# CHAPTER 1

## Diseases of *Eucalyptus* in Colombia

## ABSTRACT

*Eucalyptus* propagation encompasses approximately 33% of the Colombian forestry industry. Significant losses are, however, regularly experienced due to fungal diseases in plantations. Prior to 1993, virtually nothing was known regarding the causal agents of these diseases and consequently, detailed assessments of diseases in *Eucalyptus* plantations in Colombia have been undertaken. The most serious diseases recorded to date are Cryphonectria canker caused by *Cryphonectria cubensis*, Botryosphaeria canker caused by *Botryosphaeria dothidea* and *B. ribis*, Cylindrocladium leaf blight caused by *Cylindrocladium spathulatum* and Mycosphaerella leaf disease caused by various *Mycosphaerella* spp. A number of other less serious diseases have also been recorded. Selection and development of clonal *Eucalyptus grandis* and its hybrids with *E. urophylla* are underway to reduce the impact to these diseases. Monitoring of current disease incidence continues as does research into the interactions of pathogens with species, clones, sites and sylvicultural practices. This intensive and emerging pathology programme should ensure that diseases do not impair the future of intensively managed plantations in Colombia.

## INTRODUCTION

*Eucalyptus* species are being extensively propagated as exotics in monocultures in many parts of the world. Turnbull (2000) estimates that there are more than ten million hectares of *Eucalyptus* spp. in plantations outside Australia. Some of the most extensive forestry operations utilising these trees are in Asia, Africa and South America.

Diseases pose a serious threat to the productivity of *Eucalyptus* plantations (Wingfield 1990). Although these trees were initially separated from their natural enemies, there are numerous records of diseases that have caused substantial damage during the early period of *Eucalyptus* plantation forestry. These diseases were caused not only by pathogens known to occur on the trees in their native range, but also by pathogens native to the areas where these exotic plants were established (Wingfield *et al.* 2001a, b). In South Africa, for example, Mycosphaerella leaf blotch caused by *M. juvenis* Crous & M. J. Wingf., which has not been recorded from Australia, led to the abandonment of *E. globulus* Labill during the early phases of *Eucalyptus* propagation and continues to be an important disease on *E. nitens* Maiden (Crous & Wingfield 1996).

Propagation of *Eucalyptus* spp. in industrial plantations in Colombia is rapidly becoming a major component of this country's forestry industry (Osorio, Wright & White 1995). The most important species presently planted is *E. grandis* W. Hil ex Maiden, although hybrids of this and other species are likely to become important in future (Wright & Osorio 1996). Although seedling stands were initially established, these are rapidly being replaced with clones selected for optimum local performance. Diseases have already been recognised as an important constraint to *Eucalyptus* production in Colombia and these are being seriously considered in selection and breeding programmes (Wright 1997). In this study, we report on the occurrence of *Eucalyptus* diseases in Colombia based on three surveys conducted in May 1995, 1997 and 1998, and further observations between 2000 and 2002. The relative importance of the various pathogens is also discussed. The causal agent of Eucalyptus rust, namely *Puccinii psidii* Winter, which occurs on native hosts in Colombia, is also discussed.

## MATERIALS AND METHODS

Plantations chosen for inspection were primarily those reported by field foresters to include trees showing symptoms of disease. An effort was also made to inspect trees of a variety of age classes, those representing different seed sources or clones as well as those on a variety of different sites. Although most trees examined were those of *E. grandis*, trees of other species growing in species trials were also inspected. In some instances, stands thought to have disease symptoms had nutritional deficiencies due to site or sylviculture. This is important where pathogens that are favoured by stress.

Diseased leaf and stem tissues were collected for laboratory examination and confirmation of diagnoses. Soil samples were also collected to augment observations on the presence of *Cylindrocladium* spp. that were associated with leaf and twig blight symptoms. Where necessary for diagnosis, isolations from plant tissue were made on malt extract agar (MEA; 20 g Merck malt extract, 20 g Merck agar/ litre distilled water). Isolations from soil samples were made by placing a small amount of a representative soil sample in a Petri dish, and baiting the soil with surface-disinfested alfalfa seeds (Crous *et al.* 1997a). After 14 days, the germinating seedlings were removed, submerged in 1 % NaOCl for 30 s, rinsed in sterile $H_2O$, and plated onto MEA, amended with streptomycin sulphate (0.05 g/litre). Petri dishes were incubated for seven days at 25 °C under continuous near-ultraviolet light, after which single spores of sporulating *Cylindrocladium* isolates were plated onto carnation leaf agar (CLA) (Crous, Phillips & Wingfield 1992), and identified using the keys of Crous & Wingfield (1994) and Crous (2002).

Leaves with Mycosphaerella leaf blotch (MLB) symptoms were collected from several plantations in Colombia. Lesions were excised from leaves, and single ascospore cultures were established on MEA using the technique described by Crous (1998). Germinating ascospores were examined after 24 h, germination patterns were determined, and they were then transferred to MEA. Colonies were subcultured onto divided plates with one half containing CLA and the other MEA and incubated at 25 °C under continuous near-ultraviolet light.

## RESULTS AND DISCUSSION

Various important or potentially important *Eucalyptus* pathogens were identified in this study. These included leaf, shoot and stem pathogens. Their relative importance is deduced from the intensity of their incidence in Colombia as well as their significance in other countries.

### Cryphonectria canker

Basal stem cankers caused by *C. cubensis* (Bruner) Hodges (Figs 1a-c) were observed on one-year-old trees of *E. grandis* at low altitude and areas with high humidity and temperature. The incidence of this disease was relatively limited and only scattered trees had been girdled at the bases resulting in tree death. Perithecia and pycnidia of the pathogen occurred abundantly on the dead bark at the crown of dead or dying trees (Fig. 1d). As far as we are aware, this (Van der Merwe *et al.* 2001) was the first confirmed report of *C. cubensis* from Colombia.

Cankers caused by *C. cubensis* higher on the stems of older trees such as those found in Brazil (Ferreira 1989) and India (Sharma, Mohanan & Florence 1985) were also observed on older trees. This was especially in areas experiencing high temperatures and rainfall and particularly between 900 and 1400 masl. The basal girdling cankers on young trees were more typical of those commonly encountered in South Africa (Wingfield, Swart & Abear 1989) and in Colombia appear to be of minor importance. However, the larger stem cankers observed in high temperature sites are extremely damaging with a high proportion of trees severely damaged or killed. Strategies to select *Cryphonectria* tolerant clones for planting on these sites are being developed to avoid further damage by this pathogen.

Recently in Colombia, C. *cubensis* was found on *Tibouchina urvilleana* (DC). Logn., native to Brazil, and *T. lepidota* Baill., which is native to Colombia (Wingfield *et al.* 2001a). This discovery on Melastomataceae was unusual, as it was previously believed that the pathogen was restricted to members of the Myrtaceae. However, contemporary phylogenetic data have shown that the Melastomataceae are relatively closely related to the Myrtaceae within the order Myrtales (Conti, Litt & Sytsma 1996, Conti *et al.* 1997)

and this association might not be unusual. Consequent surveys for *C. cubensis* on native hosts in Colombia, also revealed that the fungus occurs on native *Miconia theaezans* (Bonpl.) Cogn. and *M. rubiginosa* (Bonpl.) DC. (Melastomataceae) (Chapter 3, this dissertation). The occurrence of *C. cubensis* on native hosts in Colombia suggests that the fungus might have originated in Central and South America (Wingfield *et al.* 2001a, Wingfield 2003).

Representative specimens (PREM) and living cultures used in this study: **Colombia**: *C. cubensis* on *E. grandis* bark, Vanessa, M. J. Wingfield, 2000, PREM 57294, CMW 10639; on *M. rubiginosa* bark, Vanessa, C. A. Rodas, 2001, PREM 57517, CMW 2357, CMW 9996, CMW 10022, CMW 10024, CMW 10025, CMW 10026, CMW 10028.

**Botryosphaeria canker and die-back**

Shoot blight and stem cankers were commonly observed on *E. grandis* trees of a variety of different age classes and on many different sites (Fig. 2). The species most commonly associated with this disease was *B. ribis* Grossenb. & Duggar (anamorph *Fusicoccum ribis* Slippers, Crous & Wingfield, sp. prov.) and *B. dothidea* (Moug.) Ces. & De Not. (anamorph *F. aesculi* Corda apud Sturm) (Chapter 2, this dissertation). *B. ribis* is also known to infect *Eucalyptus* spp. (Shearer, Tippett & Bartle 1987, Webb 1983) elsewhere in the world and appears to be the primary causal agent of the symptoms observed in Colombia. A similar disease of *Eucalyptus* spp. is caused by *B. dothidea* and *B. eucalyptorum* Crous, H. Smith & M.J. Wingf. in temperate and mediterranean areas of the world (Gibson 1979, Smith, Kemp & Wingfield 1994, Smith *et al.* 2001), although the relative importance of these fungi is not known.

*Botryosphaeria dothidea* is a well-recognised endophyte on *Eucalyptus* spp. that commonly causes disease on stressed trees (Smith *et al.* 1994). The fungus is a latent pathogen (Smith, Wingfield & Petrini 1996) and although this has not been shown experimentally, we assume that the same is true of *B. ribis.* In most cases, trees infected with this pathogen in Colombia had not been subjected to any noticeable stress, although high rainfall might, in some cases, have contributed to disease development. In South Africa, there is clear evidence of variation in the susceptibility of different clones of *E. grandis* to infection by *B. dothidea,* which is also considered to be one of

the most damaging pathogens of *Eucalyptus* in the country (Smith *et al.* 2001). Factors associated with disease development and the relative susceptibility of *E. grandis* clones to *B. ribis* are being considered in clonal deployment and breeding strategies for Colombia (Wright 1997).

Representative dried cultures (PREM) collected in this study: **Colombia**: *B. ribis* on *E. grandis* branches, La Estrella, C. A. Rodas, Aug. 2001, PREM 57509; La Ignacia, C. A. Rodas, Aug. 2001, PREM 57510; El Líbano, C. A. Rodas, Aug. 2001, PREM 57506. *B. dothidea* on *E grandis,* Andes, M. J. Wingfield, May. 2000, PREM 57508.

**Eucalyptus rust**

*Puccinia psidii* was commonly found in this survey in Colombia on native Myrtaceae such as *Eugenia jambos* L. (Fig. 3). The fungus has, however, not been found infecting *Eucalyptus* spp. as is commonly the case in Brazil (Ferreira 1983). The climate in Colombia is highly conducive to rust development and given the presence of the pathogen, this disease could become important in the future. This is especially if susceptible *Eucalyptus* spp. or clones are propagated.

Eucalyptus rust caused by *P. psidii* is potentially one of the most important diseases of *Eucalyptus* spp. in the world. The pathogen is known primarily from countries in South and Central America where it commonly occurs on native Myrtaceae (Coutinho *et al.* 1998). The disease is best known on *Eucalyptus* spp. such as *E. cloeziana* F. Muell. and *E. saligna* Sm. in Brazil, where the pathogen also occurs on several native plants (Coutinho *et al.* 1998). It has also been reported from *E. camaldulensis* Dehnhardt in Taiwan (Coutinho *et al.* 1998, Wang 1992), although it is uncertain whether this fungus is the same as that occurring in South America.

**Cylindrocladium shoot and leaf blight**

Numerous species of *Cylindrocladium* have been associated with nursery and plantation diseases of *Eucalyptus* spp. (Crous & Wingfield 1994). Cylindrocladium shoot and leaf blight disease of *E. grandis* plantation trees in Colombia (Fig 4) was predominantly associated with *C. reteaudii* (Bugnic.) Boesew. and *C. candelabrum*

Viégas in the past (Crous 2002). These two species, and especially *C. reteaudii*, were also the most dominant species isolated from the collected soil samples. Other species that were baited from soil under *Eucalyptus* trees, and that have been linked to diseases of this host in the past include *C. parasiticum* Crous, M.J. Wingf. & A.C. Alfenas and *C. gracile* (Bugnic.) Boesew. (Crous 2002, Ferreira 1989, Hodges & May 1972).

Cylindrocladium leaf blight on *E. grandis* in Colombia is commonly found in one-year-old plantations and the disease is prevalent in areas associated with high stand density and high rainfall and humidity. Most recent collections from leaf blight affected areas have all been of *C. spathulatum* El–Gholl, Kimbr., E. L. Barnard, Alfieri & Schoult, while none of the other species previously reported has been found (Chapter 4, this dissertation). The pathogen has not been reported in nurseries as it occurs in other parts of the world.

Representative living (STE-U) and dried cultures (PREM) used in this study: **Colombia**: *C. candelabrum* on *E. grandis* leaves, La Selva, M. J. Wingfield, Jun. 1995, STE-U 1151-1152. *C. gracile*, soil under *E. grandis*, La Paz Rodal, M. J. Wingfield, 1993, STE-U 726. *C. parasiticum*, soil under *E. grandis*, La Selva, M. J. Wingfield, 1993, STE-U 723-725. *C. reteaudii* on *E. grandis* leaves, La Selva, M. J. Wingfield, Jun. 1995, STE-U 1069-1070. *C. spathulatum* on *E. grandis* leaves, La Suiza, C. A. Rodas, July 2002, PREM 57504; Samaria, C. A. Rodas, July 2002, PREM 57505; Don Miguel, C. A. Rodas, July 2002, PREM 57502; Sta Maria, C. A. Rodas, July 2002, PREM 57503; Libano, C. A. Rodas, July 2002, PREM 57500; A. Maria, C. A. Rodas, July 2002, PREM 57505.

**Mycosphaerella leaf blotch**

Mycosphaerella leaf blotch disease (MLB) (Fig. 5) of *Eucalyptus* spp. is caused by a complex of *Mycosphaerella* spp. There are currently 29 *Mycosphaerella* spp. found on *Eucalyptus* spp., including various anamorph states for which no *Mycosphaerella* state has yet been recorded (Crous 1998). Although several species have been recorded from most countries where *Eucalyptus* spp. are grown, recent collections have shown that these identifications were mostly incorrect, and that only a few species occur on more than one continent (Crous 1998).

Several *Mycosphaerella* spp. were identified from *Eucalyptus* spp. in Colombia. *Mycosphaerella suberosa* Crous, F.A. Ferreira, Alfenas & M.J. Wingf. was associated with corky leaf spots. This fungus has also been found on *E. grandis*, *E. dunnii* Maiden and *E. molluccana* Roxb. in Brazil (Crous *et al.* 1993a), Indonesia (Crous & Wingfield 1997) and Western Australia (Carnegie, Keane & Podger 1997). In Colombia, it was observed on young and older mature foliage of *E. globulus* (M. J. Wingfield, personal communication). *Mycosphaerella parkii* Crous, M.J. Wingf., F.A. Ferreira & Alfenas, known from *E. grandis* and *E. globulus* in Brazil (Crous *et al.* 1993b) and *E. grandis* in Indonesia (Crous & Alfenas 1995), was isolated from leaf spots on juvenile leaves of *E. grandis*, and appeared to be fairly common in Colombian plantations. *Mycosphaerella africana* Crous & M.J. Wingf. that has been described from several *Eucalyptus* spp. in South Africa (Crous & Wingfield 1996) and has also been recorded from *E. globulus* in Portugal, was found on *E. grandis* in Colombia (Crous & Wingfield 1997). Other species that occur in Colombia, is *M. flexuosa* Crous & M. J. Wingf. and *M. lateralis* Crous & M. J. Wingf. (M. J. Wingfield, personal communication).

*Sonderhenia eucalypticola* (A. R. Davis) H. Swart & J. Walker (teleomorph: *Mycosphaerella walkeri* R.F. Park & Keane) was found on juvenile leaves of *E. globulus*. This fungus is known from several *Eucalyptus* spp. in Australia and New Zealand (Park *et al.* 2000). However, the teleomorph occurs on *E. globulus* and another *Eucalyptus* sp. from Australia (Peredo,1995), and has recently also been recorded on a *Eucalyptus* sp. from Chile (Wingfield, Crous & Peredo 1995). It has also recently been collected on *E. globulus* leaves from Ecuador (Crous and Wingfield, unpublished), suggesting that it occurs widely throughout South America.

Representative specimens used in this study: **Colombia**: *M. africana*, on *E. grandis* leaves, Sinai, M. J. Wingfield, Jun. 1995, PREM 54978. *M. colombiensis* on leaves of *E. urophylla,* Pinal farm, M. J. Wingfield, May 1995, PREM 54396, PREM 54397. *M. flexuosa,* on leaves of *E. globulus,* Selva, M. J. Wingfield, May 1995, PREM 54401. *M. lateralis* on leaves of *E. grandis,* Selva, M. J. Wingfield, Jun. 1994, PREM 54403; on leaves of *E. grandis,* Sinai, M. J. Wingfield, Jun. 1995, PREM 54404.

**Phaeoseptoria leaf spot and Corky leaf spot**

Phaeoseptoria leaf spot, caused by *Phaeophleospora epicoccoides* Cooke & Massee (Crous *et al.* 1997b) (Figs 6a-c) and corky leaf spot, caused by *Aulographina eucalypti* Cooke & Massee (Wall & Keane 1984) (Figs 6d-e) occur commonly and widespread on *Eucalyptus* spp. in Colombia. These pathogens are found in most areas of the world where *Eucalyptus* spp. are propagated, and they are also known in Australia (Sankaran, Sutton & Winter 1995). *Phaeophleospora epicoccoides* generally infects mature and senescent leaves, especially under conditions of severe stress. It is not considered an important pathogen (Knipscheer, Wingfield & Swart 1990).

*Aulographina eucalypti* causes a disease commonly known as corky leaf disease and is most common on mature leaves as well as on petioles and twigs. The pathogen is generally not considered to be particularly important although a single report of serious defoliation in Australia (Stefanatos 1993) might be cause for some concern (Crous & Wingfield 1997, Crous 1998).

**CONCLUSIONS**

Diseases pose a serious threat to productivity of *Eucalyptus* plantations. In Colombia, knowledge of disease presence and biology has led to significant changes in sylviculture and clonal deployment. Trials to select disease tolerant planting stock have been established and have already shown positive results. Furthermore, monitoring of plantations for disease problems is actively pursued and should make it possible to reduce the impact of diseases in the future.

The use of conventional methods is not likely to eliminate the threat of diseases in plantations in Colombia. However, with sufficient research effort and the utilisation of this information, losses to plantations can be minimized. Sustainable development of *Eucalyptus* plantations will require that diseases strategies are strongly considered in all management plans.

