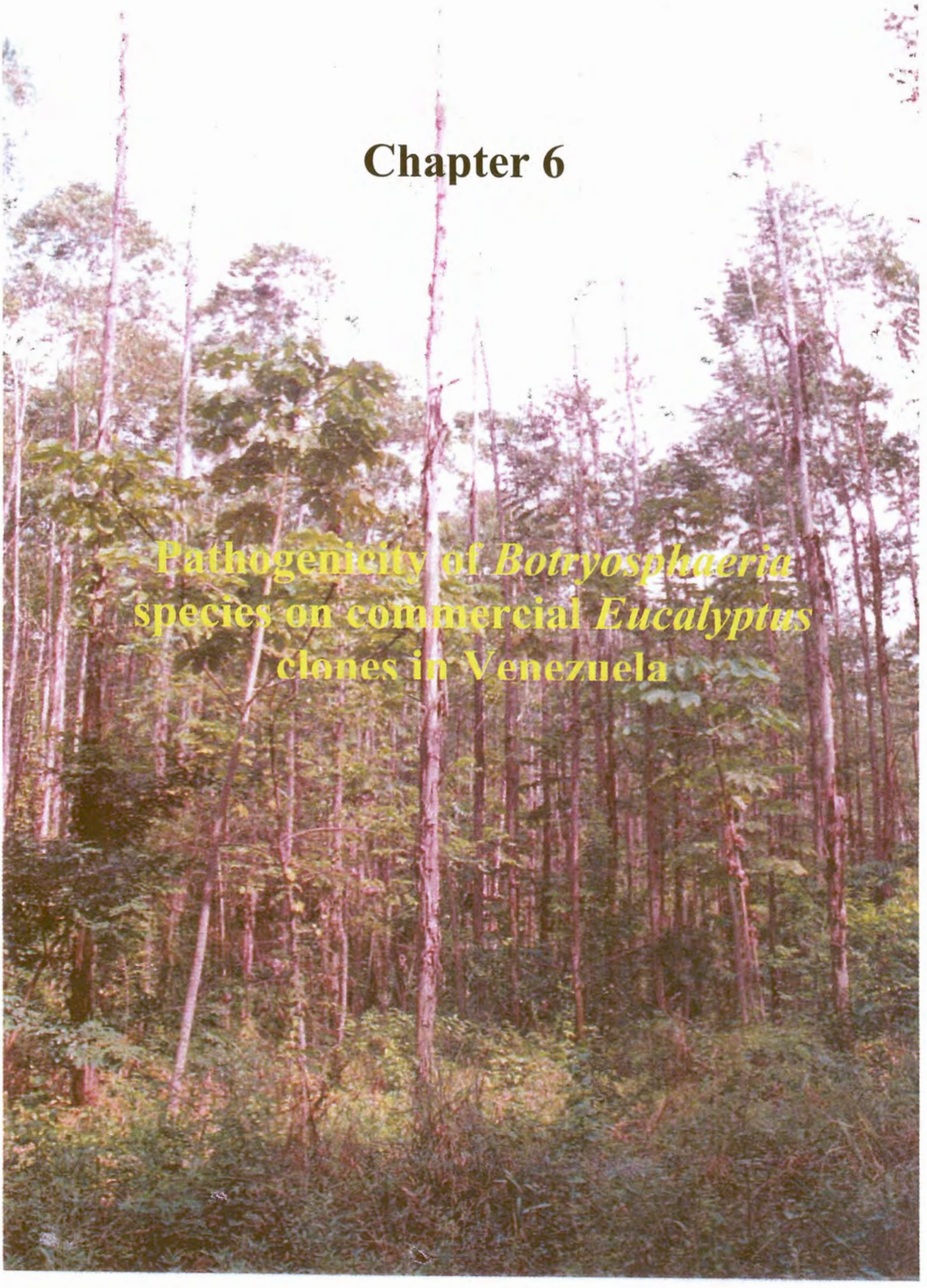

# Chapter 6

# Pathogenicity of *Botryosphaeria* species on commercial *Eucalyptus* clones in Venezuela

# Pathogenicity of *Botryosphaeria* species on commercial *Eucalyptus* clones in Venezuela

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*Botryosphaeria* spp. include a number of 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 *Botryosphaeria* species (*B. parva*, *B. ribis*, *B. mamane*, *B. rhodina*, *B. dothidea*, *Fusicoccum andinum* and *F. stromaticum*) on one commercial clone (256) of 2-year-old *Eucalyptus urophylla* x *E. grandis* hybrids in Venezuela. Stems of approximately between 25-35 cm in diam. and 7 m. in height were inoculated and lesion development recorded after seven weeks. Inoculations with *B. mamane*, *B. rhodina*, *B. dothidea*, *F. andinum* and *F. stromaticum* started to heal and produce callus around the wounds seven weeks after inoculation. *Botryosphaeria parva* and *B. ribis* caused bark swelling around the lesions and bleeding (black kino exudation) was observed when the outer bark was removed. A second inoculation trial was performed on four commercial clones to evaluate variation in their tolerance to infection, using *B. ribis* and *B. parva*, which were most pathogenic in the first trial. The clones differed in their tolerance to infection by *B. parva* and *B. ribis*. Clone 213 was the most tolerant, clones 113 and 276 were moderately tolerant, and clone 138 was least tolerant. *Botryosphaeria parva* was significantly more virulent than *B. ribis* in this clonal evaluation trial.

