoculation.

Inoculation of four E. urophylla × E. grandis *clones with* N. ribis *and* N. parvum

In the second inoculation trial, on four commercially used clones, only isolates of *N. ribis* and *N. parvum* were used. Bark swelling and exudation of black kino exudation typified the inoculation points on all of the clones treated with these fungi. In contrast, the

Fig. 2. Lesion development during inoculation trials. (*a*) Control inoculations on *Eucalyptus* hybrid stem with malt extract agar plugs produced no lesions. (*b*) Lesion produced by *Neofusicoccum parvum*. (*c*) Presence of black kino was observed when the outer bark was removed (white arrow) after inoculation with *N. parvum*.

control inoculations with sterile MEA plugs showed no lesion development.

Significant differences in lesion size were observed for lesions on the different clones ($P > 0.0001$, d.f. = 3, $F = 4.18$) after inoculation with *N. ribis* and *N. parvum*. Clone 213 was the most tolerant to infection by either fungus with an average lesion length of 31 mm. Clones 113 and 276 had average lesions length of 44 mm, and clone 138 was the most susceptible with an average lesion length of 53 mm (Fig. 3).

Significant differences in lesion size were also produced by *N. ribis* and *N. parvum* on the different clones. *N. parvum* was more pathogenic than *N. ribis* on clones 113, 138 and 213, but on clone 276 *N. ribis* was more pathogenic (Fig. 3). On average across the clones, *N. parvum* was the most pathogenic (Fig. 3).

Discussion

This study considered the relative pathogenicity of different species of Botryosphaeriaceae to *Eucalyptus* in Venezuela and then assessed the relative susceptibility of a suite of different clones to the most pathogenic species. Only two of the Botryosphaeriaceae were significantly pathogenic and it was also shown that *Eucalyptus* clones differ in their susceptibility to these fungi. This presents a potential opportunity to select clones for deployment in Venezuelan plantations, free from damage caused by Botryosphaeriaceae.

B. mamane did not produce lesions on stems of inoculated eucalypts. Before its recent discovery in Venezuela, this unusual species was known only from Hawaii where it has been associated with witches' broom on the native leguminous forest tree, *Sophora chrysophylla* (Gardner 1997). In Venezuela, *B. mamane* was isolated as an endophyte from asymptomatic *Eucalyptus* tissue and there were no signs of symptoms reported from Hawaii (Mohali *et al*. 2007). This is clearly a curious fungus of unknown origin and at least in terms of *Eucalyptus* plantation forestry, it appears to be unimportant.

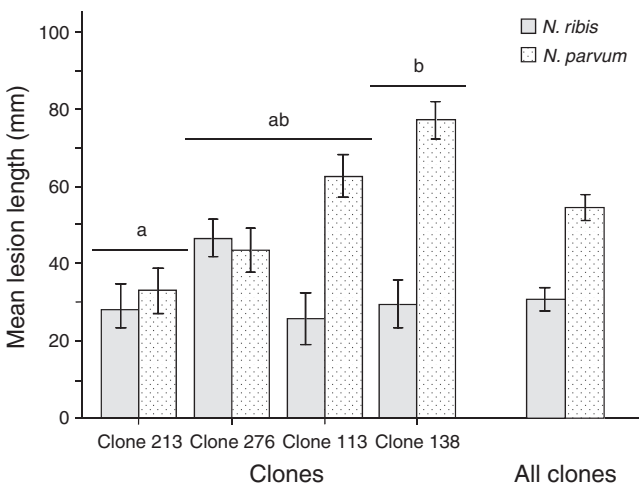

Fig. 3. Relative susceptibility (mean lesion length in mm) of four commercial clones of *Eucalyptus* to inoculation with *Neofusicoccum parvum* and *N. ribis*. Clones with the same letter above the bar do not differ significantly ($P = 0.05$).

An intriguing result of this study was that *L. theobromae* was not pathogenic. This result was unexpected as the fungus is known to be pathogenic on and associated with many diseases of trees (Sharma *et al*. 1984; Roux *et al*. 2000, 2001). In Venezuela, *L. theobromae* has been associated with the death of *Pinus* and *Eucalyptus* trees, but only when the plants were severely stressed by drought or other factors (Mohali 1993; Mohali *et al*. 2002, 2007). The isolates of *L. theobromae* used in the present study were obtained from both asymptomatic and symptomatic (blue stain) tissue and their lack of pathogenicity deserves further investigation.