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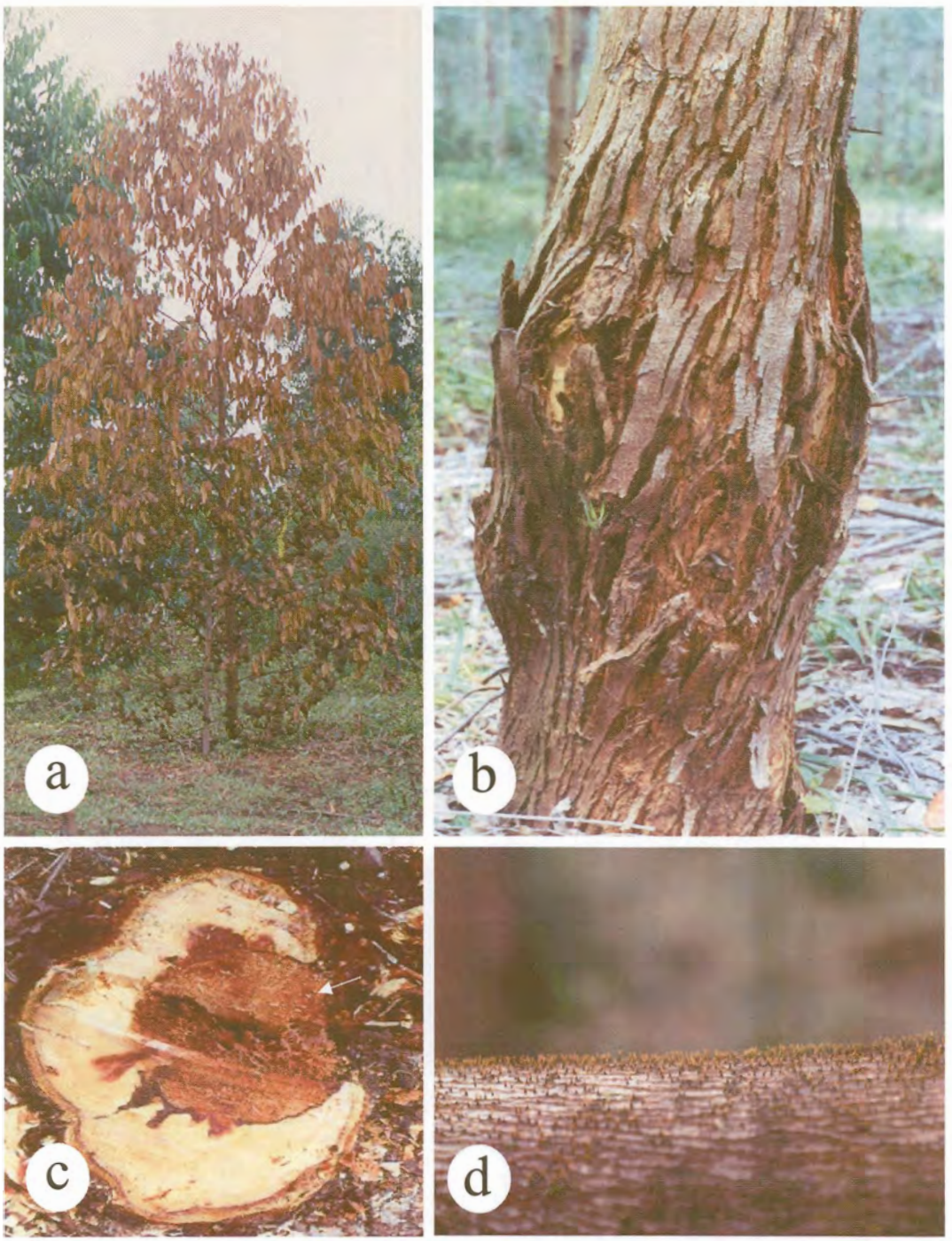
**Fig. 1.** Typical disease symptoms associated with infection by *Cryphonectria cubensis*. **(a)** Sudden wilt and death of trees. **(b)** Basal stem canker and cracked bark. **(c)** Section through a stem canker showing discoloured and dead sapwood (indicated with arrow). **(d)** Abundant presence of sexual and asexual fruiting structures on bark surrounding canker.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b
c
d

**Fig. 2.** Symptoms associated with infections by *Botryosphaeria* spp. on *Eucalyptus grandis*. **(a)** Die-back of the leader shoots. **(b-c)** Shoot blight and twig canker (indicated with arrows). **(d)** Stem and branch cankers. **(e)** Gum like kino exudation.

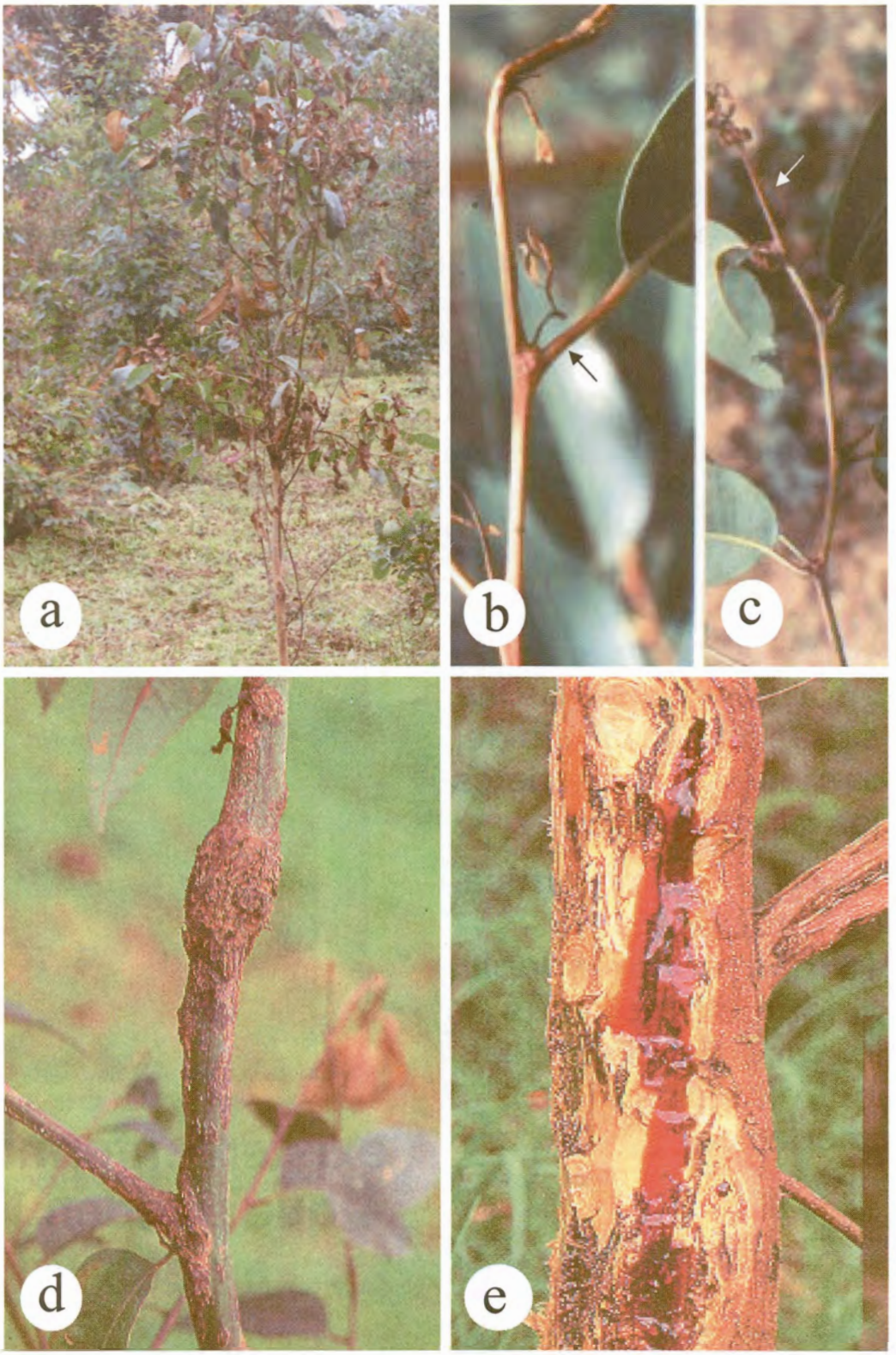

**Fig. 3.** Symptoms associated with rust caused by *Puccinia psidii*. **(a)** Uredinia formed on leaves of *Eugenia jambos*. **(b)** Characteristic egg-yolk yellow pustules developing on the top and bottom of leaf surfaces. **(c)** Leaf deformation and shoot death.



**Fig. 4.** Symptoms of infection by *Cylindrocladium* spp. on leaves of *Eucalyptus grandis*. **(a-b)** Early stage of development of leaf spots on the top and bottom of leaves. **(c-d)** Well-developed leaf spots on the top and bottom of leaves showing deformation and necrosis of substantial areas of the leaf surfaces.

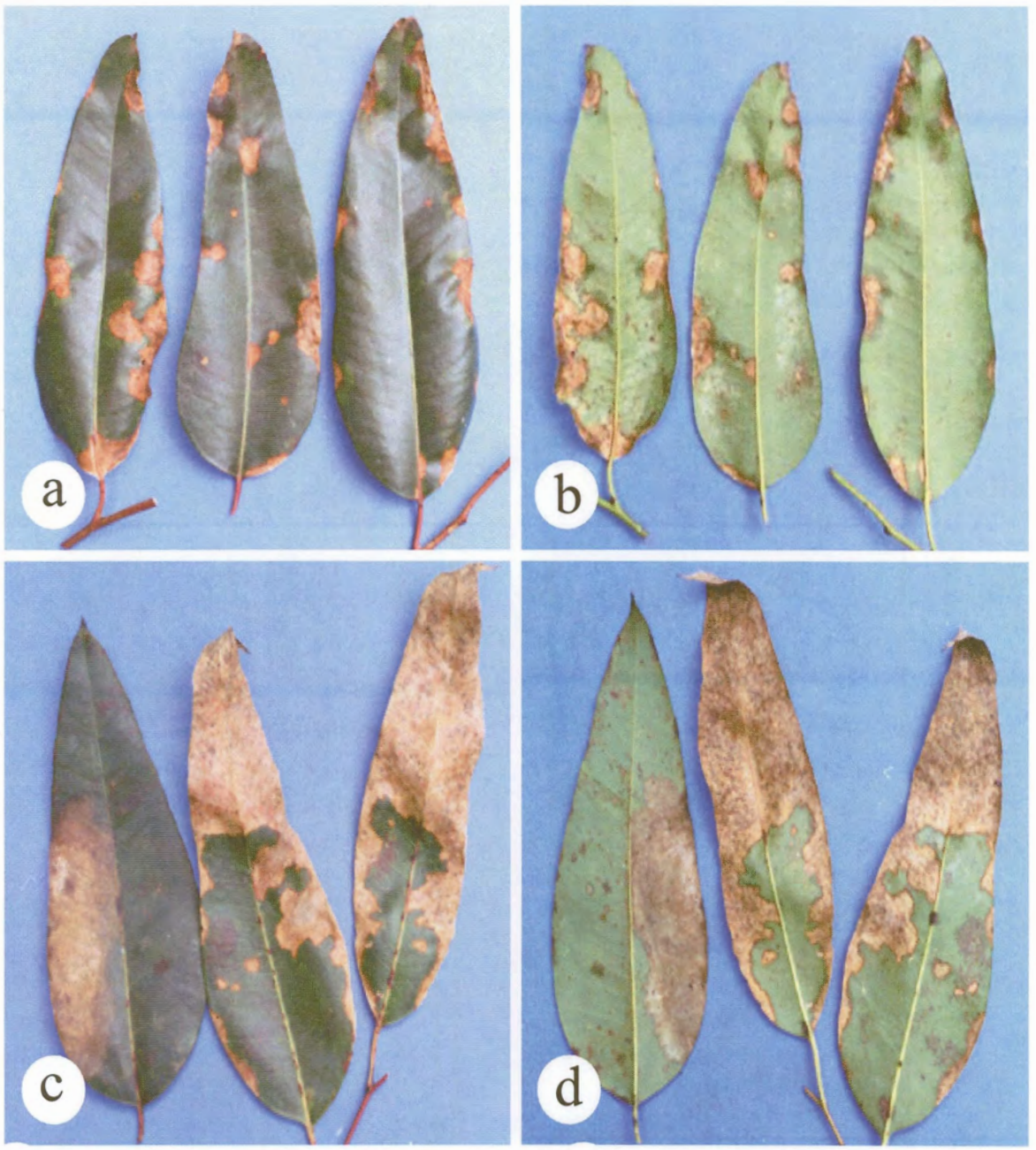
a
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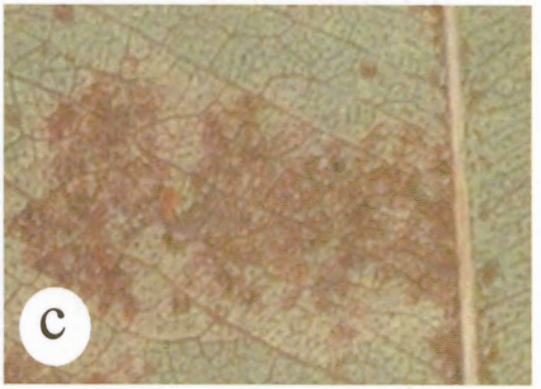
**Fig. 5.** Symptoms associated with infection by *Mycosphaerella* spp. **(a-b)** Irregular to sub-circular leaf spots on the top and bottom of leaves of 16-month-old *Eucalyptus grandis* trees.


**Fig. 6. (a-c)** Small dark and red lesions on *Eucalyptus grandis* leaves typical of infections by *Phaeophleospora epicoccoides*. **(d-e)** Corky lesions caused by *Aulographina eucalypti* on the abaxial surfaces of *E. grandis* leaves.


a


b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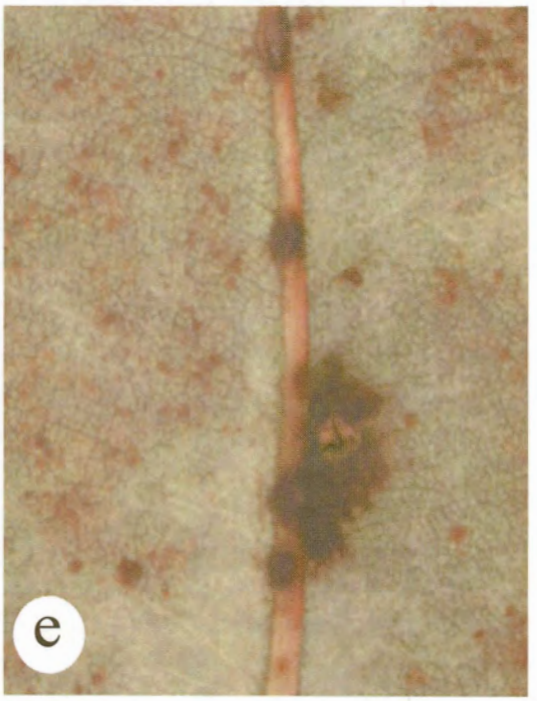
d


e

# CHAPTER 2

# Diseases of *Eucalyptus* associated with *Botryosphaeria* spp. in Colombia

**ABSTRACT**

Botryosphaeria canker is a serious disease of *Eucalyptus* trees in all major *Eucalyptus* growing areas of the world. Although Botryosphaeria canker has been known to occur in Colombia, the species associated with the disease, as well as the impact they have on *Eucalyptus* plantations in Colombia, have not been studied. In this study, the identity of the *Botryosphaeria* species were investigated through morphological and DNA sequence studies. The pathogenicity of these species was also assessed on 42 clones planted in three different sites. Two species of *Botryosphaeria* were found to occur on *E. grandis* in Colombia. *Botryosphaeria ribis* was the more common species, and also more aggressive. *Botryosphaeria dothidea* was only found in one zone, and was also athogenic than *B. ribis*. These two species could be distinguished easily based on DNA sequences of the ITS1/ITS2 rDNA region and EF1-α, as well as conidial sizes. Significant differences in resistance of clones to these pathogens were also evident from the various trials. This study has shown that of the two species present in Colombia, *B. ribis* should be the focus of future efforts to reduce the impact of this disease, and that a resistance breeding program against the pathogen will be possible.

## INTRODUCTION

Species of *Botryosphaeria* include an important group of pathogenic fungi that infect a wide range of woody plants. These fungi have a cosmopolitan distribution and are also found on many woody plants in the tropics and sub-tropics. Disease symptoms include stem and branch cankers, die-back, bleeding necrosis, coppice failure and seed capsule abortion (Smith, Kemp & Wingfield 1994, Neely 1996, Barnard *et al.* 1987, Webb 1983).

*Botryosphaeria* spp. are members of the family Botryosphaeriaceae and order Dothideales (Hawksworth *et al.* 1995). Identification of these fungi is often difficult, because teleomorph states are rarely encountered in nature. The taxonomy of anamorphs is confusing, because it is based on characters such as conidial pigmentation, septation and stromatal morphology, which show extensive plasticity and can be influenced by substrate and growth conditions (Butin 1993, Sutton 1980). More than 140 *Botryosphaeria* spp. have been described, mainly based on the hosts on which they occur (Denman *et al.* 2000). Many of these are likely to be synonyms, while others most likely represent species complexes.

The taxonomy of *Botryosphaeria* spp. has been confused and complicated for many decades. New species have tended to be described based on new hosts on which they occur. Another problem has been that names for these fungi, such as *Botryosphaeria dothidea* (Moug.) Ces. & De Not. and *B. ribis* Grossenb. & Dugg. have been used loosely and interchangeably (Denman *et al.* 2000, Slippers *et al.* 2003). Previous reports of *B. dothidea* and *B. ribis* on *Eucalyptus* could be wrong as molecular taxonomy have now shown that these species are rare on *Eucalyptus* and many new species have been described. Recently, DNA based techniques have been applied to the taxonomy of *Botryosphaeria* spp. (Jacobs & Rhener 1998, Slippers *et al.* 2003, Zhou & Stanosz 2001). This has made it possible to more accurately differentiate between species and to evaluate the taxonomic value of various identification procedures.