## INTRODUCTION

The genus *Botryosphaeria* Ces. & De Not. represents a cosmopolitan group of fungi that have an exceptionally wide host range, particularly on woody plants (von Arx, 1987). These fungi are regarded as weak pathogens that attack stressed or wounded plants after drought, hail, wind, frost damage or insect infestation (Schoeneweiss, 1984; Old, 2000; Old & Davison, 2000). It has also been shown that some *Botryosphaeria* spp. live in asymptomatic tissue of hosts including *Eucalyptus* as latent pathogens, only expressing disease when the host defence mechanisms are inactivated due to stress (Fisher *et al.*, 1993; Smith *et al.*, 1994, 1996).

Infection by *Botryosphaeria* spp. has been reported to occur via wounds (von Arx & Müller, 1954; Ciesla *et al.*, 1996; Old, 2000; Old & Davison, 2000). On healthy plants, infection can also be directly through lenticels, stomata or other openings on healthy plants, without causing apparent damage or disease symptoms (Brown & Hendrix, 1981; Michailides, 1991; Smith *et al.*, 1996). After infection, these fungi also have the ability to invade the vascular system of woody hosts.

*Eucalyptus* species, which are native to Australia, are one of the most widely planted forest species worldwide (Poynton, 1979; Turnbull, 2000). *Botryosphaeria* spp. have been reported from both native and introduced *Eucalyptus* spp. and in many cases are amongst the most important pathogens of these trees (Davison & Tay, 1983; Shearer *et al.*, 1987; Old *et al.*, 1990; Smith *et al.*, 1994, 1996; Old, 2000; Old & Davison, 2000; Slippers *et al.*, 2004b). Common symptoms on *Eucalyptus* are dieback and cankers on stems and branches. The cankers are characterized by swelling of the stems, cracking of the bark around the lesions and copious exudation of red/black kino (Ciesla *et al.*, 1996; Smith *et al.*, 1994; Old, 2000; Old & Davison, 2000).

Various *Botryosphaeria* spp. have been reported from exotic *Eucalyptus* spp. (Sankaran *et al.*, 1995; Old, 2000; Old & Davison, 2000) where they are associated with a wide range of disease. Thus, *Botryosphaeria dothidea* has been isolated from basal cankers on *E. marginata* (Davison & Tay, 1983); seed capsule abortion and twig dieback on *E. camaldulensis* induced by *B. ribis* is known in Florida (Barnard *et al.*, 1987; Webb, 1983); stem canker on *E. tereticornis* caused by *Lasiodiplodia* (=*Botryodiplodia*) *theobromae* in Kerala, India (Sharma *et al.*, 1984); stick rot and canker caused by *B. ribis* is known on *E. grandis* and *E. citriodora* in Brazil (De Arruda Silveira, 2001); cankers on stems, branch and root disease on *Eucalyptus* sp. is caused by *B. ribis* in Argentina (Frezzi, 1952) and canker and die-back on *Eucalyptus* in South Africa, Congo and Uganda is caused by *Botryosphaeria dothidea, B. parva, B. eucalyptorum* and *Lasiodiplodia theobromae* (Smith *et al.*, 1994; Smith *et al.*, 2001; Roux, *et al.*, 2000, 2001, Slippers *et al.*, 2004b)

Recent studies have led to the identification of seven *Botryosphaeria* spp. from *Eucalyptus*, *Acacia* and *Pinus* in Venezuela (Mohali *et al.*, 2005a, b). The aim of this study was to test the pathogenicity of the different species of *Botryosphaeria* in field inoculations on commercial *Eucalyptus* clones. A second aim was to determine whether a suite of commercially propagated clones are susceptible to infection by the most pathogenic of the *Botryosphaeria* spp. occurring on *Eucalyptus* in Venezuela.

## MATERIALS AND METHODS

### *Fungal isolates*

Isolates of the *Botryosphaeria* spp. used in this study (Table 1) emerged from a previous study (Mohali *et al.*, 2005a, b). These isolates originated from stems and branches of *Eucalyptus urophylla* S.T. Blake x *E. grandis* W. Hill ex Maiden hybrids and *Acacia mangium* Willd., growing in plantations in Acarigua, Portuguesa state, and from *Eucalyptus* sp. growing in the Cordillera Los Andes, Mérida state (Table 1). *Botryosphaeria* spp were collected from asymptomatic plant tissue, as well as from trees exhibiting blue stain or die-back, and from entirely dead trees. The isolates were grown on 2 % malt extract agar (MEA) (2% DIFCO Detroit, MI, USA) 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 (FABI), University of Pretoria, South Africa.