B. dothidea produced only small lesions on the inoculated *Eucalyptus* stems. This species has been associated with mortality of young transplants, dieback and cancer diseases on *Eucalyptus* in different parts of the world (Webb 1983; Barnard *et al*. 1987; Smith *et al*. 1994). However, these studies were undertaken before the taxonomic revisions of *Botryosphaeria* spp. based on DNA sequence data. References to the fungus in previous studies could thus have been references to species other than *B. dothidea* and it is impossible to make logical comparisons (Slippers *et al*. 2004*a*). In this study, isolates were collected from asymptomatic plant tissue, as well as from trees exhibiting blue stain or dieback, and from entirely dead trees that were stressed due to severe drought (Mohali *et al*. 2007). As with *L. theobromae*, it appears that *B. dothidea* is not an aggressive pathogen on vigorously growing *Eucalyptus* in Venezuela.

N. andinum and *P. stromaticum* produced only small lesions on inoculated *Eucalyptus* stems. These fungi represent newly described taxa recently discovered from Venezuela (Crous *et al*. 2006; Mohali *et al*. 2006). *N. andinum* was isolated from dried branches of old *Eucalyptus* trees without obvious diseases symptoms. *P. stromaticum* originated from asymptomatic plant tissue, as well as from trees exhibiting blue stain or dieback, and from entirely dead trees (Mohali *et al*. 2006, 2007). *N. andinum* originates from the mountain ranges near Mérida state, an area climatically very different from where the inoculations were conducted, which might have influenced the results of the inoculations. Overall, the present study has yielded the first ecological information regarding these species that are apparently non-pathogenic endophytes.

N. ribis and *N. parvum* were the most pathogenic species emerging from the present study. They produced the largest lesions as well as cracks in the bark and black kino exudation from the points of inoculation. *N. ribis* and *N. parvum* are well known pathogens of forest tree species, including *Eucalyptus* spp. (Frezzi 1952; Davison and Tay 1983; Shearer *et al*. 1987; Ahumada 2003; Rodas 2003; Slippers *et al*. 2004*b*; Slippers and Wingfield 2007). The pathogenicity of *N. ribis* and *N. parvum* on inoculated *Eucalyptus* in Venezuela suggests that they have the capacity to cause diseases when the trees are under stress.

Many Botryosphaeriaceae are known to have wide host ranges, thus once a species has been introduced into a new area, it is quite possible that it would spread to and infect other hosts (Slippers and Wingfield 2007). In a study of Botryosphaeriaceae occurring on native Myrtaceae in South Africa together with the related introduced *Eucalyptus*, two species (*N. parvum* and *L. theobromae*) were found to co-infect both hosts, and they are thus likely to move between them (Pavlic *et al*. 2007). Consequently there is a threat

of native Botryosphaeriaceae in this area causing disease on the introduced hosts, and likewise, for introduced Botryosphaeriaceae from *Eucalyptus* to cause disease on native Myrtaceae. In this regard, an investigation in Uruguay has shown that Botryosphaeriaceae on native Myrtaceae also infect *Eucalyptus* spp. (Pérez *et al*. 2007). Such host shifts may not be uncommon and where novel host–pathogen encounters follow pathogen introductions, the naïve recognition and defence systems of the newly encountered hosts could be compromised leading to the development of epidemics (Slippers *et al*. 2005).

This study represents a first attempt to understand the pathogenicity of Botryosphaeriaceae on *Eucalyptus* in Venezuela. In some instances, results were unexpected and these deserved further study. In the case of *N. ribis* and *N. parvum*, there is reason to be optimistic that clones tolerant to infection can be found and deployed in plantations, as has been the case in other countries such as South Africa (Wingfield *et al*. 2001; Smith *et al*. 2002). Based on the results of the present study, it should be possible to initiate disease screening procedures to rapidly identify clones that might be less affected by cankers associated with the Botryosphaeriaceae.

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References

Ahumada R (2003) Fungal diseases affecting *Eucalyptus* plantations in Chile. MSc Thesis, University of Pretoria, South Africa.