*Botryosphaeria* anamorphs appear to provide the best means to identify species. These states occur in two distinct groups that also have strong phylogenetic support (Crous & Palm 1999, Denman, Crous & Wingfield 1999, Denman *et al.* 2000, Slippers *et al.*

2003, Zhou, Smith & Stanosz 2001, Zhou & Stanosz 2001). These include those with hyaline conidia that are treated in *Fusicoccum* and others with dark, thick-walled conidia that are best placed in *Diplodia.*

*Botryosphaeria* spp. are well recognized as opportunistic wound and stress related pathogens (Schoeneweiss 1981). These fungi are also well adapted to live as facultative parasites or saprophytes on dead wood and other plant material (Sivanesan 1984). Under certain environmental conditions they can be virulent pathogens infecting twigs, stems, roots and leaves, which penetrate their hosts through wounds, open stomata and lenticels (Luttrell 1950, Wene & Schoeneweiss 1980). A recent understanding of these fungi is that they infect healthy plant tissue and can exist for extended periods of time in a latent form (Fisher, Petrini & Sutton 1993, Roux 1998, Smith, Wingfield & Petrini 1996). With the onset of stress, they then become active and cause serious disease.

*Botryosphaeria* spp. constitute an important group of pathogens of many hosts throughout the world (Von Arx & Muller 1954). In terms of exotic plantation forestry these species are also pathogens of *Eucalyptus* (Smith 1995, Roux 1998). *Botryosphaeria dothidea* has been reported to cause canker and die-back diseases of *Eucalyptus* species in Australia and the United States (Barnard *et al.* 1987, Davison & Tay 1983, Webb 1983). Webb (1983) reported the presence of *B. dothidea* in commercial seed of *Eucalyptus camaldulensis* Dehnh. in South Florida. Shearer *et al.* (1987) showed that this fungus was responsible for the death of *Eucalyptus radiata* Sieb. ex DC. in selection trials in Australia. Davison & Tay (1983) reported the natural occurrence of *B. dothidea* cankers in *Eucalyptus marginata* Donn. ex Sm. forests in Australia. Likewise, Barnard *et al.* (1987) showed that *B. dothidea* is involved as one of a complex of organisms that cause coppice failure of *E. grandis* W. Hill ex Maiden in Florida. Smith *et al.* (1994) found that two species of *Botryosphaeria* are associated with cankers on *Eucalyptus* in South Africa namely *B. dothidea* and *B. eucalyptorum* Crous, H. Smith & M. J. Wingf.

*Eucalyptus* plantations represent an important renewable resource for the forestry industry in Colombia. One of the most important species presently planted is *E. grandis.* This species has been used in reforestation and clonal programs by private

companies and government projects for the production of timber, pulp and protection of eroded soils.

*Botryosphaeria* spp. have been recognized as an important constraint to the productivity of *E. grandis* plantations in Colombia. Since 1994, diseases caused by *Botryosphaeria* spp. have been recorded in *E. grandis* trees in different geographic zones at the Smurfit Carton de Colombia and, *Eucalyptus grandis* plantations commonly affected by this disease range in age from 6 to 36 months, with the most susceptible trees being those between 18 to 26 months. Common symptoms include small necrotic lesions at the point of the insertion of twigs on the shoots and these develop to form large irregular cankers causing die-back of shoots. Cankers located on branches and main stems give rise to abundant production of kino which degrades the wood and weakens the stems. Wind or other external agents then lead to stem breakage and considerable loss.

Despite their importance, almost nothing is known regarding the identity or occurrence of *Botryosphaeria* spp. in Colombia. The aims of this study were, therefore, to identify the *Botryosphaeria* spp. associated with cankers on *E. grandis* in Colombian plantations and to consider their relative importance to forestry in the country.

## MATERIALS AND METHODS

### *Symptoms and collection of samples*

Isolations were made from a wide range of symptoms. These included die-back of shoots and twigs, small necrotic lesions at the insertion points of twigs on the shoots and irregular cankers formed on stems and branches (Fig. 1). In addition, isolations were made from pseudothecia on the bark of diseased *Eucalyptus* branches.

Samples of diseased *E. grandis* tissue were collected from three distinct geographical areas, namely the Andina, Valle and Cauca zones (Fig. 2). From these areas, 17 farms belonging to Smurfit Carton de Colombia were included in the collections. Diseased tissue was collected and transferred to the laboratory for detailed examination. All samples included in this study were collected between May 2000 and May 2001.

Tissue samples from diseased stems and branches were surface sterilised in 70 % ethanol for 30 s and thereafter washed in sterile distilled water. This material was placed in moisture chambers and incubated at 24 °C until fungi began to sporulate on the surface of the samples. In addition, after surface sterilisation, small (1-2 mm) pieces of tissue were placed on the surface of malt yeast extract Agar (MYA) (2 % malt extract, 0.2 % yeast extract, and 2 % agar; Biolab, Midrand, S.A.) and incubated at 25 °C for seven days.

Forty four isolates of *Botryosphaeria* spp. were obtained from preliminary isolations and single conidial subcultures were made for all of these isolates. In order to produce single conidial isolates, fungi were grown on water agar (WA) (2 % agar; Biolab) with sterilized pine needles placed on the surface. After incubation for 7–14 days at 25 °C under continuous fluorescent light, spore masses began to exude from pycnidia. Conidial masses were collected, diluted in sterile water and streaked out onto the surface of WA. After seven hours, germinating conidia were selected and transferred to MYA.

No differences in the morphology of cultures were observed between samples. All 44 single conidial isolates were transferred to 2% malt extract agar (MEA) (Biolab) slants and stored at 4 °C. These have been maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa (Table 1).

***Morphological characteristics***

The obtained isolates were inoculated on sterile pine needles on WA to induce sporulations. Conidia from pycnidia were mounted on glass microscope slides in a drop of lactophenol. Length and width measurements were made for 10 conidia per isolate using a light microscope with an Axiocam digital camera and accompanying Axiovision 3.1 software (Carl Zeiss, Mannheim, Germany). The measurements were subjected to statistical analyses and are presented as (min-)(average – std. dev.) – (average + std. dev)(-max).

***DNA extraction and amplification***

DNA was obtained using a modified version of the method of Raeder & Broda (1985). Mycelium was scraped off the surface of single conidial isolates and transferred to sterile Eppendorf tubes (1.5 ml). An amount of 400 µl of DNA extraction buffer (200 mM Tris-HCl pH 8.0, 150 mM NaCl, 25 mM EDTA pH 8.0, 0.5% SDS) were added to each tube, and cell walls were broken using a pestle. When a homogeneous solution was obtained an additional 400 µl of DNA extraction buffer was added as well as 500 µl of phenol and 300 µl of chloroform. These were then mixed using a vortex mixer and centrifuged at 10000 rpm for 60 min and at 4 (C. The DNA-containing aqueous layer was transferred to new tubes and extraction and washing were repeated until the white interface disappeared. Precipitation of DNA was achieved by adding 0.1 volume of 3 M NaAc (pH 5 to 5.5) and 2 volumes of absolute ethanol which was then centrifuged for 30 min at 4 (C and the EtOH was removed. The pelleted DNA was washed with 70% EtOH, dried under vacuum and re-suspended in sterile SABAX water (100 µl). An amount of 5 µl Rnase was added to the DNA solution that was incubated for two hours at room temperature. The DNA concentration was determined by UV light visualization after electrophoresis on a 1% agarose gel, which was stained with ethidium bromide.

The PCR amplification of a part of the nuclear rRNA operon was achieved using the primers ITS1 (5'TCCGTAGGTGAACCTGCGG3') and ITS4 (5'TCCTCCG GCTTATTGATATGC3') (White et al. 1990). The amplified region included the 3' end of the 16S (small subunit) rRNA gene, the first internal transcribed spacer (ITS 1) the complete 5.8S rRNA gene, the second ITS (ITS 2) region and the 5' end of the 26S (large subunit) of the rRNA gene. In addition, a part of the translation elongation factor 1α (EF1-α) gene was amplified using the primers EF1-728F (5'CATCGAGAAGTTCGAGAAGG) and EF1-986R (5'TACTTGAAGGAACCCTT ACC) (Carbone, Anderson & Khon 1999). The PCR reaction mixture for all was done according to Slippers et al. (2003) and contained 1X PCR reaction Buffer and MgCl2 (10 mM Tris-CHl, pH 8, 1.5 mM MgCl2, 50 mM KCL, pH 8.3) (Roche Diagnostics, Randburg, South Africa), 0.2 mM of each dNTP (Roche Diagnostics), 0.15 µM of each primer, 2.5 Units Expand High Fidelity Taq DNA polymerase (Roche Molecular

Biochemicals, Almeda, California, USA) and 10 ng of DNA. The reaction mixture was made up to a final volume of 50 µl by adding sterile deionized Sabax water. The amplification of the EF1-α region was done using the same protocol as described above, except that ExpandTM High Fidelity Taq polymerase was used (Roche Molecular Biochemicals).

The PCR reaction conditions for both regions, comprised of an initial denaturation of the DNA at 94 °C for 2 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, elongation at 72 °C for 1 $^{1}/_{2}$ min, and concluding with a final elongation at 72 °C for 5 min. All PCR products were run on 1 % agarose gels, stained with ethidium bromide, and visualized under UV light. DNA concentration was determined by comparison with 100 bp or standard λ size markers.

***DNA sequencing and analysis***

The PCR amplified fragments were purified using a High Pure PCR product Purification Kit (Roche Molecular Biochemicals). Each strand of the PCR products was sequenced in both directions with the same primers used for amplification. Sequencing reactions were carried out using the ABI PRISM $^{TM}$ Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied BioSystems, Foster City, California, USA) and capillary electrophoresis on an ABI PRISM $^{TM}$ 310 DNA Autosequencer (Applied BioSystems). All the reactions were done using protocols recommended by the manufacturers.

Sequence data were processed using Sequence Navigator version 1.0.1$^{TM}$ (Applied BioSystems). The nucleotide sequences were aligned manually by inserting gaps and phylogenetic relationships were determined from the aligned sequences using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2002).

Nucleotide substitutions were treated as unordered, unweighted characters. Maximum parsimony trees were found using the heuristic search option with unlimited random addition replicates with the tree bisection reconstruction (TBR) as branch swapping algorithm. Gaps were treated as fifth character and "Ns" are mising. Support for clades

was assessed by 1000 bootstrap replicates (Felsenstein 1985). Statistical congruence between ITS rDNA and EF1-α sequence data sets was tested using partition homogeneity test (Farris *et al.* 1995, Huelsenbeck, Bull & Cunningham 1996) in PAUP. These tests revealed that the data were combinable and all DNA sequence sets were subsequently analysed together. In order to establish the phylogenetic relationships and the identities of the *Botryosphaeria* spp. used in this study, sequences of known *Botryosphaeria* spp. from GenBank and Slippers *et al.* (2003) were included in the alignment (Table 2). Trees were rooted using a sequence of *Guignardia philoprina* (Ellis) Viala & Ravaz as outgroup taxon (Table 2).

***Pathogenicity Tests***

**Greenhouse trial.** A preliminary pathogenicity test was conducted in a greenhouse maintained at approximately 25 °C with natural light. In this trial, six isolates representing two distinct morphological groups identified in the prior taxonomic studies and referred to as Group A (CMW 8922, CMW 8925, CMW 8929) and Group B (CMW 8949, CMW 8961, CMW 8956) were used for the inoculations. The inoculations were conducted on trees of a *E. grandis* clone (ZG14), 18 months old, known to be susceptible to infection by *Botryosphaeria* spp. After two weeks of acclimatization in the greenhouse, ten trees were inoculated with each isolate. Inoculum was derived from one-week-old cultures growing on MYA. Ten trees were inoculated with sterile agar medium as negative control.

For inoculations, wounds were made in the stems of trees using a cork borer (6 mm diam) to expose the cambium. Wounds were inoculated with an agar disc of the same size bearing mycelium or not, and sealed with Parafilm (American National Can, Chicago, USA) to prevent desiccation. Lesion lengths (mm) were assessed six weeks after inoculation.

**Field trials.** Four inoculation trials were conducted in the field in Colombia. These were at four different sites chosen to represent a wide range of climate and humidity conditions where *Botryosphaeria* cankers are found. The trials were as follows:

**Trial N°1**. La Suiza farm in Restrepo, Valle, located at 1469 masl, 76° 29' 49" W, 3° 51' 45" N. This site receives an average annual precipitation of 1067 mm/y. **Trial N°2.** Cecilia farm near Darien, Valle, at 1825 masl, 1825 mm/y of precipitation, located at 76° 26' 06" W, 3° 57' 06" N. **Trial N°3.** Libano farm near Pereira, Risaralda, with 2102 masl, precipitation of 3143 mm/y and 75° 35' 49" W, 75° 35' 49" N. **Trial N° 4.** Angela Maria farm near Santa Rosa, Risaralda located at 1864 masl, with an average of 2437 mm/y precipitation and located at 75° 11' 14" W, 6° 8' 46" N.

Isolates CMW 8922 and CMW 8961 representing the two different *Botryosphaeria* morphological groups and previously shown to be most pathogenic in the greenhouse trial, were used in the field inoculations. These inoculations were on a total of 560, 30-month-old *E. grandis* trees. These trees consisted of ten clones (2, 4, 11, 12, 18, 20, 23, 27, 301, 303) and four seed lots (210, T210, 211, T 211) used in all four trials. Forty trees of each clone or seed lot, distributed in ten different blocks (four trees per block), were inoculated with each isolate. The block design consisted of two lines of trees per block, in which each clone or seed lot was planted twice. Each isolate was thus inoculated in trees in one line of each block. In order to avoid any border effect, the trial sites were surrounded by two rows of border trees. The same design was used in all four trials.

Inoculations were made as described above for the greenhouse inoculations except that the cork borer size for the inoculations was 4 mm and the inoculated areas were covered with tissue paper moistened with sterile water and secured with masking tape (Fig. 3). Trials were initiated during June 2002 and resultant external and internal lesions (lengths and widths) were measured after 12 weeks in September 2002.

Statistical analyses of greenhouse and field trial data were carried out using SAS analytical programs (1990). Analysis of variance tables were produced, as well as tables of means with the 95% confidence limits (C.L.) for each mean. Analysis of interactions between *Botryosphaeria* isolates, *E. grandis* clones and localities of the trials was assessed using an AMMI analysis (Eisenberg *et al.* 1996), because this method is uniquely able to focus completely on the interaction sums of squares.

## RESULTS

### *Morphological characteristics*

Isolates were similar in colour when grown on MYA medium at 24 °C. Initially (2-4 days) the mycelium was white and gradually darkened from the center of colonies, first having a dark-green colour and gradually becoming black with age (Figs 4a, c). No obvious or consistent differences could be detected in the colour of the two groups of isolates (Fig. 4).

Of the 44 isolates used in this study, two morphological groups were found based on conidial differences. Eight isolates belonged in one group, which we have designated Group A, and the remaining 36 isolates resided in Group B. All isolates belonging to Group A were collected from only one farm in the Andina zone while isolates of Group B were found in all three evaluated zones.

Both groups had *Fusicoccum*-type conidia that were hyaline, thin-walled, aseptate and smooth (Figs 4b, d). The conidia of these groups, however, differed in size and shape. Conidia of Group A were fusiform to fusiform-elliptical with obtuse to pointed ends and were (25.0-)27.0 – 29.0(-30.0) x 5.0(-7.0) µm (average 27.9 x 5.2 µm) (Fig. 4d, Table 3). Conidia of isolates belonging to Group B were elliptical to fusiform with pointed ends, granular contents and (18.0-)20.0 – 21.0(-23.0) x (5.0-)6.0(-7.0) µm (average length x width 20.3 x 5.6 µm) (Fig. 4b, Table 3).

### *DNA sequencing and analysis*

Sequences of approximately 557 bp were obtained from the amplified ITS 1/2 rDNA region and approximately 332 bp for EF1-α. A partition homogeneity test of the full data set indicated that they could be combined ($p = 0.60$). Alignment of the combined sequences gave rise to a data set of 889 characters (Fig. 5). Of these, 601 characters were parsimony-uninformative and 288 were parsimony-informative. Phylogenetic analyses were done on the combined data set of ribosomal DNA and the EF1-α intron region of five *Botryosphaeria* isolates from Colombia, and 14 isolates from GenBank

including *B. ribis, B. eucalyptorum, B. lutea* Phillips, *B. dothidea, B. rhodina* (Cooke) von Arx, *B. obtusa* (Schw.) Shoemaker and *B. stevensii* Shoemaker. The dataset thus consisted of 19 ingroup taxa and the outgroup taxon, *G. philoprina*. Two most parsimonious trees were obtained (length = 642 steps, consistency index = 0.822, retention index = 0.899, rescaled consistency index = 0.739, and g1 = -0.821) (Fig. 6). It showed that of the five *Botryosphaeria* isolates from Colombia, two (CMW 8922, CMW 8929), belonging to Group A, grouped in the *B. dothidea* clade with 100 % bootstrap support. The remaining three grouped closely with *B. ribis* with a 74 % bootstrap support. These three isolates (CMW 8961, CMW 8959, CMW 8949) belonged to the morphological Group B.

***Pathogenicity Tests***

**Greenhouse trial.** Six weeks after inoculation of *E. grandis* with six isolates of *Botryosphaeria* spp. representing *B. dothidea* (CMW 8922, CMW 8925, CMW 8929) and *B. ribis* (CMW 8949, CMW 8956, CMW 8961), the mean lesion lengths for isolates in the *B. ribis* group was significantly (P = 0.05) greater than that for the *B. dothidea* group (Table 4a-b, Figs 7-8). Of the *B. ribis* group isolates, CMW 8961 gave rise to the longest lesions (average lesion length 81.7 mm). Of the *B. dothidea* group isolates, CMW 8922 produced the longest lesions (average lesion length 40.7 mm). These two isolates were, therefore, selected for subsequent field pathogenicity trials.

**Field trials.** In general, results derived from the greenhouse and the four field pathogenicity trials were consistent in showing that the isolate of *B. ribis* was significantly more pathogenic than the isolate of *B. dothidea*. This fact was clearly seen in the significantly smaller (P = 0.0001) lesion lengths associated with *B. dothidea* than those caused by *B. ribis* (Figs 9-13). Lesions caused by *B. ribis* were also more variable in size on the different clones, while the lesions caused by *B. dothidea* were all similar in size (Figs. 9-13).