### *Inoculation tests*

Inoculation experiments were conducted in January 2004. Inoculations were made on trees of approximately 7 m. in height, 25-35 cm in diam. and 2-years-old, in plantations of *E. urophylla* x *E. grandis* hybrids belonging to Smurfit Carton de Venezuela, 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 on which various test *Botryosphaeria* spp. grown. The wounds were covered with the original bark discs and sealed with masking tape to prevent desiccation. The lengths of the lesions that had formed in the cambium were measured seven weeks after the inoculations were made.

A total of seven *Botryosphaeria* spp. were used in the trial, of which six were isolated from the same area where this study was conducted. These included *B. ribis*, *B. parva*, *B. dothidea*, *B. mamane* Gardner, *B. rhodina*, *Fusicoccum stromaticum prov. nom.* and *F. andinum prov. nom.* from the mountain ranges in Mérida state (Table 1).

Two isolates of each of the seven *Botryosphaeria* spp. were used in inoculations (Table 1). A total of 280 trees (20 trees per isolate / 40 per taxon) of an *Eucalyptus urophylla* x *E. grandis* hybrid clone (256) were inoculated. Twenty trees of the same clone were inoculated with sterile MEA plugs to serve as controls. Prior to inoculations, the isolates were grown on 2 % MEA in Petri dishes at 25 °C for 14 days.

A second inoculation trial was made using only *B. ribis* and *B. parva*, which were the most pathogenic species in the trial used to assess pathogenicity of the different *Botryopshaeria* spp. This inoculation was made on four commercial clones (113, 138, 213, 276) representing *Eucalyptus grandis* x *urophylla* hybrids, and 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 seven weeks after inoculation with the fungi.

***Statistical analyses***

Analyses of variance (ANOVA) were computed using the 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

There were significant differences ($P > 0.0001$, Table 2) in the lesion lengths for the different *Botryosphaeria* spp. inoculated onto clone 256. It was thus possible to separate the isolates into low, medium and high pathogenicity groups (Table 3, Fig. 1). The control inoculations with MEA plugs produced no lesions in the outer bark and had begun to heal and produce callus after seven weeks (Fig. 2).

Like the control, the isolates of *Botryosphaeria mamane* and *B. rhodina* did not produce lesions (Table 3, Fig. 1). *Botryosphaeria dothidea* produced very small lesions that did not differ significantly from the control and those of *B. mamane* and *B. rhodina* (Table 3, Fig. 1). Sites of inoculation with these three *Botryosphaeria* spp. had started to produce callus tissue around the wounds by the time the trial was terminated.

*Fusicoccum andinum prov. nom.* and *F. stromaticum prov. nom.* produced small lesions that were significantly larger than those for *Botryosphaeria mamane* and *B. rhodina* (Table 3, Fig.1). The sites of inoculation with *F. andinum* and *F. stromaticum* had started to heal and produce callus by the end of the trial.

*Botryosphaeria ribis* and *B. parva* produced significantly larger lesions than all the other *Botryosphaeria* spp. included in this study (Table 3, Fig. 1, 3). Both these species produced bark swelling around the lesions and bark cracks on some trees. Black kino exudation (Fig. 4, white arrow) was observed when the outer bark was removed.

In the second inoculation trial, on four commercial clones, only the isolates of *B. ribis* and *B. parva* were used. One isolate of *B. ribis* (CMW13409) and one of *B. parva* (CMW13355), was selected for this experiment. Bark swelling and occasional cracks and exudation of black kino were produced around the lesions on all of the clones

inoculated with these fungi. The control inoculations with sterile MEA plugs showed no lesion development.

Significant differences in lesion size were observed for lesions on the different clones ($P > 0.0001$, Table 4) after inoculations with *B. ribis* and *B. parva*. Clone 213 was the most tolerant to infection by either *B. ribis* or *B. parva* with an average lesion length of 30 mm. Clones 113 and 276 had average lesion length of 44 mm, and clone 138 was the least tolerant to infection with an average lesion length of 53 mm (Table 5, Fig. 5).

Significant differences in lesion size were also produced by *B. ribis* and *B. parva* on the different clones. *Botryosphaeria parva* was more virulent than *B. ribis* on the clones 113, 138 and 213, but on clone 276 *B. ribis* was more virulent (Fig. 6). On average across the clones, *B. parva* was more pathogenic (Fig. 6).

## DISCUSSION

In this study stems of different *E. urophylla* x *E. grandis* hybrid clones were inoculated with seven different *Botryosphaeria* spp. recently identified in Venezuela (Mohali *et al.*, 2005a, b). Only some of the seven *Botryosphaeria* species were pathogenic on these trees and others failed to produce lesions. Clearly, those species that were pathogenic have the potential to damage trees sustaining an important forestry industry in Venezuela.

