CHAPTER 5

BOTRYOSPHAERIA SPECIES ON *TIBOUCHINA* IN SOUTH AFRICA, AUSTRALIA AND NEW ZEALAND

ABSTRACT

Botryosphaeria species are important canker pathogens of woody plants including *Eucalyptus* spp. In South Africa, *B. eucalyptorum* and *B. dothidea* are important causal agents of canker and die-back diseases on *Eucalyptus* spp. The recent discovery of the *Eucalyptus* pathogen, *Cryphonectria cubensis,* on ornamental *Tibouchina* trees has raised the question as to whether *Tibouchina* spp. might be alternative hosts for other *Eucalyptus* pathogens. The aim of this study was to consider whether *Botryosphaeria* spp. occurring on *Eucalyptus* spp., might also occur on ornamental *Tibouchina* spp. Isolations were made from *Tibouchina* trees in South Africa, New Zealand and Australia. Isolates were identified based on morphological characteristics, as well as using DNA-based techniques. Two *Botryosphaeria* spp., *B. parva* and *Dothiorella mangiferae* were consequently identified. Pathogenicity trials showed that both species were pathogenic and that *D. mangiferae* was most virulent. *Botryosphaeria parva* has recently been shown to be a common pathogen of *Eucalyptus* in various parts of the world. In contrast *D. mangiferae* is a pathogen of Mango in Australia and this is the first record of the fungus in South Africa. The origin of these fungi remains unknown but there is growing evidence that they are able to move widely between hosts and to cause disease on a wide range of plants.

INTRODUCTION

Botryosphaeria spp. are important canker pathogens of at least 70 plant genera (Smith 1934, Punithalingam & Holiday 1973), including *Eucalyptus* and *Pinus* spp. (Davison & Tay 1983, Hodges 1983, Webb 1983, Barnard *et al.* 1987, Smith, Kemp & Wingfield 1994). Although *Botryosphaeria* spp. cause diseases of many economically important crops, they are generally regarded as weak pathogens causing disease only when hosts are weakened or stressed. Examples of such stress factors are competition for water, nutrients and sunlight (Toole 1963), a high water table (Pusey 1989), extreme cold (Ramos *et al.* 1991), hail (Swart, Wingfield & Knox-Davies 1987) and planting stress (Schoeneweiss 1965).

Considerable confusion has surrounded the taxonomy of *Botryosphaeria* spp. This has arisen from the fact that teleomorph structures of these fungi are very similar and they are not always found in nature (Pennycook & Samuels 1985). Thus, identification of *Botryosphaeria* spp. has most commonly been based on anamorph morphology or on host preference. The most useful morphological characteristics of the anamorphs are conidial size, shape, colour and septation (Crous & Palm 1999, Denman *et al.* 2000). Species with pigmented conidia are generally grouped in the genera *Diplodia* Fr. and *Lasiodiplodia* Ellis & Everh. Those with hyaline conidia reside in genera such as *Fusicoccum* Corda and *Dothiorella* Sacc. Similarities among the species within these broader groups have, however, hindered the accurate identification of these fungi (Sutton 1980, Morgan-Johnes & White 1987, Johnson *et al.* 1992).

Botryosphaeria spp. are well-known pathogens of *Eucalyptus* spp., although taxonomic problems have meant that names that have been used in past reports, are not always correct. In South Africa two species, *B. dothidea* (Moug.) Ces. & De Not. and *B. eucalyptorum* Crous, Smith & Wingf. have been reported to infect these trees (Smith *et al.* 1994, Smith *et al.* 2001). *Botryosphaeria rhodina* (Cooke) Von Arx and *B. parva* Pennycook & Samuels have been reported on *Eucalyptus* from Uganda, Congo and Ethiopia (Roux *et al.* 2001, Nakabonge 2002, Alemu 2003) while *B. ribis* Gorssenb. & Dugg. has been reported to cause die-back of *E. grandis* Hill & Maiden and *E. radiata* Sieber & De Gandollefrom in Australia (Barnard *et al.* 1987, Shearer, Tippett & Bartle 1987).

Symptoms of *Botryosphaeria* die-back on *Eucalyptus* spp. include tip die-back, stem cankers, cracking of the bark, kino exudation and tree mortality (Shearer *et al.* 1987, Smith *et al.* 1994). *Botryosphaeria* spp. are known to infect plants via natural openings and wounds caused by pruning, insects, sunburn, and hail (Marks & Minko 1969, Maas & Uecker 1984, Johnson 1992, Johnson 1994). It has more recently been recognised that they can infect healthy tissue and exist in a latent form until the onset of stress (Maas & Uecker 1984, Smith, Wingfield & Stanosz 1996).