Barber PA, Burgess TI, Hardy GE, St J, Slippers B, Keane PJ, Wingfield MJ (2005) *Botryosphaeria* species from *Eucalyptus* in Australia are pleoanamorphic, producing *Dichomera* synanamorphs in culture. *Mycological Research* **109**, 1347–1363. doi: 10.1017/S0953756205003989

Barnard EL, Geary T, English JT, Gilly SP (1987) Basal cankers and coppice failure of *Eucalyptus grandis* in Florida. *Plant Disease* **71**, 358–361. doi: 10.1094/PD-71-0358

Bettucci 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. doi: 10.1017/S0953756297003717

Bettucci L, Alonso R, Tiscornia S (1999) Endophytic mycobiota of healthy twigs and the assemblage of species associated with twig lesions of *Eucalyptus globulus* and *E. grandis* in Uruguay. *Mycological Research* **103**, 468–472. doi: 10.1017/S0953756298007205

Burgess TI, Barber PA, Hardy GEStJ (2005) *Botryosphaeria* spp. associated with eucalypts in Western Australia including description of *Fusicoccum macroclavatum* sp. nov. *Australasian Plant Pathology* **34**, 557–567. doi: 10.1071/AP05073

Ciesla WM, Diekmann M, Putter CAJ (1996) Technical Guidelines for the safe movement of germplasm No. 17. *Eucalyptus* spp. Food and Agriculture Organization of the United Nations, Italy.

Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WFO, Phillips AJL, Alves A, Burgess T, Barber P, Groenewald JZ (2006) Phylogenetic lineages in the *Botryosphaeriaceae*. *Studies in Mycology* **55**, 235–253.

Davison EM, Tay CS (1983) Twig, branch and upper trunk cankers of *Eucalyptus marginata*. *Plant Disease* **67**, 1285–1287. doi: 10.1094/PD-67-1285

De Arruda Silveira RLV, Higashi EN, Sgarbi F, Almeida MR (2001) Principais doenças na cultura do *Eucalyptus*. *Informações Agronómicas* **93**, 26–32.

Fisher PJ, Petrini O, Sutton BC (1993) A comparative study of fungal endophytes in leaves, xylem and bark of *Eucalyptus nitens* in Australia and England. *Sydowia* **45**, 338–345.

Frezzi MJ (1952) Presencia de '*Botryosphaeria ribis*' en la Republica Argentina y su importancia economica. *Revista de Investigaciones Agropecuarias Serie 5. Patologia Vegetal* **6**, 247–262.

Gardner DE (1997) *Botryosphaeria mamane* sp. nov. associated with witches'-brooms on the endemic forest tree *Sophora chrysophylla* in Hawaii. *Mycologia* **89**, 298–303. doi: 10.2307/3761086

Mohali S (1993) Estudio histológico de Madera de pino caribe con manchado azul causado por *Botryodiplodia theobromae*. *Fitopatologia Venezolana* **6**, 14–17.

Mohali S, Encinas O, Mora N (2002) Manchado azul en madera de *Pinus oocarpa* y *Azadirachta indica* en Venezuela. *Fitopatologia Venezolana* **15**, 30–32.

Mohali S, Slippers B, Wingfield MJ (2006) Two new *Fusicoccum* species from *Acacia* and *Eucalyptus* in Venezuela, recognised based on morphology and DNA sequences data. *Mycological Research* **110**, 405–413. doi: 10.1016/j.mycres.2006.01.006

Mohali S, Slippers B, Wingfield MJ (2007) Identification of *Botryosphaeria* species from *Eucalyptus*, *Acacia* and *Pinus* in Venezuela. *Fungal Diversity* **25**, 103–125.

Nei C, Hull J, Jenkins D, Steinberger D, Bent N (2005) 'SPSS: statistical package for the social sciences 11.0.' (McGraw-Hill: Chicago, IL)

Old KM (2000) Eucalypt diseases of complex etiology. In 'Diseases and pathogens of eucalypts'. (Eds PJ Keane, GA Kile, FD Podger, BN Brown) pp. 411–425. (CSIRO Publishing: Melbourne)

Old KM, Davison EM (2000) Canker diseases of *Eucalyptus*. In 'Diseases and pathogens of eucalypts'. (Eds PJ Keane, GA Kile, FD Podger, BN Brown) pp. 241–257. (CSIRO Publishing: Melbourne)

Pavlic D, Slippers B, Coutinho TA, Wingfield MJ (2007) Botryosphaeriaceae occurring on native *Syzygium cordatum* in South Africa and their potential threat to *Eucalyptus*. *Plant Pathology* **56**, 624–636. doi: 10.1111/j.1365-3059.2007.01608.x

Pérez CA, Altier N, Simeto S, Wingfield MJ, Slippers B, Blanchette RA (2007) Botryosphaeriaceae causing diseases on *Eucalyptus* and native Myrtaceae in Uruguay. In 'Proceedings of the IUFRO WP 2.08.03 meeting – Eucalyptus and diversity: balancing productivity and sustainability, 22–26 October, Durban, South Africa'. p. 84.