*Botryosphaeria mamane* did not produce lesions on stems of inoculated eucalypts. Prior to its recent discovery in Venezuela, this unusual species was known only from Hawaii. In that location, *B. mamane* has been associated with witches'-broom on the native leguminous forest tree, *Sophora chrysophylla* (Gardner, 1997), but these

symptoms have not been reported on *Eucalyptus*. In Venezuela, *B. mamane* was isolated as an endophyte from asymptomatic *Eucalyptus* tissue (Mohali *et al.*, 2005b). The origin of this fungus is unknown and it could be native to either Hawaii or Venezuela or it might have been introduced into either of these locations from elsewhere in the world.

*Botryosphaeria rhodina* did not produce lesions on *Eucalyptus* stems in this study. This result was somewhat unexpected as the fungus is associated with many diseases of trees. Some symptoms associated with this fungus include lesions in the inner wood, stem cankers, kino exudation and root disease on *E. tereticornis*, *E. grandis* and *Eucalyptus* spp. (Sharma *et al.*, 1984; Smith *et al.*, 1994; Roux *et al.*, 2000, 2001), as well as causing a reduction in the strength of tropical hardwoods of low density (Findlay, 1959). In Venezuela, *B. rhodina* has been associated with the death of *Pinus* and *Eucalyptus* trees, but only when plants are severely stressed by drought or other factors (Cedeño & Palacios-Pru, 1992; Mohali, 1993; Mohali *et al.*, 2002, 2005b). The isolates of *B. rhodina* used in this study were obtained from both asymptomatic and symptomatic tissue. These results suggest that *B. rhodina* is not a primary pathogen on healthy *Eucalyptus*, but based on its behaviour on other trees, it could be a serious opportunistic pathogen when trees are under environmental stress.

*Botryosphaeria 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 (Barnard *et al.*, 1987; Webb, 1983; Smith *et al.*, 1994). However, these studies were undertaken before recent studies characterizing *Botryopshaeria* spp. based on DNA sequence data, and the fungus might have represented one of a number of different species (Slippers *et al.*, 2004a). 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.*, 2005b). As with *B. rhodina*, it appears that *B. dothidea* is not a primary pathogen on vigorously growing *Eucalyptus* in Venezuela, but the fungus has the potential to be a pathogen if trees are severely stressed.

*Fusicoccum andinum prov. nom* and *F. stromaticum prov nom.* produced only small lesions on inoculated *Eucalyptus* stems. These fungi represent newly described taxa that have only recently been discovered from Venezuela and we can not say, as yet, if they are native to the region or originate elsewhere (Mohali *et al.*, 2005a). *Fusicoccum andinum prov. nom.* was isolated from dried branches of old *Eucalyptus* trees without obvious diseases symptoms. *Fusicoccum stromaticum prov. nom.* originated from asymptomatic plant tissue, as well as from trees exhibiting blue stain or die-back, and from entirely dead trees (Mohali *et al.*, 2005a, 2005b). Currently there is no indication that these species are important pathogens and they rather appear to be weakly pathogenic endophytes.

*Fusicoccum andinum* was isolated from the mountain ranges (Mohali *et al.*, 2005a) where the temperature and humidity conditions are significantly different from those where this inoculation study was conducted. It is possible that the differences in conditions between the area of occurrence and the site of inoculation could have influenced the pathogenicity of this fungus in this study. In order to consider this question, pathogenicity trials would need to be conducted on eucalypts in the mountain ranges.

*Botryosphaeria ribis* and *B. parva* were the most pathogenic species in this study, where they produced the largest lesions as well as cracks in the bark and black kino exudation from the points of inoculation. *Botryosphaeria ribis* and *B. parva* are well-known pathogens of forest tree species, including *Eucalyptus* spp. (Frezzi, 1952;

Davison & Tay, 1983; Shearer *et al.*, 1987; Slippers *et al.*, 2004b; Ahumada 2003; Rodas, 2003). The pathogenicity of *B. ribis* and *B. parva* on inoculated *Eucalyptus* in Venezuela suggests that they have the capacity to cause diseases when the trees are under stress.

This study represents the first pathogenicity tests of different *Botryosphaeria* species of *Eucalyptus* in plantations in Venezuela. Future control efforts should clearly focus on the pathogenic species, *B. ribis* and *B. parva*. The tolerance of certain clones to inoculations with these fungi suggests that forestry enterprises will be able to capitalise on disease-tolerant *Eucalyptus* clones to avoid disease. This approach has been used in other countries such as South Africa (Wingfield *et al.*, 2001; Smith *et al.*, 2002). Based on the results of this study, it should be possible to initiate commercial disease screening trials to ensure that clones highly susceptible to infection by *Botryosphaeria* spp. are not planted on a wide scales.