Very little is known regarding the geographic origin of *Botryosphaeria* spp. They are generally regarded as having wide host ranges and distribution. However, this view is tainted by the fact that many collections have been identified based on morphology and are probably incorrect. In terms of *Eucalyptus*, the areas of origin of *Botryosphaeria* spp. is an intriguing question with different species of *Botryosphaeria* found in different parts of the world, often on the same *Eucalyptus* spp. (Shearer *et al.* 1987, Smith *et al.* 1994, 1996, Roux *et al.* 2001, Nakabonge 2002). It is not known whether these have originated from the areas of origin of *Eucalyptus*, or if they have crossed from native plants to exotic *Eucalyptus* spp.

Recently, the *Eucalyptus* canker pathogen *Cryphonectria cubensis* (Bruner) Hodges, was shown to occur naturally on a number of genera in the Myrtales (Hodges, Alfenas & Ferreira 1986, Wingfield *et al.* 2001, Myburg *et al.* 2002a,b, Chapter 2, *this thesis*). In 2001, *C. cubensis* was reported on native *Tibouchina urvilleana* (CD.) Long. and *T. lepidota* Baill. in Colombia, where the pathogen also occurs on *Eucalyptus* spp. (Wingfield *et al.* 2001). This was followed shortly, thereafter, by a report of the fungus on *T. urvilleana* in South Africa (Myburg *et al.* 2002a). The fact that *Tibouchina* spp. resides in the Melastomataceae, which is closely related to the Myrtaceae (Conti *et al.* 1997) makes this unusual discovery of *C. cubensis* on *Tibouchina*, more plausible (Wingfield 2003).

The fact that *C. cubensis* is a pathogen of both *Eucalyptus* spp. and *Tibouchina* spp. has led us to question whether other *Eucalyptus* pathogens might occur on *Tibouchina* spp. *Tibouchina* spp. produce attractive flowers and are commonly planted in southern hemisphere countries such as South Africa and Australia as ornamentals. These trees are also commonly found in areas where *Eucalyptus* spp. occur as natives or are grown in plantations. The aim of this study was, thus, to determine whether *Botryosphaeria* spp. occur on *Tibouchina* spp. and if so, whether these fungi represent similar species to those on *Eucalyptus* spp. in the same areas.

MATERIALS AND METHODS

Disease symptoms and collection of samples

Branches were collected from *T. urvilleana* growing as ornamentals in South African gardens in the KwaZulu-Natal Province (Table 1). Samples were collected from healthy as well as dying branches and returned to the laboratory for isolation. Samples were incubated at 5 °C for one week prior to isolation. Samples were then surface disinfested with 70% (v/v) ethanol and left to air dry. Pieces of wood (4 mm^2) were cut from the centres of the branches and transferred to 2% Malt Extract Agar (MEA) (20 g Biolab Malt Extract, 15 g Biolab Agar, 1 litre water) amended with streptomycin (100 mg) (Sigma Chemical Company, St. Louis, America) and incubated at 25 °C. All fungi growing from these samples with a culture morphology resembling *Botryosphaeria* spp. were transferred to Petri dishes containing fresh MEA.

Isolates from Australia and New Zealand were collected from various *Tibouchina* spp. (Table 1). These were from dead and dying branches and stems and were isolated either by direct transfer of spores from sporocarps or by using the isolation technique described above.

Morphological characterisation

Cultures resembling those of *Botryosphaeria* spp. were induced to sporulate by transferring them to water agar (WA) (20 g Biolab Agar, 1 litre distilled water), to which sterile pine needles or *Tibouchina* twigs had been added to the agar surface. Cultures were incubated at 25 °C for 2 weeks under near UV light (280 nm). Fruiting structures produced on the pine needles or *Tibouchina* twigs were dissected by hand to expose conidia or ascospores. Spores were mounted in lactophenol and examined using Nomarski differentiation interference contrast microscopy. Isolates were grouped based on conidial morphology. For each isolate the average length and width (upper third of the spore) of 25 conidia was measured using a light microscope (Carl Zeiss) and these are presented as (min-)(mean-SD) - (mean+SD)(-max) µm. Images were captured electronically with an Axiovision digital camera system (Carl Zeiss).