Lesions on the different clones inoculated in the different trials, formed a continuum of values. The smaller lesions, however, usually differed significantly from the larger lesions especially in the case of the *B. ribis* inoculations (Figs 10-13). The clones that were the least and the most resistant, however, was different in each trial. In the La

Suiza farm trial, clone 303 had the largest lesions while clone 20 was most resistant when inoculated with *B. ribis*. In contrast, for clones inoculated with *B. dothidea* clone 23 had the longest lesions and clone 303 had the shortest lesions (Table 5a-b, Fig. 10). Unfortunately, clones T210 and T211 inoculated with *B. dothidea*, and T210 and T211 inoculated with *B. ribis*, could not be included in this trial because an undue number of these trees had died due to other causes prior to the completion of the experiment. In the Cecilia farm trial, clone 2 was most susceptible while clone T211 was the most resistant to *B. ribis*. For inoculation with *B. dothidea* clone 4 was more susceptible than clone 27 (Table 6a-b, Fig. 11). In the Libano farm trial, lesion length in clone 2 was significantly higher than in clone 301 when they were inoculated with *B. ribis*. In addition, clone 18 was more susceptible than clone 11 when inoculated with *B. dothidea* (Table 7a-b, Fig. 12). In the Angela Maria farm trial, the susceptibility of clone 020 was significantly greater than that of clone T211 when inoculated with *B. ribis*. For inoculations with *B. dothidea,* clone 301 was more resistant than clone 23 (Table 8a-b, Fig. 13).

Results from the AMMI analysis of the interaction between *Botryosphaeria* isolates, *E. grandis* clones and sites, showed that for the four localities (La Suiza, Cecilia, Libano and Angela Maria) studied, differences were present in the amount of interaction at each trial site. Thus, Libano and Angela Maria had the lowest lesion lengths, but were nevertheless highly interactive as seen from their high positive scores (Fig. 14). In the analysis Cecilia is situated closer to the zero score line and this locality is expected to exhibit more stable pathogenicity around the 60 to 80 mm length. La Suiza had the highest average lesion lengths and was also highly interactive. Therefore, it can be expected to produce more variable results.

Data for the inoculated clones are scattered in a line from smaller lesions and relatively low scores through increasing lesion length and higher scores (Fig. 14). Clones inoculated with *B. dothidea* had the smallest lesions and their positive scores may be multiplied with the positive scores at Libano and Angela Maria to give a greater positive value. This implies that *B. dothidea* may result in greater pathogenicity at these localities. Conversely, the scores for these clones inoculated with *B. dothidea,* when multiplied with the negative scores of Cecilia and La Suiza would result in negative values. This implies that their lesion development is expected to be reduced in these

environments; therefore *B. dothidea* is not expected to become an important pathogen in the Cecilia and La Suiza environments. The same can be applied to the score values obtained for the different clones inoculated with *B. ribis* (Fig. 14) at the various locations.

## DISCUSSION

*Botryosphaeria* spp. were first recognised to be associated with cankers on *Eucalyptus* in Colombia approximately ten years prior to this study. However, the identity of the fungus had not been determined conclusively. Pathogenicity tests to determine the role of the fungus in disease had also not been conducted. This study represents the first detailed investigation of *Botryosphaeria* spp. on *Eucalyptus* in Colombia. Results have shown that two species of the fungus are associated with serious die-back of planted *Eucalyptus* in this country. They have also provided an enhanced understanding of the role of these fungi in disease.

*Botryosphaeria ribis* and *B. dothidea* were shown to both be associated with stem cankers and shoot die-back. These species have previously been associated with *Eucalyptus* diseases (Barnard *et al.* 1987, Davison & Tay 1983, Shearer *et al.* 1987, Smith *et al.* 2001, Webb 1983). Previous studies have, however, treated *B. dothidea* and *B. ribis* as aggregate species and the identity of the causal agent might not have been consistent with modern taxonomic treatments of the group (Slippers *et al.* 2003).

This study has shown that *B. ribis* is more common and substantially more pathogenic than *B. dothidea* on *Eucalyptus* in Colombia. To the best of our knowledge, this is the first definitive record of *B. ribis* causing serious disease of this important plantation species. Although it was previously recognized as an important pathogen of *Eucalyptus* in the past, it now seems that *B. dothidea* is relatively unimportant (Slippers *et al.* 2003). Future research aimed at reducing the impact of *Botryosphaeria* canker in Colombia should clearly focus on *B. ribis*.

In this study, we have included isolates from three different zones (Cauca, Valle and Andina) in Colombia, in which *Botryosphaeria* canker is an important disease of *E. grandis*. These areas differ substantially in climate but *B. ribis* was found to occur in

both cooler and warmer areas. Temperature, therefore, does not appear to be an important factor limiting the occurrence of the fungus. However, because *Botryosphaeria* spp. are typically stress related pathogens (Schoeneweiss 1980), climate may affect susceptibility of trees.

The AMMI analyses have shown that there are distinct interactions between site, clones inoculated and the two *Botryosphaeria* spp. present. Thus, at La Suiza and Cecilia *B. ribis* is clearly the most severe pathogen across clones. Although *B. dothidea* was, in general, only mildly pathogenic at Angela Maria, the high environmental score for this site indicates that it does seem to have the capacity to increase pathogenicity of the disease should conditions or the fungus change.

DNA sequence data were essential to identify the *Botryosphaeria* spp. recognised in this study. This has also been true in a number of recent studies considering species of the fungus (Jacobs & Rhener 1998, Slippers *et al.* 2003, Zhou & Stanosz 2001). However, once these species had been clearly defined, it was also possible to show that they could be distinguished based on morphology. This was best achieved based on conidial size, where *B. dothidea* has longer spores than those of *B. ribis*. This morphological characteristic should facilitate rapid identification of the two species in the future.

In this study, we found significant differences in pathogenicity between *B. dothidea* and *B. ribis*. Lesion lengths associated with *B. dothidea* inoculations were significantly smaller than those associated with *B. ribis,* in both greenhouse and field trials. In addition, this result was confirmed at sites across three major climatic zones where *E. grandis* and its hybrids are currently being planted. The consistent pathogenicity of *B. ribis* in all areas shows that it has the capacity to infect trees, assuming climatic conditions are conducive to infection.

Results of this study clearly show that different clones differ substantially in their susceptibility to infection by *B. ribis*. This is also consistent with observations of the natural occurrence of *Botryosphaeria* canker on different clones. Our results suggest that it will be possible to conduct field inoculation trials to select clones that are tolerant

to infection by the fungus. This would have many advantages for *Eucalyptus* forestry in Colombia.

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**Table 1.** *Botryosphaeria* isolates from *Eucalyptus grandis* used in this study.

| Isolate number (CMW) [a] | Locality / Zone | Altitude (masl) | Collector |
|---|---|---|---|
| 8922 | Andes / Andina | 2102 | M. J. Wingfield |
| 8923 | Andes / Andina | 2102 | M. J. Wingfield |
| 8924 | Andes / Andina | 2102 | M. J. Wingfield |
| 8925 | Andes / Andina | 2102 | M. J. Wingfield |
| 8926 | Andes / Andina | 2102 | M. J. Wingfield |
| 8927 | Andes / Andina | 2102 | M. J. Wingfield |
| 8928 | Andes / Andina | 2102 | M. J. Wingfield |
| 8929 | Andes / Andina | 2102 | M. J. Wingfield |
| 8930 | Carolina / Andina | 1700 | C. A. Rodas |
| 8931 | Carolina / Andina | 1700 | C. A. Rodas |
| 8932 | Selva / Andina | 2048 | C. A. Rodas |
| 8933 | Selva / Andina | 2048 | C. A. Rodas |
| 8934 | Selva / Andina | 2048 | C. A. Rodas |
| 8935 | Carolina / Andina | 1700 | C. A. Rodas |
| 8936 | Carolina / Andina | 1700 | C. A. Rodas |
| 8937 | Carolina / Andina | 1700 | C. A. Rodas |
| 8938 | Carolina / Andina | 1700 | C. A. Rodas |
| 8939 | Carolina / Andina | 1700 | C. A. Rodas |
| 8940 | Carolina / Andina | 1700 | C. A. Rodas |
| 8941 | Carolina / Andina | 1700 | C. A. Rodas |
| 8942 | Carolina / Andina | 1700 | C. A. Rodas |
| 8943 | Carolina / Andina | 1700 | C. A. Rodas |
| 8944 | Carolina / Andina | 1700 | C. A. Rodas |
| 8945 | Carolina / Andina | 1700 | C. A. Rodas |
| 8946 | Carolina / Andina | 1700 | C. A. Rodas |
| 8947 | Carolina / Andina | 1700 | C. A. Rodas |
| 8948 | Carolina / Andina | 1700 | C. A. Rodas |
| 8949 | Libano / Andina | 2102 | C. A. Rodas |
| 8950 | Sta Rosa / Cauca | 1750 | C. A. Rodas |
| 8951 | Cabuyerita / Cauca | 1750 | C. A. Rodas |
| 8952 | Guineal / Cauca | 1650 | C. A. Rodas |
| 8953 | Sta Maria / Cauca | 1850 | C. A. Rodas |
| 8955 | HatoFrio / Cauca | 2000 | C. A. Rodas |
| 8956 | HatoFrio / Cauca | 2000 | C. A. Rodas |
| 8957 | Vanessa / Cauca | 1000 | C. A. Rodas |
| 8958 | Ignacia / Cauca | 2000 | C. A. Rodas |
| 8959 | Ignacia / Cauca | 2000 | C. A. Rodas |
| 8960 | Alaska / Valle | 1870 | C. A. Rodas |
| 8961 | Estrella / Valle | 1469 | C. A. Rodas |
| 8962 | Volconda / Valle | 1700 | C. A. Rodas |
| 8963 | Cedral / Andina | 1839 | C. A. Rodas |
| 8964 | Carolina / Andina | 1700 | C. A. Rodas |
| 8965 | Aguabonita / Andina | 1950 | C. A. Rodas |
| 8966 | Buenos Aires/ Andina | 1900 | C. A. Rodas |

[a] Isolate numbers are those of the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

**Table 2.** Isolates of different *Botryosphaeria* species used in the phylogenetic studies. Isolates sequenced in this study are in bold.

| Isolate number [a] | Species | Host | Origin | Isolator | Genbank ITS | EF1-α |
|---|---|---|---|---|---|---|
| CMW 7772 | *Botryosphaeria ribis* | *Ribes* sp. | New York | B. Slippers/ G. Hudler | AY236935 | AY236877 |
| CMW 7773 | *B. ribis* | *Ribes* sp. | New York | B. Slippers/ G. Hudler | AY236936 | AY236878 |
| **CMW 8961** | ***B. ribis*** | ***Eucalyptus grandis*** | **Estrella, Colombia** | **C. A. Rodas** | **n.a.** | **n.a.** |
| **CMW 8959** | ***B. ribis*** | ***E. grandis*** | **Ignacia, Colombia** | **C. A. Rodas** | **n.a.** | **n.a.** |
| **CMW 8949** | ***B. ribis*** | ***E. grandis*** | **Libano, Colombia** | **C. A. Rodas** | **n.a.** | **n.a.** |
| CMW 9078 | *B. parva* | *Actinidia deliciosa* | New Zealand | S. R. Pennycook | AY236940 | AY236885 |
| CMW 9080 | *B. parva* | *Populus nigra* | New Zealand | G. J. Samuels | AY236942 | AY236887 |
| CMW 10125 | *B. eucalyptorum* | *E. grandis* | Mpumalanga, S. A. | H. Smith | AF283686 | AY236891 |
| CMW10126 | *B .eucalyptorum* | *E. grandis* | Mpumalanga, S. A. | H. Smith | AF283687 | AY236892 |
| CMW 9076 | *B. lutea* | *Malus x domestica* | New Zeland | S. R. Pennycook | AY236946 | AY236893 |
| CMW 992 | *F. luteum* | *A. deliciosa* | New Zeland | G. J. Samuels | AF027745 | AY236894 |
| **CMW 8922** | ***B. dothidea*** | ***E. grandis*** | **Andes, Colombia** | **C. A. Rodas** | **n.a.** | **n.a.** |
| **CMW 8929** | ***B. dothidea*** | ***E. grandis*** | **Andes, Colombia** | **C. A. Rodas** | **n.a.** | **n.a.** |
| CMW 8000 | *B. dothidea* | *Prunus* sp. | Crocifisso, Switzerland | B. Slippers | AY236949 | AY236898 |
| CMW 7999 | *B. dothidea* | *Ostrya* sp. | Crocifisso, Switzerland | B. Slippers | AY236948 | AY236897 |
| CMW 0130 | *B. rhodina* | *Vitex doniana* | Uganda | J. Roux | AY236952 | AY236901 |
| CMW 9074 | *B. rhodina* | *Pinus* sp. | Mexico | T. Burgess | AY236952 | AY236901 |
| CMW 7774 | B. obtusa | *Ribes* sp. | New York | B. Slippers/ G. Hudler | AY236953 | AY236902 |
| CMW 7060 | B. stevensii | *Fraxinus excelsior* | Netherlands | H. A van der Aa | AY236955 | AY236904 |
| CMW 7063 | Guignardia philoprina | *Taxus baccata* | Netherlands | H. A. van der Aa | AY236956 | AY236905 |

[a] Designation of culture collections: CMW = Culture collection of the Forestry and Agricultural Biotechnology Institute, (FABI), University of Pretoria, Pretoria, S.A.

**Table 3.** Differences in conidial size of isolates of *Botryosphaeria* spp. from Colombia.

| Isolate[a] Group | CMW[b] | Length Min. | Length Average | Length Max. | Width Min | Width Average | Width Max. |
|---|---|---|---|---|---|---|---|
| ***Botryosphaeria dothidea*** | 8922 | 27.5 | 29.2 | 30.0 | 4.1 | 5.0 | 6.8 |
| **(Group A)** | 8923 | 25.0 | 27.0 | 30.0 | 5.0 | 5.5 | 7.5 |
| | 8924 | 25.0 | 26.0 | 27.5 | 5.0 | 5.0 | 5.0 |
| | 8925 | 25.0 | 26.7 | 27.5 | 5.0 | 5.0 | 5.0 |
| | 8926 | 27.5 | 29.0 | 32.5 | 5.0 | 5.5 | 7.5 |
| | 8927 | 27.5 | 31.2 | 35.0 | 5.0 | 5.2 | 7.5 |
| | 8928 | 25.0 | 28.2 | 32.5 | 5.0 | 5.5 | 7.5 |
| | 8929 | 17.5 | 26.5 | 27.5 | 5.0 | 5.2 | 7.5 |
| **Average of 80 conidia** | | **25.0** | **27.9** | **30.3** | **4.8** | **5.2** | **6.7** |
| ***B. ribis*** **(Group B)** | 8930 | 17.5 | 19.5 | 22.5 | 5.0 | 5.4 | 5.1 |
| | 8932 | 20.9 | 22.7 | 25.2 | 5.1 | 5.4 | 5.2 |
| | 8933 | 16.0 | 16.8 | 19.0 | 4.6 | 5.3 | 6.6 |
| | 8934 | 22.6 | 25.2 | 28.6 | 5.0 | 5.8 | 6.6 |
| | 8935 | 18.8 | 20.1 | 21.4 | 4.7 | 5.4 | 6.0 |
| | 8936 | 18.1 | 19.5 | 22.1 | 4.9 | 5.5 | 6.3 |
| | 8937 | 15.6 | 17.5 | 18.5 | 4.9 | 5.3 | 6.1 |
| | 8938 | 15.6 | 19.0 | 21.5 | 4.3 | 5.6 | 6.8 |
| | 8939 | 17.0 | 19.3 | 21.4 | 3.7 | 4.3 | 5.0 |
| | 8940 | 17.2 | 18.9 | 20.6 | 3.7 | 4.7 | 5.1 |
| | 8941 | 23.4 | 26.1 | 30.8 | 6.1 | 5.6 | 9.1 |
| | 8942 | 20.7 | 22.9 | 25.5 | 5.1 | 6.6 | 7.6 |
| | 8944 | 19.9 | 22.9 | 24.7 | 5.3 | 5.8 | 6.4 |
| | 8945 | 17.6 | 19.5 | 22.1 | 5.2 | 5.6 | 6.2 |
| | 8946 | 16.6 | 19.2 | 20.5 | 4.3 | 5.6 | 6.8 |
| | 8947 | 17.2 | 19.8 | 21.2 | 5.1 | 5.8 | 6.7 |
| | 8948 | 15.0 | 16.5 | 17.5 | 5.0 | 5.2 | 7.5 |
| | 8949 | 17.5 | 19.2 | 22.5 | 5.0 | 5.5 | 7.5 |
| | 8950 | 22.5 | 24.5 | 30.0 | 5.0 | 6.0 | 7.5 |
| | 8951 | 20.0 | 23.5 | 25.0 | 5.0 | 5.3 | 7.5 |
| | 8952 | 20.0 | 24.0 | 25.0 | 5.0 | 6.2 | 7.5 |
| | 8956 | 22.5 | 24 | 25.0 | 5.0 | 7.0 | 7.5 |
| | 8957 | 20.0 | 21.2 | 22.5 | 5.0 | 6.0 | 7.5 |
| | 8958 | 17.5 | 21.0 | 22.5 | 4.7 | 5.8 | 7.5 |
| | 8959 | 15.6 | 17.6 | 20.0 | 4.5 | 5.3 | 6.4 |
| | 8960 | 15.0 | 18.5 | 22.5 | 5.0 | 5.2 | 7.5 |
| | 8961 | 17.3 | 18.6 | 19.8 | 4.0 | 5.0 | 5.7 |
| | 8962 | 17.5 | 19.7 | 22.5 | 5.0 | 7.0 | 7.5 |
| | 8963 | 17.5 | 19.0 | 22.5 | 5.0 | 6.2 | 7.5 |
| | 8965 | 12.5 | 15.5 | 20.0 | 5.0 | 5.5 | 7.5 |
| | 8966 | 17.5 | 18.7 | 22.5 | 5.0 | 6.7 | 7.5 |
| **Average of 310 conidia** | | **18.1** | **20.3** | **22.7** | **4.8** | **5.6** | **6.8** |

[a] Ten conidia were measured for each isolate.

[b] Designation of the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute, (FABI), University of Pretoria.

**Table 4.** Comparison of lesion lengths (mm) **(a)** and analysis of variance **(b)** of internal lesion lengths on an *Eucalyptus grandis* clone (ZG 14) inoculated with six isolates of *Botryosphaeria ribis* and *B. dothidea* in the greenhouse.

**(a)**

| ***Botryosphaeria dothidea*** | | ***Botryosphaeria ribis*** | |
|---|---|---|---|
| **Isolate** | **Mean** | **Isolate** | **Mean** |
| CMW 8922 | 40.7 | CMW 8949 | 76.8 |
| CMW 8925 | 30.1 | CMW 8956 | 76.4 |
| CMW 8929 | 40.9 | CMW 8961 | 81.7 |
| **Average** | **37.2** | | **78.3** |
| Control | 12.9 | | 12.9 |
| S.E.M of trial | 13.46 | | |

The difference between average of *B. dothidea* and *B. ribis* is statistically significant at $P < 0.05$.