## REFERENCES

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

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

Arx, J. A. von & Müller, E. (1954) Die gattuge der amerosporen Pyrenomyceten. Beitr Kryptogamenfl Scheiz 11: 1-434.

Barnard, E. L., Geary, T., English, J. T. & Gilly, S. P. (1987) Basal cankers and coppice failure of *Eucalyptus grandis* in Florida. *Plant Disease*. 71: 358-361.

Brown, E. A. & Hendrix, F. F. (1981) Pathogenicity and histopathology of *Botryosphaeria dothidea* on apple stems. *Phytopathology* 71: 375-379.

Cedeño, L. & Palacios-Pru, E. (1992) Identificación de *Botryodiplodia theobromae* como la causa de las lesiones y gomosis en cítricos. *Fitopatologia Venezolana* 5: 10-13.

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

Davison, E. M. & Tay, C. S. (1983) Twig, branch and upper trunk cankers of *Eucalyptus marginata*. *Plant Disease* 67: 1285-1287.

De Arruda Silveira, R. L. V., Higashi, E. N., Sgarbi, F. & Almeida, M. R. (2001) Principais doencas na cultura do *Eucalyptus*. *Informacoes Agronomicas*. No 93: 26-32.

Findlay, W. P. K. (1959) Sap-stain of timber. *Forestry Abstracts* 20: 1-7.

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

Frezzi, M. J. (1952) Presencia de "*Botryosphaeria ribis*" en la Republica Argentina y su importancia economica. *Revista de Investigacion Agricola* 6: 247-262.

Gardner, D. E. (1997) *Botryosphaeria mamane* sp. nov. associated with witches'-brooms on the endemic forest tree *Sophora chrysophylla* in Hawaii. *Mycologia* 89: 298-303.

Michailides, T. J. (1991) Pathogenicity, distribution, sources of inoculum, and infection courts of *Botryosphaeria dothidea* on Pistacia. *Phytopathology* 81: 566-573.

Mohali, S. (1993) Estudio histologico 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, M. J. (2005a) Two new *Fusicoccum* species from *Acacia* and *Eucalyptus* in Venezuela, recognised based on morphology and DNA sequences data. *Mycological Research* (in press).

Mohali, S., Slippers, B. & Wingfield, M. J. (2005b) Identification of *Botryosphaeria* species from *Eucalyptus*, *Acacia* and *Pinus* in Venezuela. *Fungal Diversity* (Submitted).

Nie, C., Hull, J., Jenkins, D., Steinberger, D. & Bent, N. (2005) SPSS: Statistical Package for the Social Sciences 11.0. McGraw-Hill. Chicago, Illinois. USA.

Old, K. M. (2000) Eucalypt diseases of complex etiology. In: Diseases and pathogens of eucalypts, by Keane, P. J., Kile, G. A., Podger, F. D. & Brown, B. N, 411-425, CSIRO Publishing, Collingwood, Australia.

Old, K. M. & Davison, E. M. (2000) Canker Diseases of *Eucalyptus*. In: Diseases and pathogens of eucalypts, by Keane, P. J., Kile, G. A., Podger, F. D. & Brown, B. N, 241-257, CSIRO Publishing, Collingwood, Australia.

Old, K. M., Gibbs, R., Craig, I., Myers, B. J. & Yaun, Z. Q. (1990) Effect of drought and defoliation on the susceptibility of *Eucalyptus* to cankers caused by *Endothia gyrosa* and *Botryosphaeria ribis*. *Australian Journal of Botany* 38: 571-581.

Poynton, R. J. (1979) Tree planting in southern Africa. Vol. 2. The Eucalypts. Department of Forestry, South Africa.

Rodas, C. (2003) Diseases of *Eucalyptus* in Colombia. M.Sc. thesis. University of Pretoria, Pretoria, South Africa.

Roux, J., Coutinho, T. A., Mujuni Byabashaija, D. & Wingfield, M. J. (2001) Diseases of plantation *Eucalyptus* in Uganda. *South African Journal Science* 97: 16-18.

Roux, J., Coutinho, T. A., Wingfield, M. J. & Bouillet, J. P. (2000) Diseases of plantation *Eucalyptus* in the Republic of Congo. *South African Journal Science* 96: 454-456.

Sankaran, K. V., Sutton, B. C. & Minter, D. W. (1995) A checklist of fungi recorded on *Eucalyptus*. *Mycological Papers* 170: 1-376.

Sharma, J. K., Mohanan, C. & Maria Florence, E. J. (1984) A new stem canker disease of *Eucalyptus* caused by *Botryodiplodia theobromae* in India. *Transaction of the British Mycological Society* 83: 162-163.

Shearer, B. L., Tippett, J. T. & Bartle, J. R. (1987) *Botryosphaeria ribis* infection associated with death of *Eucalyptus radiata* in species selection trials. *Plant Disease* 71: 140-145.