DNA isolation and amplification

DNA was extracted from a representative set of isolates (Table 2) selected based on conidium morphology. Mycelium from actively growing cultures (Table 2) was placed in 1.5 ml microcentifuge tubes containing 750 µl, 3% Malt Extract Broth and incubated at 25 °C for four days. DNA was isolated using the method described by Murray and Thompson (1980). The internally transcribed spacer (ITS) regions, including the 5.8S gene, were amplified using the primer pair ITS 1 (5'-TTTCCGTAGGTGAAACCTGC-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.* 1990).

Polymerase chain reactions (PCR) contained 5 ng DNA, 0.2 mM of each dNTP (Promega, Madison, Wisconsin, U.S.A.), 0.15 µM of primers ITS1 and ITS4, 0.5 units Taq polymerase (Roche Molecular Biochemicals, Almeda, California) and 1x Buffer with $MgCl_2$ (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl). Sterile water was used to adjust the final volume to 25 µl. Amplification reactions were performed on a Perkin Elmer GeneAmp PCR System 9700 thermocycler (Perkin-Elmer Applied Biosystems Inc., Foster City, California). The conditions for all PCR reactions were an initial denaturation at 96 °C for 1 min followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing for one min at 54 °C, followed by extension at 72 °C for 90 sec. A step of 5 sec elongation was added with each cycle after the first twenty-five cycles. The process was completed with a final extension at 72 °C for 10 min. PCR products were visualised on 2% agarose-ethidium bromide gels using ultra violet light. Product sizes were estimated with a 100 bp standard size marker (Promega).

Restriction Fragment Length Polymorphisms (RFLPs)

Restriction fragment length polymorphisms (RFLPs) were used to identify the groups of isolates that had been distinguished based on morphology. Amplified PCR products of the ITS regions were digested with the restriction enzyme *Cfo* I. This enzyme has been identified as one of three enzymes able to distinguish between six *Botryosphaeria* spp. occurring in South Africa (Jacobs 2002). Reactions consisted of 0.3 μl *Cfo* I enzyme, 2.2 μl Buffer L (Roche), 2.5 μl sterile water and 20 μl PCR product. The reaction was incubated at 37 °C for 3 hrs. The digested amplicons were separated by electrophoresis on a 3% agarose-ethidium bromide gel using a Tris-acetate (TAE) buffer electrophoresis system (Maniatis, Fritsch & Sambrook 1982) and visualized under ultra violet light.

DNA sequencing and analyses

A representative set of seven isolates identified based on morphology and RFLP analysis was chosen for DNA sequence comparison. PCR products were purified using a High Pure PCR Product Purification Kit (Roche Diagnostic GmbH, Mannheim, Germany). DNA fragments were sequenced with the same primer pairs used in the amplification reactions. An ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS (Perkin-Elmer, Warrington, United Kingdom) was used for sequencing on an ABI PRISM 3100™ automated sequencer. Sequences were aligned using ClustalX (Thompson *et al.* 1997) and manually adjusted using Sequence Navigator version 1.0.1 (Perkin-Elmer Applied BioSystems Inc., Foster City, California). All sequences obtained in this study have been deposited in GenBank (Table 2).

Data analyses were performed using PAUP* (Phylogenetic Analysis Using Parsimony) version 4.0b (Swofford 1998). Analyses were done using the heuristic search option with TBR (tree-bisection-reconnection) branch swapping. Gaps inserted during sequence alignment, were treated as fifth base (NEWSTATE). A bootstrap analysis (50% majority rule, 1000 replications) was done to determine the confidence levels of the tree branching points (Felsenstein 1995). Previously published sequences of various commonly known *Botryosphaeria* spp. as well as those from Slippers *et al.* (2003) were included for comparative purposes (Table 2). *Mycosphaerella africana* Crous & Wingf., a genus known to be closely related to *Botryosphaeria* spp., was used as a monophyletic outgroup taxon to root the phylogenetic tree. The stringency of the branch nodes were tested using Markov Chain Monte Carlo Algorithms (MCMC) (Larget & Simon 1999) in Bayesian Analysis (Lutzoni, Pagel & Reeb 2001). Random trees were obtained through 100 000 generations with every 10th tree sampled. The first 1500 trees were discarded as the burnin period. A general time reversal model was used and four MCMC chains were run simultaneously in the analysis. The sampled trees were summarised in a consensus tree showing posterior probabilities of the branches.