**(b)**

| **Source** | **DF** | **MS** | **F value** | ***P* value** |
|---|---|---|---|---|
| Isolates | 6 | 7207.8 | 7.96 | 0.0001 |
| Error | 61 | 905.8 | | |

**Table 5.** Mean lesion lengths (mm) **(a)** and analysis of variance **(b)** resulting from inoculation of *Eucalyptus grandis* clones with isolates of *Botryosphaeria dothidea* and *B. ribis* at the farm La Suiza.

**(a)**

| *Botryosphaeria dothidea* | | | *Botryosphaeria ribis* | | |
|---|---|---|---|---|---|
| **Clone** | **Mean** | **% Susceptibility [a]** | **Clone** | **Mean** | **% Susceptibility [a]** |
| 23 | 35.8 | 100 | 303 | 217.6 | 100 |
| 2 | 34.8 | 97.2 | 4 | 195.5 | 89.8 |
| 27 | 32.8 | 91.5 | 2 | 191.4 | 88.0 |
| 12 | 31.3 | 87.3 | 12 | 189.9 | 87.3 |
| 20 | 27.5 | 76.8 | 20 | 177.3 | 81.5 |
| 18 | 26.3 | 73.4 | 11 | 172.1 | 79.1 |
| 11 | 25.9 | 72.4 | 210 | 167.3 | 76.9 |
| 210 | 20.8 | 58.2 | 23 | 165.0 | 75.8 |
| 211 | 18.9 | 52.8 | 211 | 162.0 | 74.4 |
| 303 | 18.9 | 52.7 | 18 | 137.9 | 63.4 |
| 301 | 17.9 | 50.0 | 27 | 108.2 | 49.7 |
| 4 | 17.9 | 49.8 | 301 | 86.3 | 39.7 |
| **S.E.M** | **4.22** | | **S.E.M** | **17.7** | |

[a] % Susceptibility = lesion lengths as a percent of most susceptible clone. eg Clone N$^0$ 23 = 35.8 / 35.8 = 100%.

**(b)**

| **Source** | **DF** | **MS** | **F value** | ***P* value** |
|---|---|---|---|---|
| Blocks | 9 | 3836.9 | 1.33 | 0.2191 |
| Isolates | 1 | 1025195.5 | 355.4 | 0.0001 |
| Clones | 11 | 12440.0 | 4.31 | 0.0001 |
| Isolates x clones | 11 | 15845.9 | 5.49 | 0.0001 |
| Error | 408 | 2884.7 | | |

**Table 6.** Mean lesion lengths (mm) **(a)** and analysis of variance **(b)** resulting from inoculation of *Eucalyptus grandis* clones with isolates of *Botryosphaeria dothidea* and *B. ribis* at the farm Cecilia.

**(a)**

| *Botryosphaeria dothidea* | | | *Botryosphaeria ribis* | | |
|---|---|---|---|---|---|
| **Clone** | **Mean** | **% Susceptibility [a]** | **Clone** | **Mean** | **% Susceptibility [a]** |
| 27 | 45.6 | 100 | 2 | 157.0 | 100 |
| 23 | 42.5 | 93.2 | 303 | 152.0 | 96.8 |
| T210 | 35.0 | 76.8 | 12 | 134.6 | 85.7 |
| 210 | 32.8 | 71.8 | 23 | 127.8 | 81.4 |
| 12 | 30.2 | 66.3 | 211 | 125.7 | 80.1 |
| T211 | 30.2 | 66.1 | 27 | 125.7 | 80.1 |
| 18 | 27.5 | 60.3 | 4 | 124.6 | 79.4 |
| 2 | 26.0 | 57.1 | 210 | 116.9 | 74.4 |
| 211 | 23.4 | 51.2 | 18 | 114.7 | 73.1 |
| 301 | 23.3 | 51.0 | 11 | 108.1 | 68.9 |
| 20 | 22.1 | 48.5 | 20 | 107.2 | 68.3 |
| 303 | 21.2 | 46.5 | 301 | 82.7 | 52.7 |
| 11 | 17.1 | 37.5 | T210 | 36.7 | 23.4 |
| 4 | 15.2 | 33.3 | T211 | 22.9 | 14.6 |
| **S.E.M** | **3.56** | | **S.E.M** | **4.83** | |

[a] % Susceptibility = lesion lengths as a percent of most susceptible clone.

**(b)**

| **Source** | **DF** | **MS** | **F value** | ***P* value** |
|---|---|---|---|---|
| Blocks | 9 | 10924.5 | 5.53 | 0.0001 |
| Isolates | 1 | 602729.7 | 305.0 | 0.0001 |
| Clones | 13 | 7500.8 | 380 | 0.0001 |
| Isolates x clones | 13 | 7615.14 | 390 | 0.0001 |
| Error | 370 | 1976.2 | | |

The interaction between isolates and clones indicates that the clones which are susceptible to *B. dothidea* are not necessarily also susceptible to *B. ribis*.

**Table 7.** Mean lesion lengths (mm) **(a)** and analysis of variance **(b)** resulting from inoculation of *Eucalyptus grandis* clones with isolates of *Botryosphaeria dothidea* and *B. ribis* at the farm Libano.

(a)

| *Botryosphaeria dothidea* | | | *Botryosphaeria ribis* | | |
|---|---|---|---|---|---|
| **Clone** | **Mean** | **% Susceptibility [a]** | **Clone** | **Mean** | **% Susceptibility [a]** |
| 18 | 19.0 | 100 | 2 | 46.5 | 100 |
| 20 | 18.9 | 99.4 | 20 | 45.4 | 97.6 |
| 303 | 18.0 | 94.9 | 18 | 45.2 | 97.3 |
| 210 | 17.6 | 92.4 | 12 | 39.9 | 85.9 |
| 211 | 17.1 | 90.0 | 303 | 39.1 | 84.2 |
| 27 | 15.5 | 81.7 | 23 | 38.5 | 82.8 |
| 23 | 15.3 | 80.7 | 11 | 37.9 | 81.5 |
| 12 | 14.8 | 77.9 | 4 | 35.4 | 76.1 |
| 4 | 14.7 | 77.6 | 27 | 35.0 | 75.3 |
| 301 | 14.7 | 77.3 | 210 | 30.5 | 65.6 |
| T210 | 14.6 | 77.0 | T211 | 26.8 | 57.7 |
| T211 | 14.5 | 76.1 | 211 | 26.5 | 57.0 |
| 2 | 13.7 | 72.1 | T210 | 22.2 | 47.8 |
| 11 | 12.8 | 67.3 | 301 | 17.3 | 37.3 |
| **S.E.M** | **1.73** | | **S.E.M** | **5.43** | |

[a] % Susceptibility = lesion lengths as a percent of most susceptible clone.

(b)

| **Source** | **DF** | **MS** | **F value** | ***P* value** |
|---|---|---|---|---|
| Blocks | 9 | 2929.2 | 8.03 | 0.0001 |
| Isolates | 1 | 44605.3 | 122.27 | 0.0001 |
| Clones | 13 | 869.5 | 2.38 | 0.0042 |
| Isolates x clones | 13 | 695.9 | 1.91 | 0.0273 |
| Error | 473 | 364.8 | | |

Isolates and clones interaction significant because of rank differences of clones under two isolates.

**Table 8.** Mean lesion lengths (mm) **(a)** and analysis of variance **(b)** resulting from inoculation of *Eucalyptus grandis* clones with isolates of *Botryosphaeria dothidea* and *B. ribis* at the farm Angela Maria.

**(a)**

| *Botryosphaeria dothidea* | | | *Botryosphaeria ribis* | | |
|---|---|---|---|---|---|
| **Clone** | **Mean** | **% Susceptibility [a]** | **Clone** | **Mean** | **% Susceptibility [a]** |
| 23 | 36.4 | 100 | 20 | 81.2 | 100 |
| T211 | 28.6 | 78.5 | 2 | 75.2 | 92.6 |
| 18 | 25.9 | 71.1 | 4 | 73.1 | 90.1 |
| 210 | 24.8 | 68.1 | 12 | 66.6 | 82.1 |
| 2 | 24.3 | 66.6 | 210 | 66.2 | 81.5 |
| 12 | 23.3 | 64.1 | 27 | 65.9 | 81.1 |
| T210 | 22.4 | 61.4 | 11 | 59.2 | 73 |
| 27 | 21.5 | 59.0 | 211 | 50.1 | 61.7 |
| 20 | 20.8 | 57.1 | 3 | 48.0 | 59.2 |
| 11 | 19.5 | 53.6 | 18 | 46.9 | 57.7 |
| 211 | 18.0 | 49.3 | 23 | 43.9 | 54.1 |
| 4 | 17.3 | 47.6 | 301 | 33.2 | 40.9 |
| 303 | 15.5 | 42.4 | T-210 | 26.6 | 32.7 |
| 301 | 14.2 | 39.1 | T-211 | 21.9 | 27 |
| **S.E.M** | **3.86** | | **S.E.M** | **8.52** | |

[a] % Susceptibility = lesion lengths as a percent of most susceptible clone.

**(b)**

| **Source** | **DF** | **MS** | **F value** | ***P* value** |
|---|---|---|---|---|
| Blocks | 9 | 1442.80 | 1.27 | 0.2525 |
| Isolates | 1 | 112951.9 | 233.74 | 0.0001 |
| Clones | 13 | 3041.65 | 395 | 0.0001 |
| Isolates x clones | 13 | 3693.31 | 3.82 | 0.0001 |
| Error | 459 | 890.96 | | |

The interaction is highly significant indicating that the rank of clones under one isolate differs from that under the other isolate.

**Fig. 1.** Disease symptoms associated with *Botryosphaeria* infection on *Eucalyptus grandis*. **(a)** Die-back of the leader shoots. **(b)** Lesion beginning at branching points of twigs. **(c-d)** Cankers on the stems and branches. **(e-f)** Internal lesions in sapwood associated with die-back.

**Fig. 2.** Geographic areas in Colombia where samples of diseased *Eucalyptus grandis* were collected.


Andina Zone
Valle Zone
Cauca Zone
50 0 50 100 150 200 250 Kilometers

**Fig. 3.** Procedures involved in the inoculation of *Botryosphaeria* spp. on *Eucalyptus grandis*. **(a)** Wounding of stem with cork borer. **(b)** Culture of fungus to be inoculated showing discs of mycelium of equal size to wounds on stem. **(c)** Mycelial disc being placed in a wound. **(d)** Inoculated area covered with moistened filter paper and secured with masking tape.

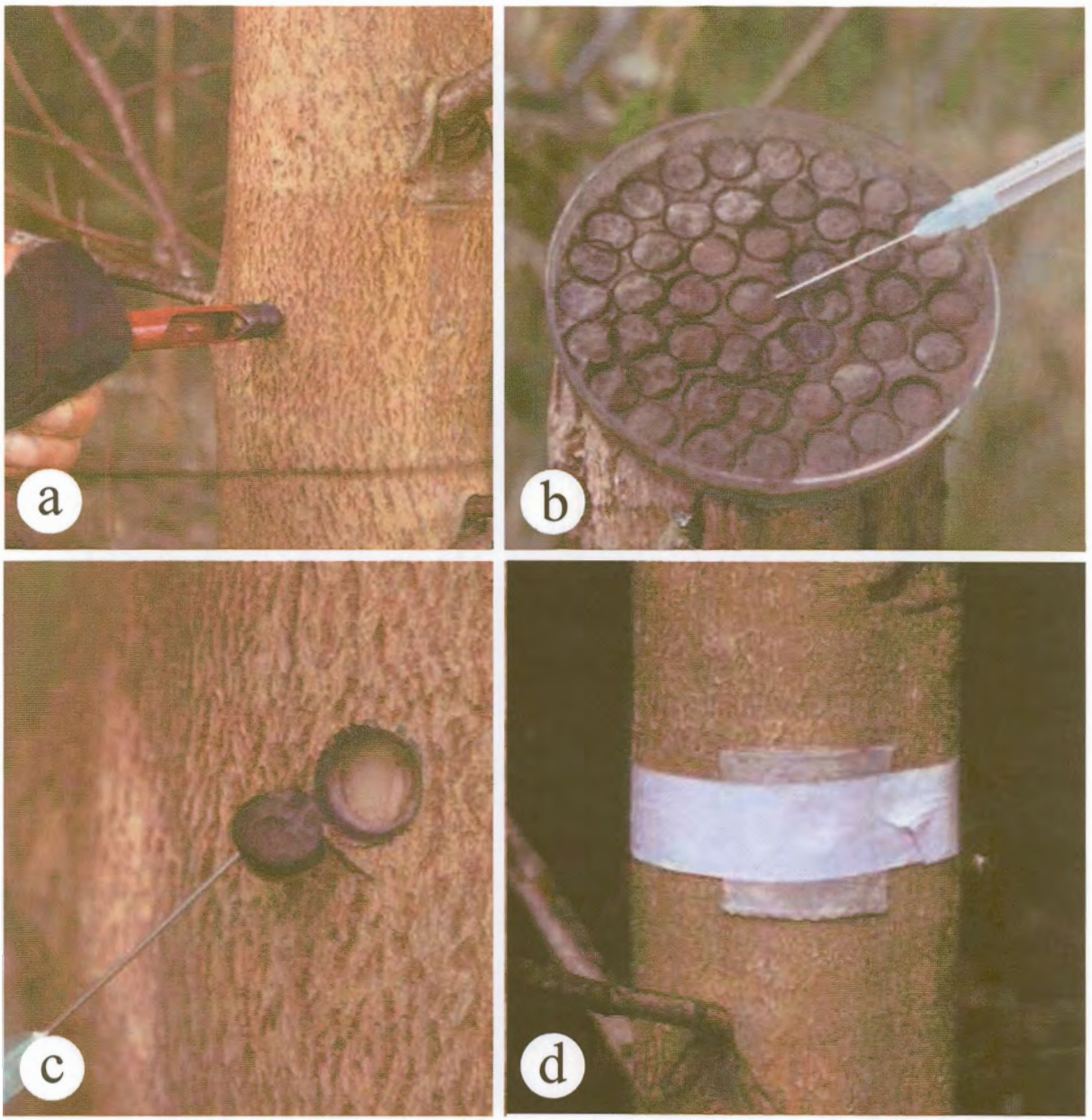


**Fig. 4.** Cultural and conidial characteristics of two *Botryosphaeria* spp. from *Eucalyptus grandis* in Colombia. **(a)** Growth of *B. ribis* in culture after 4 days (left) and 20 days (right). **(b)** Conidia of *B. ribis*. **(c)** Growth of *B. dothidea* in culture after 4 days (left) and 20 days (right). **(d)** Conidia of *B. dothidea*. Bars Figs. b, d = 10 µm.