Shoeneweiss, D. F. (1984) The role of environmental stress in diseases of woody plants. *Plant Disease* 56: 308-314.

Slippers, B., Crous, P. W., Denman, S., Coutinho, T. A., Wingfield, B. D. & Wingfield M. J. (2004a) Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. *Mycologia* 96: 83-101.

Slippers, B., Fourie, G., Crous, P. W., Coutinho, T. A., Wingfield, B. D. Carnegie, A. J. & Wingfield, M. J. (2004b) Speciation and distribution of *Botryosphaeria* spp. on native and introduced *Eucalyptus* trees in Australia and South Africa. *Studies in Mycology* 50: 343-358.

Smith, H., Coutinho, T. A., Wolfaardt, F. W. & Wingfield, M. J. (2002) Relative susceptibility of northern provenances of *Pinus greggii* to infection by *Sphaeropsis sapinea*. *Forest Ecology and* Management 166: 331-336.

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, 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, H., Wingfield, M. J. & Petrini, O. (1996) *Botryosphaeria dothidea* endophytic in *Eucalyptus grandis* and *Eucalyptus nitens* in South Africa. *Forest Ecology and Management* 89: 189-195.

Turnbull, J. W. (2000) The eucalypts-their importance, diversity and biology. In: Diseases and pathogens of eucalypts, by Keane, P. J., Kile, G. A., Podger, F. D. & Brown, B. N, 1-9, CSIRO Publishing, Collingwood, Australia.

Webb, R. S. (1983) Seed capsule abortion and twig dieback of *Eucalyptus camaldulensis* in South Florida induced by *Botryosphaeria ribis*. *Plant Disease* 67: 108-109.

Wingfield, M. J., Roux, J., Coutinho, T., Govender, P. & Wingfield, B. D. (2001) Plantation disease and pest management in the next century. *South African Forestry Journal* 190: 67-71.

**Table 1.** Isolates of different *Botryosphaeria* species from Venezuela used in the inoculations trials. All isolates were collected by S. Mohali in 2003.

| Isolates No | *Botryosphaeria* spp. | Host | Origin |
|---|---|---|---|
| CMW13355 | *Botryosphaeria parva* | *E. urophylla* x *E. grandis* | Acarigua, Portuguesa state |
| CMW13419 | *B. parva* | *E. urophylla* x *E. grandis* | Acarigua, Portuguesa state |
| CMW13362 | *B. ribis* | *E. urophylla* x *E. grandis* | Acarigua, Portuguesa state |
| CMW13409 | *B. ribis* | *E. urophylla* x *E. grandis* | Acarigua, Portuguesa state |
| CMW13373 | *B. dothidea* | *E. urophylla* x *E. grandis* | Acarigua, Portuguesa state |
| CMW13381 | *B. dothidea* | *E. urophylla* x *E. grandis* | Acarigua, Portuguesa state |
| CMW13370 | *B. mamane* | *E. urophylla* x *E. grandis* | Acarigua, Portuguesa state |
| CMW13397 | *B. mamane* | *E. urophylla* x *E. grandis* | Acarigua, Portuguesa state |
| CMW13487 | *B. rhodina* | *E. urophylla* x *E. grandis* | Acarigua, Portuguesa state |
| CMW13488 | *B. rhodina* | *E. urophylla* x *E. grandis* | Acarigua, Portuguesa state |
| CMW13366 | *Fusicoccum stromaticum prov. nom* | *E. urophylla* x *E. grandis* | Acarigua, Portuguesa state |
| CMW13426 | *F. stromaticum* | *Acacia mangium* | Acarigua, Portuguesa state |
| CMW13444 | *F. andinum prov. nom* | *Eucalyptus* sp. | Mérida, Mérida state |
| CMW13455 | *F. andinum* | *Eucalyptus* sp. | Mérida, Mérida state |

**Table 2.** ANOVA for the lesion length associated with different *Botryosphaeria* spp. inoculated on *E. urophylla* x *E. grandis* hybrid (clone 256) in Venezuela.

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Species | 382,830 | 7 | 54,690 | 62,483 | 0,0001 |
| Isolates | 4,705 | 1 | 4,705 | 5,375 | 0,0210 |
| Species x Isolates | 59,029 | 7 | 8,433 | 9,634 | 0,0001 |

Dependent Variable: Lesion length (mm)

**Table 3.** Mean lesion length (mm) of *Botryosphaeria* spp. inoculated on *E. urophylla* x *E. grandis* hybrid (clone 256) in Venezuela presented in groups representing three levels of pathogenicity (a, b, c).

| Fungus | Low a | Medium b | High C |
|---|---|---|---|
| Control | 0 | | |
| *B. mamane* | 0 | | |
| *B. rhodina* | 0 | | |
| *B. dothidea* | 2 | | |
| *F. andinum* | | 6 | |
| *F. stromaticum* | | 9 | |
| *B. ribis* | | | 27 |
| *B. parva* | | | 27 |

Tukey' multiple range test (P = 0.05)

**Table 4.** ANOVA for the lesion length associated with *Botryosphaeria parva* and *B. ribis* inoculated on different *Eucalyptus* commercial clones in Venezuela.