Pathogenicity

To determine the relative pathogenicity of the *Botryosphaeria* spp. isolated from *Tibouchina* spp., inoculations were performed in a greenhouse. All trees were maintained under greenhouse conditions for two weeks to acclimatise them to these conditions, prior to inoculation. The greenhouse was subjected to natural day/night conditions and had a temperature setting of ~25 °C. Tree diameters varied from 10 to 15 mm. Two most vigorously growing isolates of the two *Botryosphaeria* spp. identified from the seven selected isolates from *Tibouchina* from South Africa were inoculated onto 20 *T. urvilleana* trees each. Ten additional trees were inoculated with sterile MEA plugs, which served as controls.

Wounds were made on tree stems using a cork borer (9 mm diam) in such a way that a disc of bark was removed to expose the cambium. Mycelial plugs of a similar size were taken from the edges of 7-day-old actively growing cultures and placed in the wounds with the mycelium facing the cambium. Wounds were sealed with laboratory film (Parafilm "M", American National Can^{TM} Chicargo, Illinois.) to protect the inoculated fungus and the cambium from desiccation. Lesion lengths were measured six weeks after the trees were inoculated. Data were subjected to analysis of variance (ANOVA) using the General Linear Model procedure of SAS (SAS/STAT Users guide, Version 6, 1989).

RESULTS

Disease symptoms and collection of samples

Symptoms commonly observed during sampling were branch die-back and in some cases death of trees. *Botryosphaeria* spp. were also isolated from healthy tree tissue confirming the endophytic nature of these fungi. A total of 23 isolates, seven from Pietermaritzburg and sixteen isolates from KwaMbonambi were collected from 52 trees sampled in South Africa. A total of 9 of these isolates were from healthy tree tissue. Six isolates were obtained from the same number of *T. lepidota* trees in Auckland, New Zealand and four isolates from the same number of *T. lepidota* trees from Brisbane, Australia (Table 1). All cultures are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

Morphological characterisation

All isolates included in this study produced hyaline, smooth-walled conidia and no teleomorph states were observed. Isolates represented two groups, which could be distinguished based on conidial wall thickness and shape. One group had fusiform, thin-walled, aseptate conidia with prominent truncate bases and rounded apices (Fig. 1A-C). The average size of these conidia was (13.71-) 14.95-17.05 (-17.46) x (3.4-) 3.8-4.8 (-5.34). The other group had conidia which were ovoid, aseptate, thick-walled, with prominent truncate bases and obtuse apices (Fig 1D). The average size of these conidia was (9.08-) 9.74-12.26 (-13.19) x (3.8-) 3.64-4.36 (-5.5). Only one isolate, originating from South Africa, resided in this group.

Restriction Fragment Length Polymorphisms (RFLPs)

Digestion of isolates with restriction enzyme *Cfo* I supported the separation of isolates into two groups (Fig. 2). Thus, all but one isolate from South Africa showed banding patterns corresponding to those of *B. parva*, with bands at 121 bp and 182 bp. A single isolate produced a banding pattern that could not be assigned to a species, but corresponded with patterns produced by *F. indigoticum* Jacobs, Slippers & Wingf. (prov. nom.), *Dothiorella mangiferae* Syd. & P. Syd. (Slippers *et al.* 2001), *F. aesculi* Corda and *F. bacilliforme* Jacobs, Slippers & Wingf. (prov. nom.) with fragment sizes of 121 bp, 148 bp and 182 bp respectively (Jacobs 2002).

DNA sequencing and analyses

PCR amplification of DNA from the seven representative isolates resulted in fragments of ~500 bp for the ITS and 5.8S regions of the rDNA operon. The fragments resulted in sequences of ~526 bp, before alignment. Aligned sequences resulted in a data set of 540 characters (Appendix 1), consisting of 540 constant characters, 109 parsimony informative characters and 431 variable characters that were parsimony uninformative. The heuristic search produced five most parsimonious trees of which one was chosen for presentation (Fig. 3). A strict Bootstrap consensus tree [length of tree = 217 steps, Consistency index (CI) = 0.8062, Retention index (RI) = 0.869, Rescaled consistency index (RC) = 0.701 and Homoplasy index (HI) = 0.194] was generated from the 109 variable characters and most branches were well supported with high bootstrap values (Fig. 3). Posterior probability values calculated for the branch nodes supported the bootstrap values.

The isolates from *Tibouchina* spp. resided in two distinct clades. The one clade included *B. parva* isolates from various hosts and geographic regions and the other clade was typified by *D. mangiferae* (Slippers *et al.* 2001) isolates from mango (*Mangifera indica* L.) in Australia.