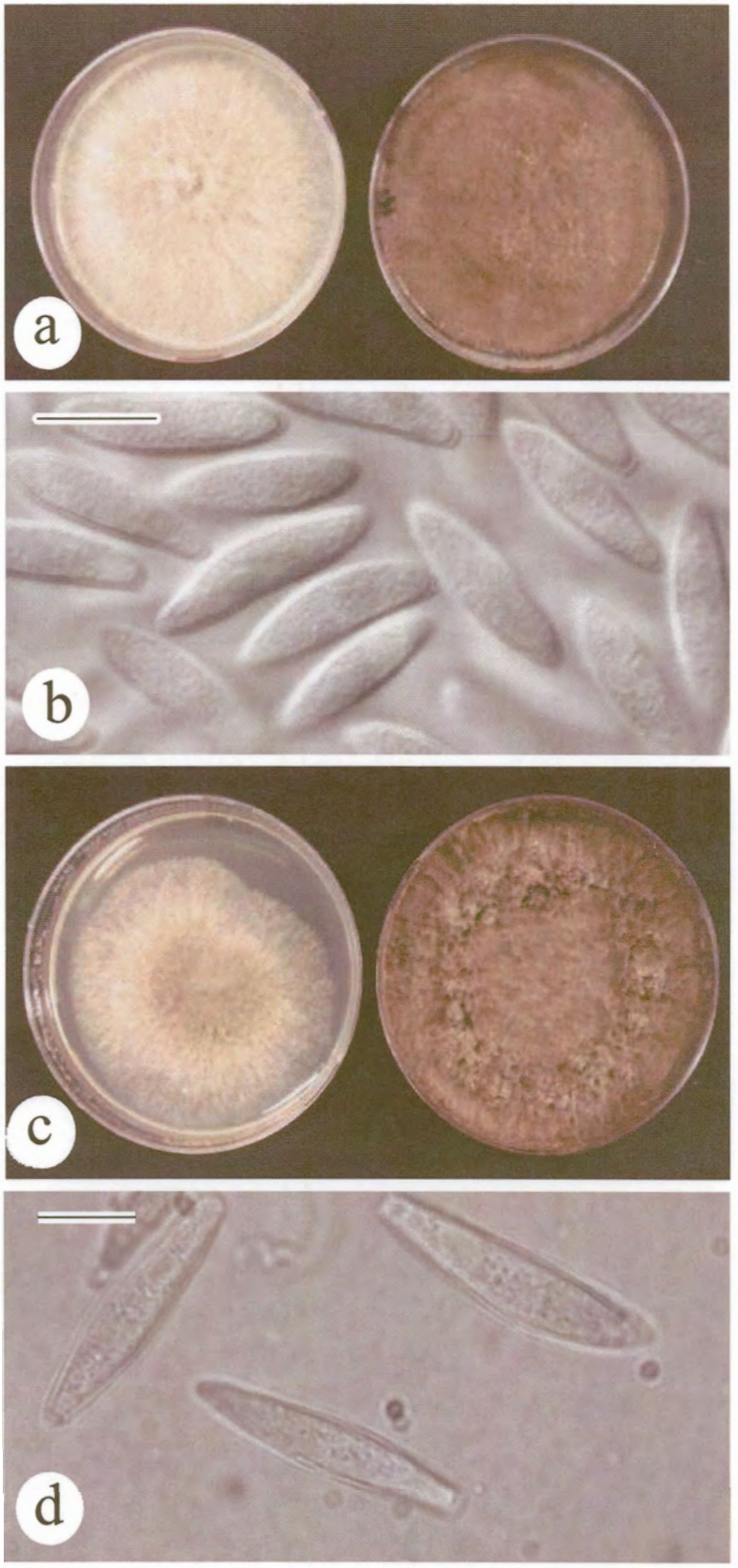

a
b
c
d

**Fig. 5.** Raw sequence data of the ITS 1/2 region and 5.8S rRNA gene and EF1-α gene and intron region for various *Botryosphaeria* spp. Unknown sequence characters are indicated with a "**N**", while gaps inserted to achieve sequences alignment are indicated with "-". Bases matching those of CMW 7772 are indicated with a ".".

```
[                  10         20         30         40         50]

CMW7772     GAAGGATCAT TACCGAGTTG ATTCGAGCTC CGGCTCGACN -TC-TCCCAC
CMW7773     .......... .......... .......... .........N ..........
CMW8959     .......... .......... .......... .........N ..........
CMW8961     NNNNNNNNNN NNNNNNNN.. .......... .........N ..........
CMW8949     NNNNNNN... .......... .......... .........N ..........
CMW9078     .......... .......... .......... .........N ..........
CMW9080     .......... .......... .......... .........N ..........
CMW10125    .......... .......... .C........ .........N ..........
CMW10126    .......... .......... .C........ .........N ..........
CMW9076     .......... .......... .......... .........N ..........
CMW992      .......... .......... .......... .........N ..........
CMW8922     .......... .......... .....G.... ....C...-N ...C......
CMW8929     .......... .......... .....G.... ....C...-N ...C......
CMW8000     .......... .......... .....G.... ....C...-N ...C......
CMW7999     .......... .......... .....G.... ....C...-N ...C......
CMW10130    .......... .........- -....G...T .........N .....N....
CMW9074     .......... .........- -......... .........N ..........
CMW7774     ....N..... .........- C.-..G...T .........AN ..........
CMW7060     .......... .........- C.-.......T .........AN ..........
CMW7063     .......... .........- ---------- -----.AC.AN C...-..A.A

[                  60         70         80         90        100]

CMW7772     CCAATGTGTA CCTACCTCTG TTGCTTTGGC GGGCCGCGGT CCT--CCGC-
CMW7773     .......... .......... .......... .......... ..........
CMW8959     ........C. .......... .......... .......... ..........
CMW8961     .......... .......... .......... .......... ..........
CMW8949     .......... .......... .......... .......... ..........
CMW9078     ..T....... .......... .......... .......... ..........
CMW9080     ..T....... .......... .......... .......... ..........
CMW10125    ..T....... .......... .......... .......... ..........
CMW10126    ..T....... .......... .......... .......... ..........
CMW9076     ..C....... .......... .......... .......... ..........
CMW992      ..C....... .......... .......... .......... ..........
CMW8922     ..TT...... .......... .......... .......... T........G
CMW8929     ..TT...... .......... .......... .......... .........G
CMW8000     ..TT...... .......... .......... .......... .........G
CMW7999     ..TT...... .......... .......... .......... .........G
CMW10130    ..TT....A. .G........ .......... ..-.-----. .--..---.N
CMW9074     ..TT....A. .G........ .......... ..-.-----. .--..---.G
CMW7774     ..TT....A. .A........ .......... ..-.-----. .T.TG....G
CMW7060     ..TT....A. .A........ .......... ..-.-----. .T..G....G
CMW7063     ..C.....A. .A.....A.T G.TGC..C.G C..G------ AT..G..C.G
```

```
[                  110         120         130         140         150]

CMW7772      ACCGG-CGCC C-TT--CG-G GGGGGCTGGC CA----GCGC ---CCGCCAG
CMW7773      .......... .......... .......... .......... ..........
CMW8959      .......... .......... ....-..... .......... ..........
CMW8961      .......... .......... .......... .......... ..........
CMW8949      .......... .......... ....-..... .......... ..........
CMW9078      .......... .......... ....-..... .......... ..........
CMW9080      .......... .......... ....-..... .......... ..........
CMW10125     .......T.. .T....-... ....-..... .........T ..........
CMW10126     .......T.. .T....-... ....-..... .........T ..........
CMW9076      ....AC.C.. G......... .....-.C... .......... ..........
CMW992       ....AC.C.. G......... .....-.C... .......... ..........
CMW8922      G...-C.C.. ...CCC.... .....--...T .......... ..........
CMW8929      G...-C.C.. ...CCC.... .....--...T .......... ..........
CMW8000      G....C.C.. ...CCC.... .....--.... .......... ..........
CMW7999      G....C.C.. ...CCC.... .....--.... .......... ..........
CMW10130     G----.---- -.--.--.- ---------- --......... .........A
CMW9074      G----.---- -.--.--.- ---------- --......... .........A
CMW7774      .--..AG... ...-....C. ..CC--CCC. .-..GC.... TTT.......
CMW7060      T--..AG... ...-...AAA AA.C--CCC. .C..GT.... T.T.......
CMW7063      ---..C-... -..CGTGT.- --.C--CCCG G.TCAG.... .........TA

[                  160         170         180         190         200]

CMW7772      AGGACCAT-A AAACTCCAGT CAGTGAAC-T TCGCAGTCTG AAAAA-C-AA
CMW7773      .......... .......... .......... .......... ..........
CMW8959      .......... .......... .......... .......... ..........
CMW8961      .......... .......... .......... .......... ..........
CMW8949      .......... .......... .......... .......... ..........
CMW9078      .......... .......... .......... .......... ..........
CMW9080      .......... .......... .......... .......... ..........
CMW10125     .......-C. .......... ....A...G. .-........ ..........
CMW10126     .......-C. .......... ....A...G. .-........ ..........
CMW9076      .......-C. .......... ....A...G- .......... .G........
CMW992       .......-C. .......... ....A...G- .......... .G........
CMW8922      ........C. ..-....... ....A...GA C-........ .....A...T
CMW8929      ........C. ..-....... ....A...GA C-........ .....A...T
CMW8000      ........C. ..-....... ....A...GA .-........ .........T
CMW7999      ........C. ..-....... ....A...GA .-........ .........T
CMW10130     ......T.C. ..-....... ....A...GC A-.AC..... .T........
CMW9074      ......T.C. ..-....... ....A...GC A-.AC..... .T........
CMW7774      ......T.C. ..-....... ....A...G- ...AC..... .T........
CMW7060      ......T.C. ..-....... ....A...G- ...AC..... ..........
CMW7063      G.A.A.T..- ..-...TT.. TTTATTTTG- GAATCT.... .GT.GTTTTT
```

```
[                  210         220         230         240         250]

CMW7772      GTTAATAAAC TAAAACTTTC AACAACGGAT CTCTTGGTTC TGGCATCGAT
CMW7773      .......... .......... .......... .......... ..........
CMW8959      .......... .......... .......... .......... ..........
CMW8961      .......... .......... .......... .......... ..........
CMW8949      .......... .......... .......... .......... ..........
CMW9078      .......... .......... .......... .......... ..........
CMW9080      .......... .......... .......... .......... ..........
CMW10125     .......... .......... .......... .......... ..........
CMW10126     .......... .......... .......... .......... ..........
CMW9076      .......... .......... .......... .......... ..........
CMW992       .......... .......... .......... .......... ..........
CMW8922      -......... .......... .......... .......... ..........
CMW8929      -......... .......... .......... .......... ..........
CMW8000      -......... .......... .......... .......... ..........
CMW7999      -......... .......... .......... .......... ..........
CMW10130     .......... .......... .......... .......... ..........
CMW9074      .......... .......... .......... .......... ..........
CMW7774      .......... .......... .......... .......... ..........
CMW7060      .......... .......... .......... .......... ..........
CMW7063      ACA......T A......... .......... .......... ..........

[                  260         270         280         290         300]

CMW7772      GAAGAACGCA GCGAAATGCG ATAAGTAATG TGAATTGCAG AATTCAGTGA
CMW7773      .......... .......... .......... .......... ..........
CMW8959      .......... .......... .......... .......... ..........
CMW8961      .......... .......... .......... .......... ..........
CMW8949      .......... .......... .......... .......... ..........
CMW9078      .......... .......... .......... .......... ..........
CMW9080      .......... .......... .......... .......... ..........
CMW10125     .......... .......... .......... .......... ..........
CMW10126     .......... .......... .......... .......... ..........
CMW9076      .......... .......... .......... .......... ..........
CMW992       .......... .......... .......... .......... ..........
CMW8922      .......... .......... .......... .......... ..........
CMW8929      .......... .......... .......... .......... ..........
CMW8000      .......... .......... .......... .......... ..........
CMW7999      .......... .......... .......... .......... ..........
CMW10130     .......... .......... .......... .......... ..........
CMW9074      .......... .......... .......... .......... ..........
CMW7774      .......... .......... .......... .......... ..........
CMW7060      .......... .......... .......... .......... ..........
CMW7063      .......... .......... .......... .......... ..........
```

```
[                  310         320         330         340         350]

CMW7772      ATCATCGAAT CTTTGAACGC ACATTGCGCC CCTTGGTATT CCGAGGGGCA
CMW7773      .......... .......... .......... .......... ..........
CMW8959      .......... .......... .......... .......... ..........
CMW8961      .......... .......... .......... .......... ..........
CMW8949      .......... .......... .......... .......... ..........
CMW9078      .......... .......... .......... .......... ..........
CMW9080      .......... .......... .......... .......... ..........
CMW10125     .......... .......... .......... .......... ..........
CMW10126     .......... .......... .......... .......... ..........
CMW9076      .......... .......... .......... .......... ..........
CMW992       .......... .......... .......... .......... ..........
CMW8922      .......... .......... .......... .T........ ....A.....
CMW8929      .......... .......... .......... .T........ ....A.....
CMW8000      .......... .......... .......... .T........ ....A.....
CMW7999      .......... .......... .......... .T........ ....A.....
CMW10130     .......... .......... .......... .......... ...G......
CMW9074      .......... .......... .......... .......... ...G......
CMW7774      .......... .......... .......... ..C...C... ...G......
CMW7060      .......... .......... .......... ......C... ..........
CMW7063      .......... .......... .......... .GCCA..... .T.GC.....

[                  360         370         380         390         400]

CMW7772      TGCCTGTTCG AGCGTCATTT CAACCCTCAA GCT-CT---G CTTGGTATTG
CMW7773      .......... .......... .......... .......... ..........
CMW8959      .......... .......... .......... .......... ..........
CMW8961      .......... .......... .......... .......... ..........
CMW8949      .......... .......... .......... .......... ..........
CMW9078      .......... .......... .......... .......... ..........
CMW9080      .......... .......... .......... .......... ..........
CMW10125     .......... .......... .......... ...T-..... ..........
CMW10126     .......... .......... .......... ...T-..... ..........
CMW9076      .......... .......... .......... .......... ..........
CMW992       .......... .......... .......... .......... ..........
CMW8922      .......... .........A .......... .......... ..........
CMW8929      .......... .........A .......... .......... ..........
CMW8000      .......... .........A .......... .......... ..........
CMW7999      .......... .........A .......... .......... ..........
CMW10130     .......... .........A .......... .......... .....A....
CMW9074      .......... .........A .......... .......... .....A....
CMW7774      .......... .........A .......... .......... ..........
CMW7060      .......... .........A .......... .......... ..........
CMW7063      .......CT. .......... .........T ..CC..AGG. .G....G...
```

```
[                  410         420         430         440         450]

CMW7772      GGCTCCGTCC TCCA---CGG ACGCGCC--T TAAAGACCTC GGCGGTGGC-
CMW7773      .......... .......... .......... .......... ..........
CMW8959      ....T..... .......... .......... .......... ..........
CMW8961      .......... .......... .......... .......... ..........
CMW8949      ....T..... .......... .......... .......... ..........
CMW9078      ...C...... .......... .......... .......... ..........
CMW9080      ...C...... .......... .......... .......... ..........
CMW10125     ...C...... .-.T..GT.. .......... C......... ..........
CMW10126     ...C...... .-.T..GT.. .......... C......... ..........
CMW9076      .......... .-.T..GT.. .......... CG........ ..........
CMW992       .......... .-.T..GT.. .......... CG........ ..........
CMW8922      ...A...... .--T.TG... G......... C......... ..........
CMW8929      ...A...... .--T.TG... G......... C......... ..........
CMW8000      ...A...... .--T.TG... G......... C......... ..........
CMW7999      ...A...... .--T.TG... G......... C......... ..........
CMW10130     ...A...... .-..CTG... .......... C......... .........T
CMW9074      ...A...... .-..CTG... .......... C......... .........T
CMW7774      ...G...... .-.TCTG... .......... .......... .........T
CMW7060      ...GA..... .-.TCTG... .......... C......... .........T
CMW7063      ..GAT..G.. AAAGCCCGC. .G.GA.GGCC GGCCCCTAAA TCTA.....G

[                  460         470         480         490         500]

CMW7772      GTCTTGCC-T CAAGCGTAGT AGAAAA--CA CCTCGCTTTG GAGCGCACGG
CMW7773      .......... .......... .......... .......... ..........
CMW8959      .......... .......... .......... .......... ..........
CMW8961      .......... .......... .......... .......... ..........
CMW8949      .......... .......... .......... .......... ..........
CMW9078      .......... .......... .......... .......... ..........
CMW9080      .......... .......... .......... .......... ..........
CMW10125     .......... .......... ....-.T... .......... .......T..
CMW10126     .......... .......... ....-.T... .......... .......T..
CMW9076      .......... .......... .......... .......... ..........
CMW992       .......... .......... .......... .......... ..........
CMW8922      .......... .......... ....C.TA.. T.......C. .......G..
CMW8929      .......... .......... ....C.TA.. T.......C. .......G..
CMW8000      .......... .......... ....C.TA.. T.......C. .......G..
CMW7999      .......... .......... ....C.TA.. T.......C. .......G..
CMW10130     ..TCA...C. .......... .....--TA.. .......... .....GTT..
CMW9074      ..TCA...C. .......... .....--TA.. .......... .....GTT..
CMW7774      ..TCA...C. .......... .....--TA.. .......... .....GTT..
CMW7060      ..TCA...C. ........A. .....--TA.. .......... .....GTT..
CMW7063      .A.CC.T.G. GGCCTCCTC. GCG..GTAGT GA.ATTCCGC ATCG.AGA.C
```

```
[                  510         520         530         540         550]

CMW7772      -CGTCG-CCC GCCGGACGAA CCTT-TGAAT TATTTCTCAA GGTTGACCTC
CMW7773      .......... .......... .......... .......... ..........
CMW8959      .......... .......... .......... .......... ..........
CMW8961      .......... ...NNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
CMW8949      .......... .......... .......... .......... ..........
CMW9078      .......... .......... .......... .......... ..........
CMW9080      .......... .......... .......... .......... ..........
CMW10125     .......... .......... .......... .-........ ..........
CMW10126     .......... .......... .......... .-........ ..........
CMW9076      .......... .......... .......... .-........ ..........
CMW992       .......... .......... .......... .-........ ..........
CMW8922      .......... .......... ....C....C .-........ ..........
CMW8929      .......... .......... ....C....C .-........ ..........
CMW8000      .......... .......... ....C....C .-........ ..........
CMW7999      .......... .......... ....C....C .-........ ..........
CMW10130     .......... .......... ....C....C .-........ ..........
CMW9074      .......... .......... ....C....C .-........ ..........
CMW7774      .......... .......... ....C....C .-........ ..........
CMW7060      .......... .......... ....C....C .-........ ..........
CMW7063      GACGA.C... TG.C.TTA.. ..CCC--..C .-..-.-... ..........

[                  560         570         580         590         600]

CMW7772      GGATGAAGTT CGAGAAGGTA AGA------- -A-AG-TTTT TCC-TTCC-G
CMW7773      .......... .......... .......... .......... ..........
CMW8959      .......... .......... .......... .......... ..........
CMW8961      NNNN...... .......... .......... .......... ..........
CMW8949      .......... .......... .......... .......... ..........
CMW9078      .......... .......... .......... .......... ..........
CMW9080      .......... .......... .......... .......... ..........
CMW10125     .......... .......... .......... .....-A.... ..........
CMW10126     .......... .......... .......... .....-A.... ..........
CMW9076      .......... .......... .......... .-...A.... ..-G......
CMW992       .......... .......... .......... .-...A.... ..-G......
CMW8922      ....NNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNN.. -.TA.G..T.
CMW8929      ....NNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNN.. -.TA.G..T.
CMW8000      .......... .......... ..-.CA..CA .-.-CA.... -.TG.G..T.
CMW7999      .......... .......... ..-.CA..CA .-.-CA.... -.TG.G..T.
CMW10130     .......... .........C C.TGCACGCA TGTC...... .-TAAC..CT
CMW9074      .....G.... .........C C.TGCACGCA TGTC...... .-TAAC..CT
CMW7774      .......... .......... ...G...... .-.-.A.... ...TGCT.CC
CMW7060      .......... .......... ...G...... .-.-.A.... ...CG.T.CC
CMW7063      A......... .......... ...ACAGC.. .-.-CAC.CC .TTGA.A.CC
```

```
[                610        620        630        640        650]

CMW7772     -CTGCACGCG C--TGGGTGC TGGGTGCTGG GTGCTGGGTG CTGGGTTCCC
CMW7773     .......... .......... .......... .......... ..........
CMW8959     .......... .......... CA........ ..-------- ------....
CMW8961     .......... .......... .......... ..-------- ------....
CMW8949     .......... .......... CA........ ..-------- ------....
CMW9078     .......... .......... CA........ ..-------- ------....
CMW9080     .......... .......... CA........ ..-------- ------....
CMW10125    .........T TCC....... ---------... ..-------- ------....
CMW10126    .........T TCC....... ---------... ..-------- ------....
CMW9076     .....C.... .GA...-... ---------... .A-------- ------.G..
CMW992      .....C.... .GA...-... ---------... .A-------- ------.G..
CMW8922     .---....T- -...---... ---------... ..-------- ------...T
CMW8929     .---....T- -...---... ---------... ..-------- ------...T
CMW8000     .---....-- -...---... ---------... ..-------- ------...T
CMW7999     .---....-- -...---... ---------... ..-------- ------...T
CMW10130    ATC.AC-TTC GGCGC--... A--.C....C .---------- ----------
CMW9074     CTC.AC-TTC GGCGC--... A--.C....C .---------- ----------
CMW7774     G.AC.G..T- -..------- ---------- ---------- ----------
CMW7060     G.AC-GT.T. .AG..C-... ..C-----..C .---------- ----------
CMW7063     AGAT.GT... GCG.CTCGCA .CTCACACCT .GCA.TCTGT GCCCC.NTTA

[                660        670        680        690        700]

CMW7772     GCACTCAATT TGCCTTATC- GCTTC---GG TGAGGGGCA- TTT--TGGTG
CMW7773     .......... .......... .......... .......... ..........
CMW8959     .......... .......... .......... .......... ..........
CMW8961     .......... .......... .......... .......... ..........
CMW8949     .......... .......... .......... .......... ..........
CMW9078     .......... .......... .......... .......... ..........
CMW9080     .......... .......... .......... .......... ..........
CMW10125    .......... .......... ........A. .......... ..........
CMW10126    .......... .......... ........A. .......... ..........
CMW9076     .......... ..T....... .......... .......... ..........
CMW992      .......... ..T....... .......... .......... ..........
CMW8922     ..G.CG.... .........A -..-...T.. .........A ...CT.....
CMW8929     ..G.CG.... .........A -..-...T.. .........A ...CT.....
CMW8000     ..G.CG.... .........A -..-...T.. .........A ...CT.....
CMW7999     ..G.CG.... .........A -..-...T.. .........A ...CT.....
CMW10130    ..G.A..G.C C......... ...-...T.. .......... ....T.C...
CMW9074     ..G.A..G.C C......... ...-...T.. .......... ....T.C...
CMW7774     --G.A..GCC CA........ ...-...... .......... ....T.C...
CMW7060     ---------- ---......T ..CG.CCT.. .......... ....T.C...
CMW7063     C.C...CT-- ----------. ------...-- ----------. ---..-----
```

```
[                  710         720         730         740         750]

CMW7772      GTGGGGT-TG GCCCGCGCTA AGCCTCGTTC GGGCT-CGGC AAAATGTCCG
CMW7773      .......... .......... .......... .......... ..........
CMW8959      .......... .......... .........T .......... ..........
CMW8961      .......... .......... .......... .......... ..........
CMW8949      .......... .......... .........T .......... ..........
CMW9078      .......... .......... .........T .......... ..........
CMW9080      .......... .......... .........T .......... ..........
CMW10125     .---...C-. .......... .T...G...T .T...T.... .....C....
CMW10126     .---...C-. .......... .T...G...T .T...T.... .....C....
CMW9076      .......C-. .......... .........T .T...T.... .....C....
CMW992       .......C-. .......... .........T .T...T.... .....C....
CMW8922      .-..C--... .......... .........T ..T..T.... .....C....
CMW8929      .-..C--... .......... .........T ..T..T.... .....C....
CMW8000      .-..C--... .......... .........T ..T..T.... .....C....
CMW7999      .-..C--... .......... .........T ..T..T.... .....C....
CMW10130     .......T.. .......... -........CT ...-.T.... ......A...
CMW9074      .......T.. .......... -........CT ...-.T.... ......A...
CMW7774      .......T.. .......... .A......CT .A........ ...C..G...
CMW7060      .......T.. .......... .A......CT .A........ ...C..G...
CMW7063      --------.-- ---------- ---------- -----.---. ...--------

[                  760         770         780         790         800]

CMW7772      CATCTGGTTT TTTTGCGACC GGCGTGCGAC CGAAGCG--C ACCCCTCGCC
CMW7773      .......... .......... .......... .......... ..........
CMW8959      .......... .......... .......... .......... G.........
CMW8961      .......... .......... .......... .......... G.........
CMW8949      .......... .......... .......... .......... G.........
CMW9078      .......... .......... .......... .......... G.........
CMW9080      .......... .......... .......... .......... G.........
CMW10125     T......... ...-...... .......... ....AT.... G.........
CMW10126     T......... ...-...... .......... ....AT.... G.........
CMW9076      .......... ...-...... .......... ...C...... ..........
CMW992       .......... ...-...... .......... ...C...... ..........
CMW8922      .......A.. .....T.... .........G. ...C...AA. ......TA..
CMW8929      .......A.. .....T.... .........G. ...C...AA. ......TA..
CMW8000      .......A.. .....T.... .......... ...C...AA. .......A..
CMW7999      .......A.. .....T.... .......... ...C...AA. .......A..
CMW10130     ..CT...... .......... .....CT.G. ...C...CCT C...ACTAG.
CMW9074      ..CT...... .......... .....CT.G. ...C...CCT C...ACTAG.
CMW7774      ..CT...... ...-...... .....CA.G. ...T...CC. C-T.ACTAG.
CMW7060      ..CT...... ..--...... ..T...A.G. ...T...CC. C.T.ACTAG.
CMW7063      -.ATCAA... ....TGTGG. CCTT.TTAGT G.GGC.ACAA C...GC.AGA
```

```
[                  810         820         830         840         850]

CMW7772      AGA-----CA CG--CCACGC A--------T GTGCGACCAG ACGCTAACGG
CMW7773      .......... .......... .......... .......... ..........
CMW8959      .......... .......... .......... .......... ..........
CMW8961      .......... .......... .......... .......... ..........
CMW8949      .......... .......... .......... .......... ..........
CMW9078      .......... .......... .......... .......... ........A.
CMW9080      .......... .......... .......... .......... ........A.
CMW10125     .--..T.G.. --.------- -......... .C...G.... .......T.C
CMW10126     .--..T.G.. --.------- -......... .C...G.... .......T.C
CMW9076      ....CTCG.. -.......A. -.........- ......T.G. ..........A
CMW992       ....CTCG.. -.......A. -.........- ......T.G. ..........A
CMW8922      .ACGCTTC.. G-......T. .CGTTCGTC. A........T .T......CA
CMW8929      .ACGCTTC.. G-......T. .CGTTCGTC. A........T .T......CA
CMW8000      .ACGCTTC.. G-......T. .CGTTCGTC. A........T .T......CA
CMW7999      .ACGCTTC.. G-......T. .CGTTCGTC. A........T .T......CA
CMW10130     GA.AAATG.T .TGA....T. .TGTACCGTC ..A...G..A .G.......C
CMW9074      GA.AAATG.T .TGA....T. .TGTACCGTC ..A...G..A .G.......C
CMW7774      GAGCAATG.C .CGA....T. .TGTGCTCTC .........C .G.......C
CMW7060      GA.AAATG.C .CGA....T. .TGTGCTCTC ..A......C .G.......C
CMW7063      GTTCTCGAT. GCAT.TCAAG GAAGGCACGC .CTGACAGTC C.AAA.T---

[                  860         870         880          ]

CMW7772      CCATCC---- CAGGAAGCCG CCGAGCTCGG TAAGGGTTC
CMW7773      .......... .......... .......... .........
CMW8959      ..C....... .......... .......... .........
CMW8961      .......... .......... .......... .........
CMW8949      ..C....... .NNNNNNNNN NNNNNNNNNN NNNNNNNNN
CMW9078      .......... .......... .......... .........
CMW9080      .......... .......... .......... .........
CMW10125     .......... .......... ....A..... .........
CMW10126     .......... .......... ....A..... .........
CMW9076      ..G..T.... .......... .......... .........
CMW992       ..G..T.... .......... N......... .........
CMW8922      ..G-..ACAA .......... .T........ .........
CMW8929      ..G-..ACAA .......... .T........ .........
CMW8000      ..G-..ACAA .......... .T........ .........
CMW7999      ..G-..ACAA .......... .T........ .........
CMW10130     G.C-..ACTA .......... .T........ .........
CMW9074      G.C-..ACTA .......... .T........ .........
CMW7774      A.G-..ACAA .......... .......... .........
CMW7060      G.G-..ACAA .......... .T........ .........
CMW7063      ---------- -......... ....A..... .........
```