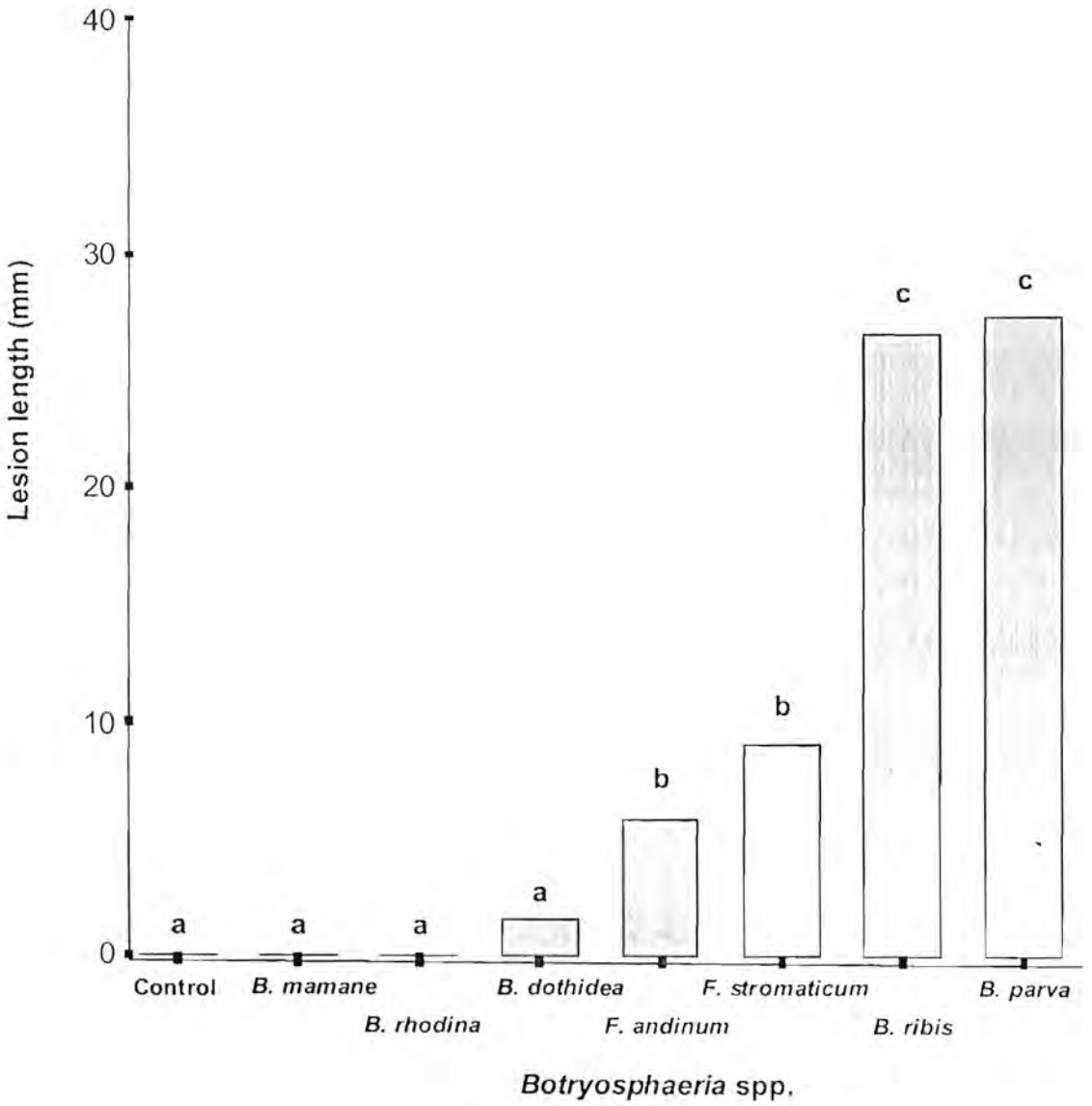
| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Isolate | 176,344 | 1 | 176,344 | 31,346 | 0,0001 |
| Clones | 145,732 | 4 | 36,433 | 6,476 | 0,0001 |
| Isolate x Clone | 179,784 | 4 | 44,946 | 7,989 | 0,0001 |

**Table 5.** Mean lesion length (mm) from commercial different clones inoculated with *Botryosphaeria parva* and *B. ribis* in Venezuela presented in groups according to level of pathogenicity ( a, b, c).

| Clones | Low a | Medium b | High c |
|---|---|---|---|
| Clone 213 | 30 | | |
| Clone 113 | | 43 | |
| Clone 276 | | 45 | |
| Clone 138 | | | 53 |

Tukey' multiple range test (P = 0.05)

**Fig. 1** Mean lesion length values (mm) of different *Botryosphaeria* spp. inoculated on *E. urophylla* x *E. grandis* hybrid (clone 256) in Venezuela. Lesions were measured seven weeks after inoculation. The isolates of *Botryosphaeria* species differ significantly according to Tukey's multiple range test (P = 0.05).