Pathogenicity

Greenhouse inoculations on *T. urvilleana* plants resulted in distinct lesions within 6 weeks (Fig. 4) whereas the control inoculations produced no lesions. The single *D. mangiferae* isolate (CMW 10332) produced significantly longer lesions ($P>0.0001$) than those associated with the two *B. parva* isolates with an average lesion length of 28.8 mm. The two *B. parva* isolates (CMW 10337, CMW 10328) produced a mean lesion length of 13.9 mm and 14.3 mm, respectively. Lesions associated with all isolates differed significantly from the control inoculations ($P<0.0001$) (Fig. 5).

DISCUSSION

Results of this study present the first record of *Botryosphaeria* spp. on *Tibouchina* spp. and thus the first report of these fungi on trees widely planted as ornamentals in South Africa, Australia and New Zealand. *Botryosphaeria parva* is shown to be the most common species on this tree and is apparently not very pathogenic. It was interesting that a single isolate of *D. mangiferae*, a fungus previously only known from Mango in Australia, was also found in South Africa and that this fungus displayed high levels of pathogenicity.

Botryosphaeria parva, the fungus most common on *Tibouchina* spp. in this study is a species that is relatively poorly understood in terms of distribution and host range. Based on morphology, this fungus is very similar to *B. dothidea* and *B. ribis*. Thus, reports of these fungi use names that in many cases are probably not correct, as has been shown by Slippers *et al.* (2003). *Botryosphaeria dothidea*, for example, is known to infect a number of myrtaceous hosts such as *Heteropyxis* spp. (Smith *et al.* 2001) and *Eucalyptus* spp. (Davison & Tay 1983, Webb 1983) in various countries. Therefore, the confusion over the taxonomy of *Botryosphaeria* spp. in the past, could have led to the confusion of *B. parva* isolates with species such as *B. dothidea*, *B. ribis*, *B. lutea* and their *Fusicoccum* anamorphs (Slippers *et al.* 2003). Based on this, and the wide host range of previously identified *B. dothidea* and *B. ribis* isolates (Smith 1934, Punithalingam & Holiday 1973, Davison & Tay 1983, Hodges 1983, Webb 1983, Barnard *et al.* 1987, Smith *et al.* 1994), the discovery of *B. parva* on *Tibouchina* spp. is not surprising.

The collection of a single isolate of *D. mangiferae* from *Tibouchina* spp. in South Africa is unusual. *Dothiorella mangiferae* is an important pathogen responsible for substantial losses to mango orchards in Australia where it causes pre and post harvest infection of fruit and decline and die-back of trees (Johnson *et al.* 1992). Extensive surveys of *Botryosphaeria* spp. in mango plantations in South Africa have not yielded any reports of this fungus (Jacobs 2002). Its discovery on *Tibouchina* spp. is difficult to explain, although many species of *Botryosphaeria* have wide host ranges and *D. mangiferae* is obviously one of these. Its limited occurrence on *Tibouchina* might indicate that this tree is not a preferred host of the fungus, but it also might be related to the relatively small sample of *Botryosphaeria* isolates collected in this study.

RFLPs have previously been used to rapidly distinguish between isolates of *Botryosphaeria* spp. (Jacobs 2002). Although this technique is rapid, simple and relatively inexpensive, it is not always possible to distinguish between closely related species. This was also the case in this study where it was possible only to distinguish between *B. parva* and a group of *Fusicoccum* spp., which included *F. indigoticum*, *D. mangiferae*, *F. aesculi* and *F. bacilliforme* (Slippers *et al.* 2001). The species in the latter group all display the same RFLP banding profile (Slippers *et al.* 2001). The use of double digests might be considered in future investigations in order to provide higher resolution and to accurately distinguish between species.

In contrast to RFLP banding patterns, DNA sequence data based on the ITS and 5.8S regions of the rDNA operon provided an accurate means to differentiate between species collected in this study. In the tree resulting from the sequence data comparisons, the isolates from this study were grouped into two clades representing *B. parva* and *D. mangiferae*, respectively. This study adds to a number of recent reports (Crous & Palm 1999, Denman *et al.* 2000, Smith *et al.* 2001, Smith & Stanoz 2001, Jacobs 2002, Nakabonge 2002, Slippers *et al.* 2003) showing that DNA sequence data comparisons provide a level of resolution for the identification of *Botryosphaeria* spp., that was previously not possible.