Poynton RJ (1979) 'Tree planting in southern Africa. Vol. 2. The Eucalypts.' (Department of Forestry: Johannesburg, South Africa)

Rodas C (2003) Diseases of *Eucalyptus* in Colombia. MSc Thesis, University of Pretoria, South Africa.

Roux J, Coutinho TA, Wingfield MJ, Bouillet JP (2000) Diseases of plantation *Eucalyptus* in the Republic of Congo. *South African Journal of Science* **96**, 454–456.

Roux J, Coutinho TA, Mujuni Byabashaija D, Wingfield MJ (2001) Diseases of plantation *Eucalyptus* in Uganda. *South African Journal of Science* **97**, 16–18.

Sankaran KV, Sutton BC, Minter DW (1995) A checklist of fungi recorded on *Eucalyptus*. *Mycological Papers* **170**, 1–376.

Schoeneweiss DF (1984) The role of environmental stress in diseases of woody plants. *Plant Disease* **56**, 308–314.

Sharma JK, Mohanan C, Maria Florence EJ (1984) A new stem canker disease of *Eucalyptus* caused by *Botryodiplodia theobromae* in India. *Transactions of the British Mycological Society* **83**, 162–163.

Shearer BL, Tippett JT, Bartle JR (1987) *Botryosphaeria ribis* infection associated with death of *Eucalyptus radiata* in species selection trials. *Plant Disease* **71**, 140–145. doi: 10.1094/PD-71-0140

Slippers B, Wingfield MJ (2007) Botryosphaeriaceae as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biology Reviews* **21**, 90–106. doi: 10.1016/j.fbr.2007.06.002

Slippers B, Crous PW, Denman S, Coutinho TA, Wingfield BD, Wingfield MJ (2004*a*) Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. *Mycologia* **96**, 83–101. doi: 10.2307/3761991

Slippers B, Fourie G, Crous PW, Coutinho TA, Wingfield BD, Carnegie AJ, Wingfield MJ (2004*b*) Speciation and distribution of *Botryosphaeria* spp. on native and introduced *Eucalyptus* trees in Australia and South Africa. *Studies in Mycology* **50**, 343–358.

Slippers B, Stenlid J, Wingfield MJ (2005) Emerging pathogens: fungal host jumps following anthropogenic introduction. *Trends in Ecology & Evolution* **20**, 420–421. doi: 10.1016/j.tree.2005.05.002

Slippers B, Burgess T, Pavlic D, Ahumada R, Maleme H, Mohali SR, Rodas CA, Wingfield MJ (2009) A diverse assemblage of Botryosphaeriaceae infect *Eucalyptus* in native and non-native environments. *Southern Forests*, in press.

Smith H, Kemp GHJ, Wingfield MJ (1994) Canker and die-back of *Eucalyptus* in South Africa caused by *Botryosphaeria dothidea*. *Plant Pathology* **43**, 1031–1034. doi: 10.1111/j.1365-3059.1994.tb01653.x

Smith H, Wingfield MJ, Petrini O (1996) *Botryosphaeria dothidea* endophytic in *Eucalyptus grandis* and *Eucalyptus nitens* in South Africa. *Forest Ecology and Management* **89**, 189–195. doi: 10.1016/S0378-1127(96)03847-9

Smith H, Coutinho TA, Wolfaardt FW, Wingfield MJ (2002) Relative susceptibility of northern provenances of *Pinus greggii* to infection by *Sphaeropsis sapinea*. *Forest Ecology and Management* **166**, 331–336. doi: 10.1016/S0378-1127(01)00667-3

Turnbull JW (2000) Economic and social importance of Eucalypts. In 'Diseases and pathogens of eucalypts'. (Eds PJ Keane, GA Kile, FD Podger, BN Brown) pp. 1–9. (CSIRO Publishing: Melbourne)

von Arx JA (1987) 'Plant pathogenic fungi. Beheifte zur Nova Hedwigia 87.' (J. Cramer: Berlin, Germany)

von Arx JA, Müller E (1954) Die gattuge der amerosporen Pyrenomyceten. *Beitr Kryptogamenfl Scheiz* **11**, 1–434.

Webb RS (1983) Seed capsule abortion and twig dieback of *Eucalyptus camaldulensis* in South Florida induced by *Botryosphaeria ribis*. *Plant Disease* **67**, 108–109. doi: 10.1094/PD-67-108

Wingfield MJ, Roux J, Coutinho TA, Govender P, Wingfield BD (2001) Plantation disease and pest management in the next century. *South African Forestry Journal* **190**, 67–71.

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