**Fig. 6.** Phylogenetic tree generated from a combined data set of the ITS 1/2 region and 5.8S rRNA gene and EF1-α gene and intron region for various *Botryosphaeria* spp. One most parsimonious tree was generated using the heuristic search option. The bootstrap values (1000 replications) > 50 % are indicate above the branches. The tree includes 19 ingroup taxa and the outgroup taxon *G. philoprina*.

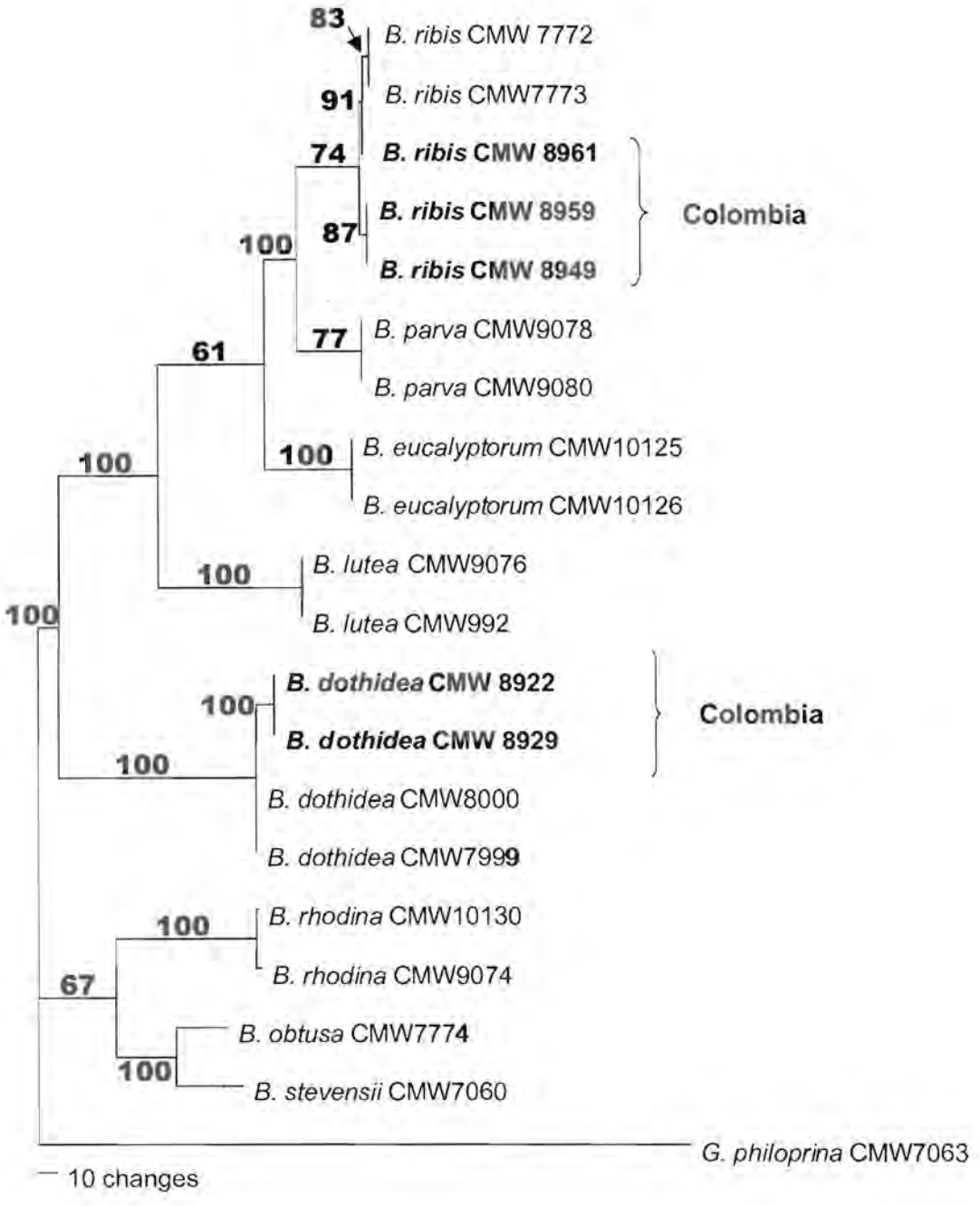

83
B. ribis CMW 7772
91
B. ribis CMW7773
74
B. ribis CMW 8961
B. ribis CMW 8959
87
B. ribis CMW 8949
Colombia
100
77
B. parva CMW9078
61
B. parva CMW9080
100
B. eucalyptorum CMW10125
100
B. eucalyptorum CMW10126
100
B. lutea CMW9076
100
B. lutea CMW992
B. dothidea CMW 8922
100
B. dothidea CMW 8929
Colombia
100
B. dothidea CMW8000
B. dothidea CMW7999
100
B. rhodina CMW10130
67
B. rhodina CMW9074
B. obtusa CMW7774
100
B. stevensii CMW7060
G. philoprina CMW7063
10 changes

**Fig. 7.** Lesions formed after inoculation with six isolates of *Botryosphaeria* spp. and one control on a *Eucalyptus grandis* clone (ZG14) in the greenhouse.

**Fig. 8.** Mean lesion length after inoculation with *Botryosphaeria dothidea* and *B. ribis* on *Eucalyptus grandis,* clone ZG14 in a greenhouse trial. Bars represent confidence limits for each isolate.

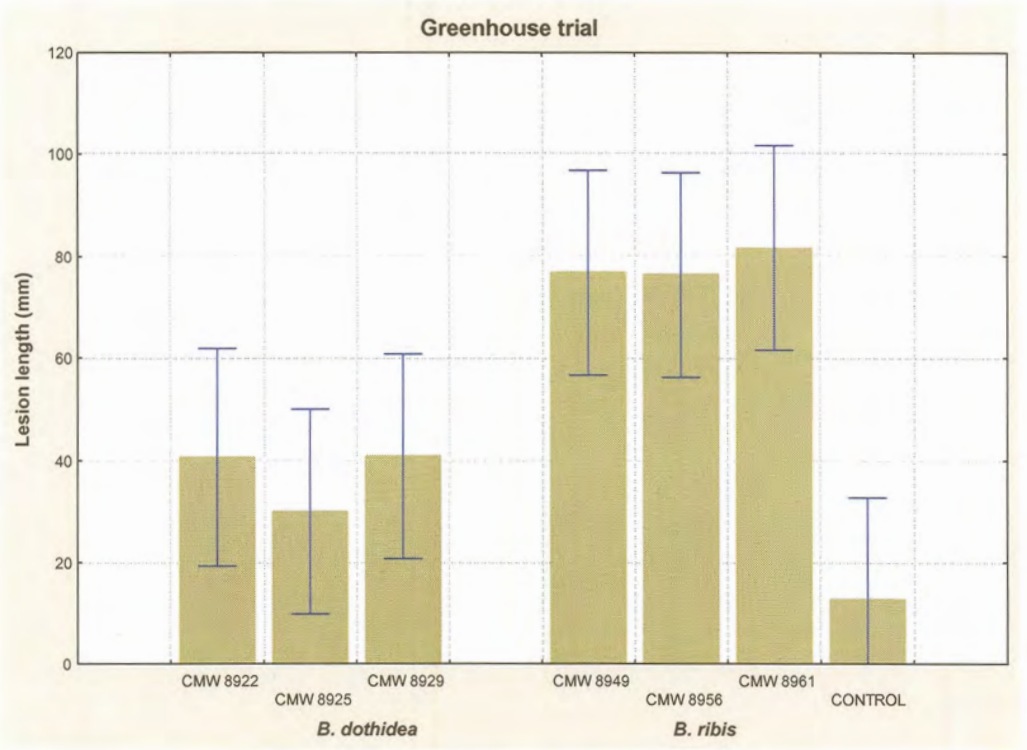

Greenhouse trial
Lesion length (mm)
120
100
80
60
40
20
0
CMW 8922
CMW 8925
CMW 8929
CMW 8949
CMW 8956
CMW 8961
CONTROL
B. dothidea
B. ribis

**Fig. 9.** Lesions associated with inoculation of *Botryosphaeria* spp. on old *Eucalyptus grandis* trees at La Suiza farm. **(a)** Control (arrow shows part of inoculation). **(b)** Lesion associated with *B. dothidea*. **(c)** Lesion associated with *B. ribis*.

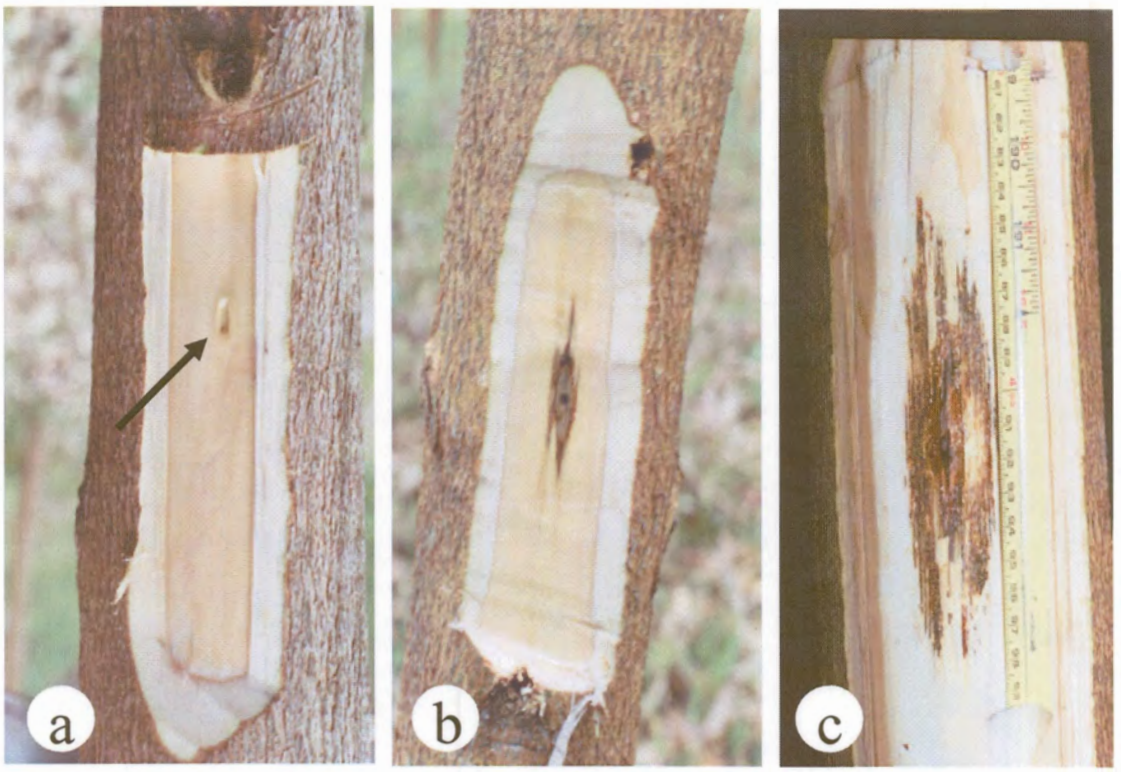

a
b
c

**Fig. 10.** Mean lesion length after inoculation with *Botryosphaeria dothidea* and *B. ribis* on 12 clones of *Eucalyptus grandis* at La Suiza farm. Bars represent confidence limits for each isolate on all clones inoculated.