Pathogenicity tests showed that *B. parva* was pathogenic on *T. urvilleana*, but the level of pathogenicity was low. In comparison, *D. mangiferae* was highly pathogenic and it would now be interesting to determine whether this fungus can infect *Eucalyptus* spp. *B. parva* has been proven to be the second most pathogenic *Botryosphaeria* sp. in Uganda (Nakabonge 2002), however, it seems to be a weak pathogen on *Tibouchina* spp.

Both *Botryosphaeria* spp. collected in this study were isolated from symptomatic as well as asymptomatic tissue. The single *D. mangiferae* isolate was isolated from asymptomatic tissue and various isolates of *B. parva* were also from healthy tree parts. This indicates that both species are able to exist as latent pathogens. This is consistent with the biology of other *Botryosphaeria* spp. that are known to exist as endophytes and latent pathogens (Toole 1963, Schoeneweiss 1965, Neely 1968, Pusey 1989, Ramos *et al.* 1991, Johnson 1994, Johnson *et al.* 1992, Smith *et al.* 1994, Stanoz *et al.* 1997). The endophytic nature of the fungi on these hosts could have facilitated their spread to new environments, as they would have been difficult to detect using typical quarantine systems.

The presence of *D. mangiferae* on *Tibouchina* spp. in South Africa does not provide any information of the origin of this fungus. *Dothiorella mangiferae* was previously known as a host-specific fungus infecting mango trees in Australia. This could indicate that the fungus might have crossed to the introduced *Tibouchina* spp. in Australia, and later moved with the *Tibouchina* spp. as they were spread as ornamental plants. However, no conclusion can be formulated until a survey of *Botryosphaeria* spp. infecting *Tibouchina* spp. in its area of origin has been undertaken. Similarly, the surveys of Mango in the area of origin of this tree would be required to determine the origin of *D. mangiferae*.

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Table 1. List of *Botryosphaeria* spp. collected from *Tibouchina* spp. in South Africa, Australia and New Zealand.

[a]Culture number	Isolate identity	Host	Location	Collector
CMW 6236-CMW 6237	*B. parva*	*T. lepidota*	Brisbane, Australia	M. J. Wingfield
CMW 6944	*B. parva*	*T. lepidota*	Brisbane, Australia	M. J. Wingfield
CMW 9947-CMW 9952	*B. parva*	*T. lepidota*	Auckland, New Zealand	M. J. Wingfield
CMW 6996	*B. parva*	*T. lepidota*	Brisbane, Australia	M. J. Wingfield
CMW 10323-CMW 10326	*B. parva*	*T. urvilleana*	Pietermaritzburg, South Africa	R. N. Heath
CMW 10327-CMW 10331	*B. parva*	*T. urvilleana*	KwaMbonambi, South Africa	R. N. Heath
CMW 10333	*B. parva*	*T. urvilleana*	KwaMbonambi, South Africa	R. N. Heath
CMW 10334-CMW 10335	*B. parva*	*T. urvilleana*	Pietermaritzburg, South Africa	R. N. Heath
CMW 10336-CMW 10339	*B. parva*	*T. urvilleana*	KwaMbonambi, South Africa	R. N. Heath
CMW 10332	*D. mangiferae*	*T. urvilleana*	KwaMbonambi, South Africa	R. N. Heath

[a]CMW represents the culture collection of the Forestry and Agricultural Biotechnology Institute, University of Pretoria.

Table 2. Species of *Botryosphaeria* included in the phylogenetic study.