**Figs 2-4.** Lesion development during inoculation trials. **Fig. 2.** Control inoculations on *Eucalyptus* hybrid stem with MEA plugs produced no lesions. **Fig. 3.** Lesion produced by *B. parva*. **Fig. 4.** Presence of black kino was observed when the outer bark was removed (white arrow) after inoculation with *B. parva*.

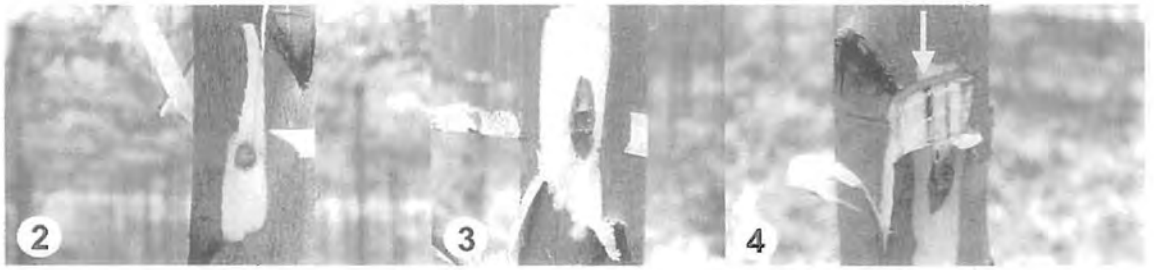


**Fig. 5** Mean lesion length (mm) on stem of different clones of *Eucalyptus urophylla* x *E. grandis* hybrids inoculated to assess the tolerance of different commercial clones of this hybrid to *Botryospharia parva* and *B. ribis*.

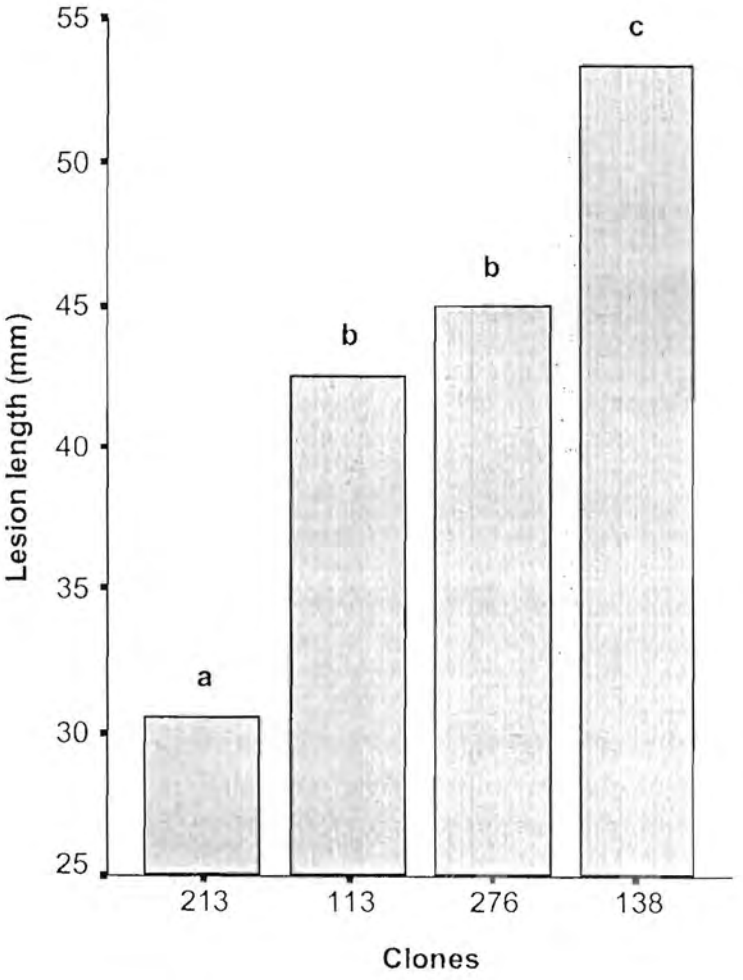


**Fig. 6** Relative susceptibility (mean lesion length in mm) of 4 commercial clones of *Eucalyptus* to inoculation with *Botryosphaeria parva* and *B. ribis*. Values in the histogram differ significantly (Table 4, $P > 0.0001$). Relative virulence of *B. parva* and *B. ribis* on all commercial clones.