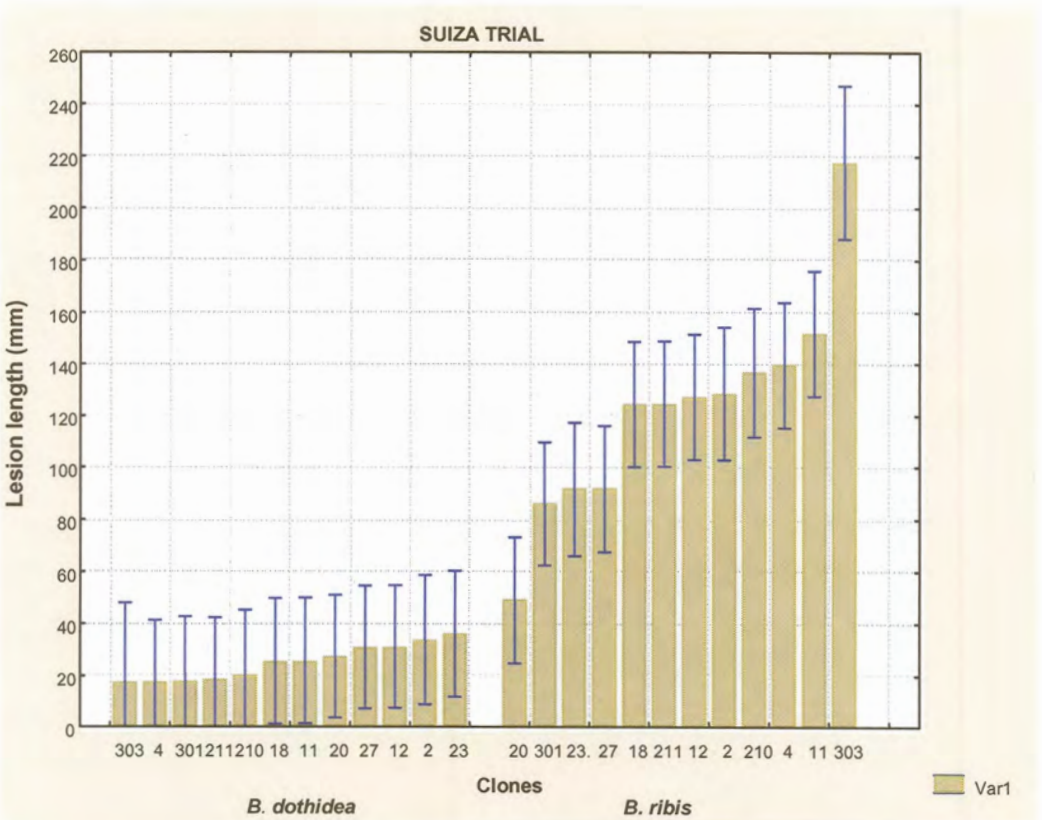

SUIZA TRIAL
Lesion length (mm)
260
240
220
200
180
160
140
120
100
80
60
40
20
0
303 4 301 211 210 18 11 20 27 12 2 23
20 301 23. 27 18 211 12 2 210 4 11 303
Clones
B. dothidea
B. ribis
Var1

**Fig. 11.** Mean lesion lengths after inoculation with *Botryosphaeria dothidea* and *B. ribis* on 14 clones of *Eucalyptus grandis* at Cecilia farm. Bars represent confidence limits for each isolate on all clones inoculated.

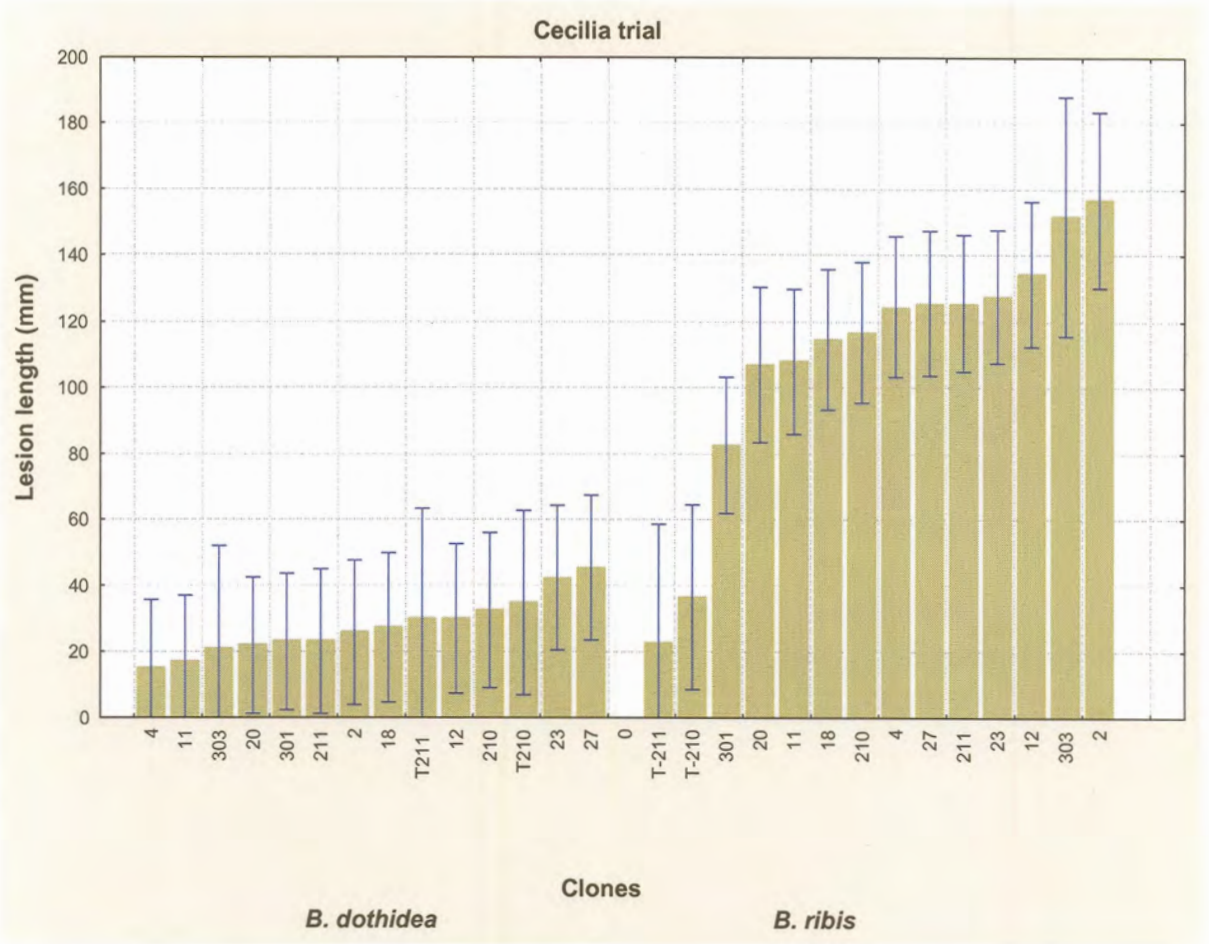

Cecilia trial
Lesion length (mm)
200
180
160
140
120
100
80
60
40
20
0
4
11
303
20
301
211
2
18
T211
12
210
T210
23
27
0
T-211
T-210
301
20
11
18
210
4
27
211
23
12
303
2
Clones
B. dothidea
B. ribis

**Fig. 12.** Mean lesion lengths after inoculation with *Botryosphaeria dothidea* and *B. ribis* on 14 clones of *Eucalyptus grandis* at Libano farm. Bars represent confidence limits for each isolate on all clones inoculated.

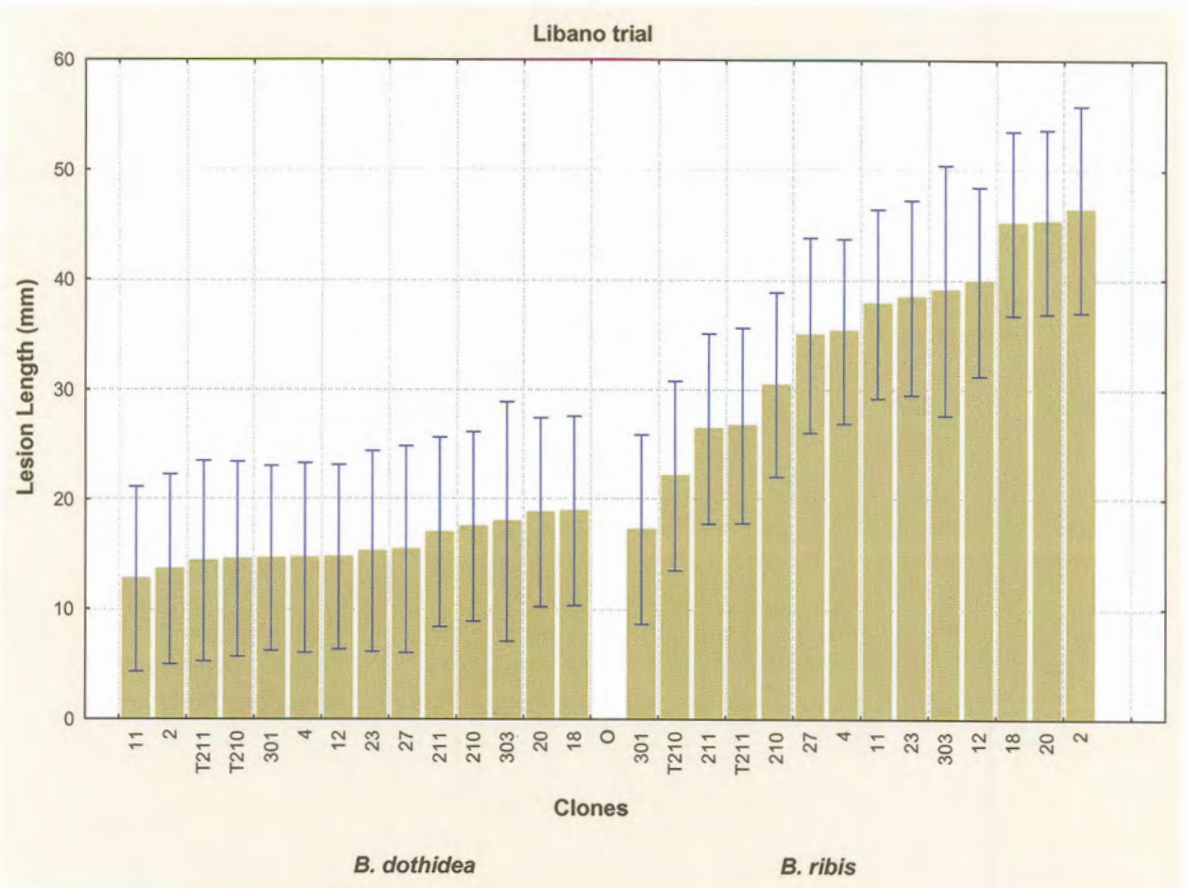

Libano trial
Lesion Length (mm)
0
10
20
30
40
50
60
11
2
T211
T210
301
4
12
23
27
211
210
303
20
18
O
301
T210
211
T211
210
27
4
11
23
303
12
18
20
2
Clones
B. dothidea
B. ribis

**Fig. 13.** Mean lesion lengths after inoculation with *Botryosphaeria dothidea* and *B. ribis* on 14 clones of *Eucalyptus grandis* at Angela Maria farm. Bars represent confidence limits for each isolate on all clones inoculated.

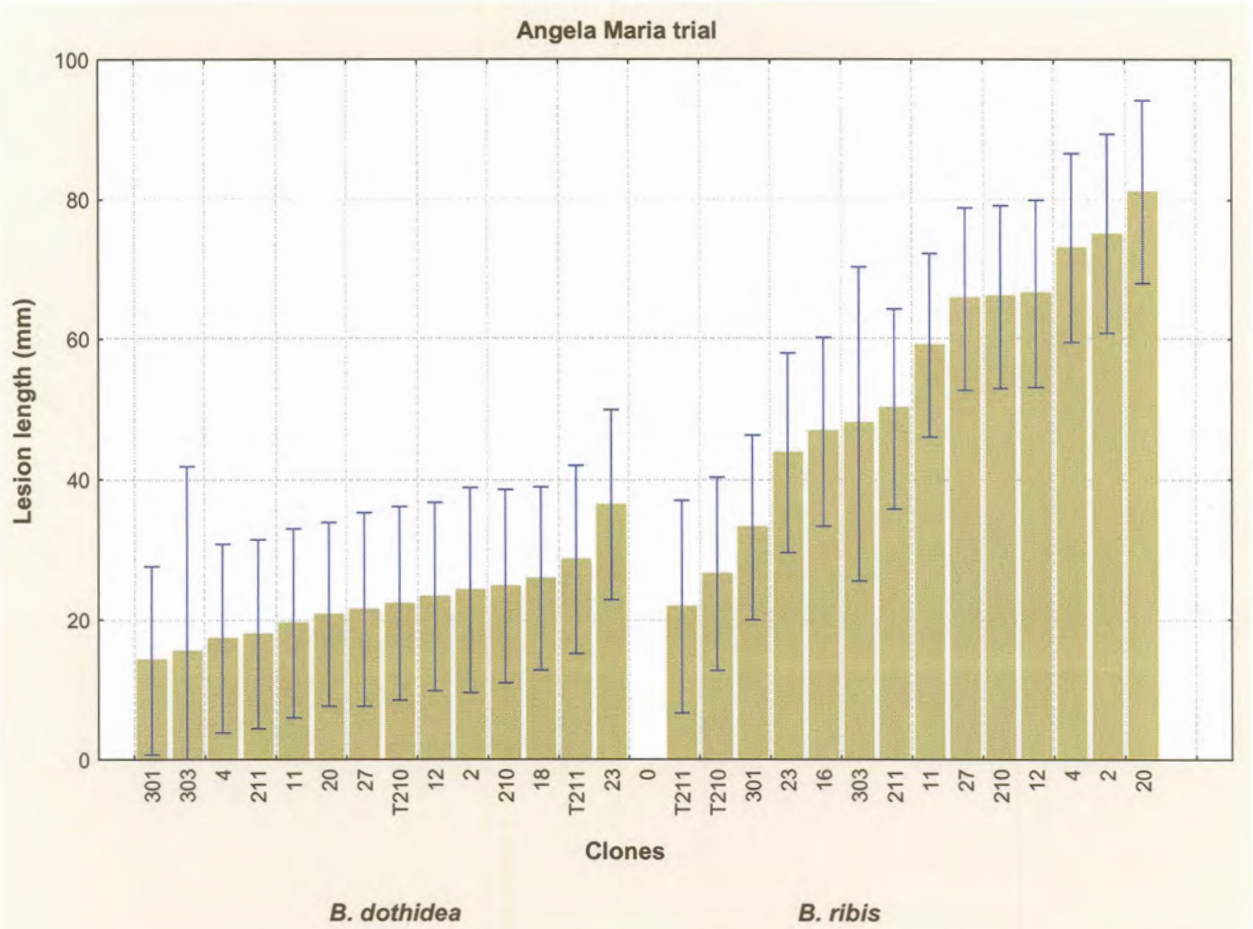
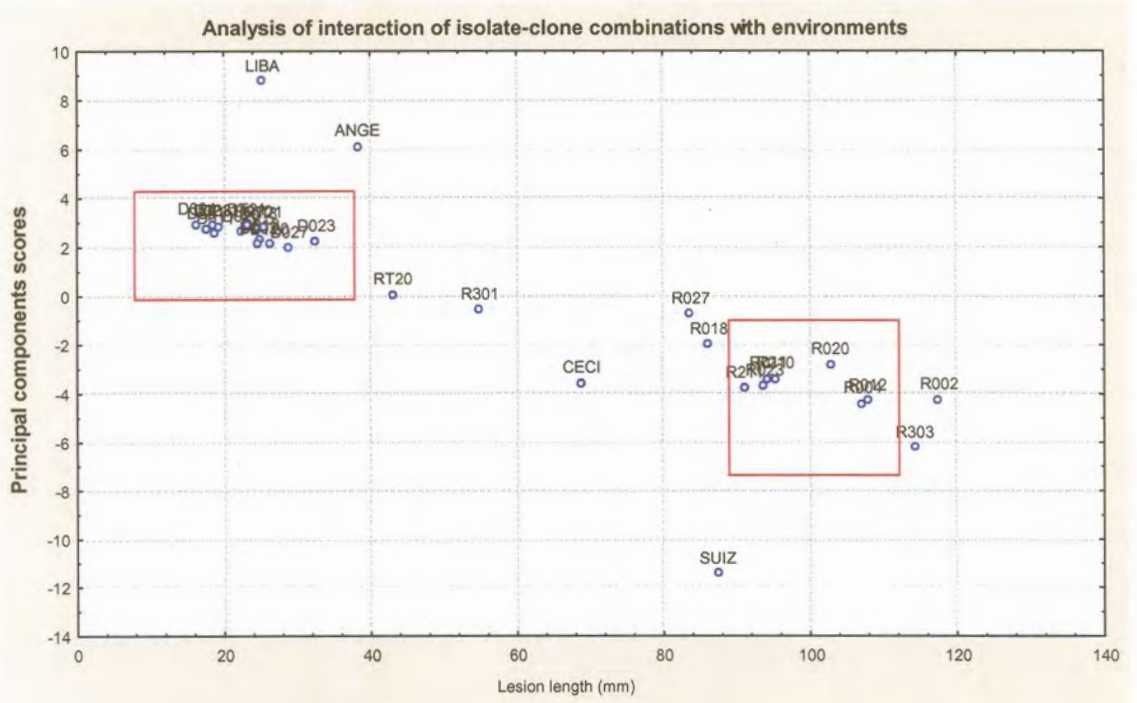

Angela Maria trial
Lesion length (mm)
100
80
60
40
20
0
301
303
4
211
11
20
27
T210
12
2
210
18
T211
23
0
T211
T210
301
23
16
303
211
11
27
210
12
4
2
20
Clones
B. dothidea
B. ribis

**Fig. 14.** Score values obtained from an AMMI analysis plotted against the overall lesion length for isolates of *Botryosphaeria dothidea* and *B. ribis*, susceptibility of clones and location interaction.




Clones marked with a 'D' = *B. dothidea*
Clones marked with a 'R' = *B. ribis*
The rectangle al lef marks clone exclusively infected with *B. dothidea*
The square al right marks clone exclusively infected with *B. ribis*