[a]Culture no.	Isolate identity	Host	Location	GenBank accession no
CMW 8000	*B. dothidea*	*Prunus* spp.	Switzerland	[d]
CMW 7780	*B. dothidea*	*Fraxinus excelsior*	Switzerland	[d]
CMW 10125	*B. eucalyptorum*	*E. grandis*	South Africa	AF 283686[b]
CMW 10126	*B. eucalyptorum*	*E. grandis*	South Africa	AF 283687[b]
CMW 7775	*B. obtusa*	*F. excelsior*	U.S.A.	[d]
CMW 994	*B. parva*	*Malus sylvestris*	New Zealand	AF 243395[b]
CMW 9081	*B. parva*	*Populus nigra*	New Zealand	[d]
CMW 10328	*B. parva*	*T. urvilleana.*	South Africa	AY194467[c]
CMW 10337	*B. parva*	*T. urvilleana.*	South Africa	AY194469[c]
CMW 9947	*B. parva*	*Tibouchina* spp.	New Zealand	AY194470[c]
CMW 9952	*B. parva*	*Tibouchina* spp.	New Zealand	AY194471[c]
CMW 6236	*B. parva*	*Tibouchina* spp.	Australia	AY194472[c]
CMW 6996	*B. parva*	*Tibouchina* spp.	Australia	AY194473[c]
CMW 9074	*B. rhodina*	*Ribis* spp.	Mexico	[d]
CMW 7054	*B. ribis*	*Ribis rubrum*	U.S.A.	AF 241177[b]
CMW 7772	*B. ribis*	*Ribis* spp.	U.S.A.	[d]
CMW 7060	*B. stevensii*	*Malus domestica*	Netherlands	[d]
ATCC 58194	*F. luteum*	*Actinidia chenensus*	New Zealand	AF 243396[b]
CMW 992	*F. luteum*	*A. deliciosa*	New Zealand	AF 027745[b]
CMW 7801	*D. mangiferae*	*Mangiferae indica*	Australia	[e]
CMW 7024	*D. mangiferae*	*M. indica*	Australia	[e]
CMW 10332	*D. mangiferae*	*Tibouchina* spp.	South Africa	AY194468[c]
CMW 3025	*M. africana*	*Eucalyptus* spp.	South Africa	AF283690[b]

[a]CMW represents the culture collection of the Forestry and Agricultural Biotechnology Institute

ATCC represents the American Type Culture Collection.

[b] Sequences obtained from GenBank.

[c] Sequences generated in this study.

[d] Sequences generated by Slippers *et al.* (2003).

[e] Sequences generated by Slippers (pers com.)

Figure 1. Light micrographs of conidia of *Botryosphaeria* spp. isolated from *Tibouchina* spp. in this study (a) *B. parva*, South Africa, (b) *B. parva*, Australia, (c) *B. parva*, New Zealand, (d) *D. mangiferae*, South Africa.

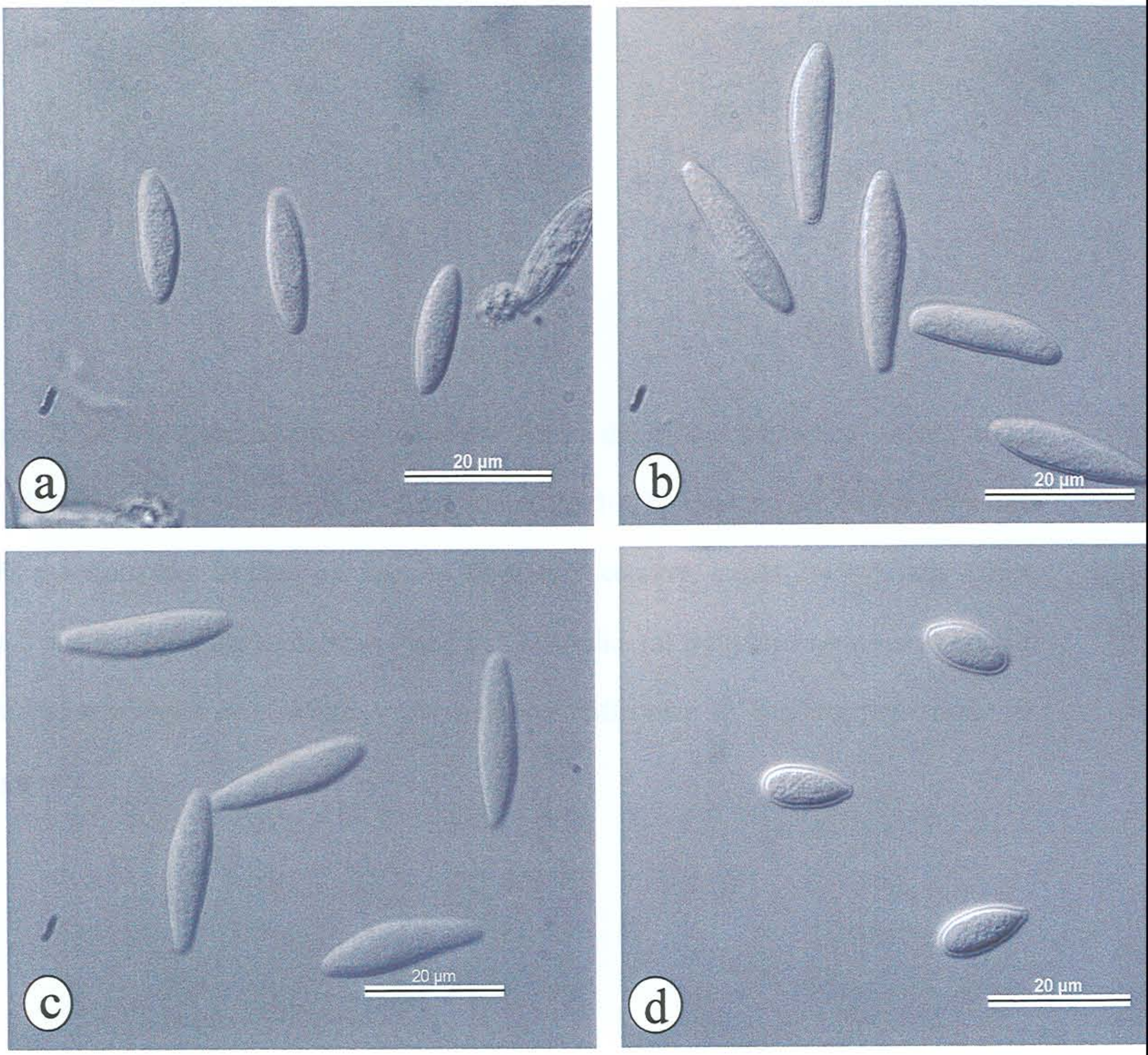

a
20 µm
b
20 µm
c
20 µm
d
20 µm

Figure 2. A 3% Agarose-ethidium bromide gel of the different banding paterns of the *Cfo* I digested ITS amplicons. Lane 1 represents the 100 bp marker. Lanes 2-8 represents amplicons from two isolates per species from each country. Lanes 2-4 = South Africa, lanes 5 and 6 = New Zealand, lanes 7 and 8 = Australia. (a) *Botryosphaeria parva* and (b) *Dothiorella mangiferae*. White arrow indicates difference in banding pattern for *D. mangiferae*.

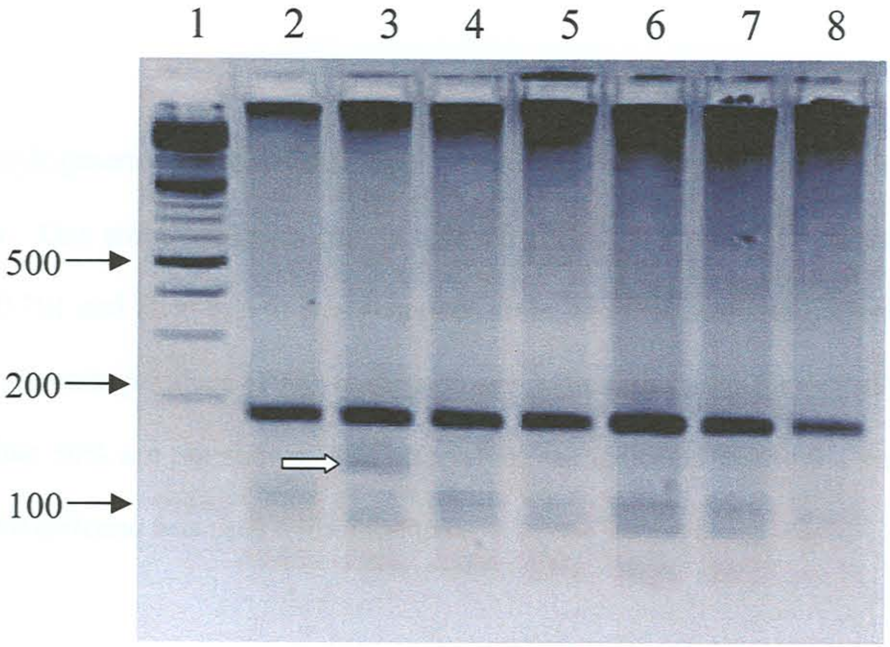

1
2
3
4
5
6
7
8
500
200
100

Figure 3. Phylogenetic tree generated from a data set including ITS and 5.8S gene sequence data. One strict consensus tree (length of tree = 217 steps, CI = 0.806, RI = 0.869, RC = 0.701 and HI = 0.194) was generated from heuristic searches performed on the data set. Bootstrap values (1000 replicates) are indicated above the branches and those lower than 50% are not shown. MCMC values are indicated below the branches. *Mycosphaerella africana* was used to root the tree.

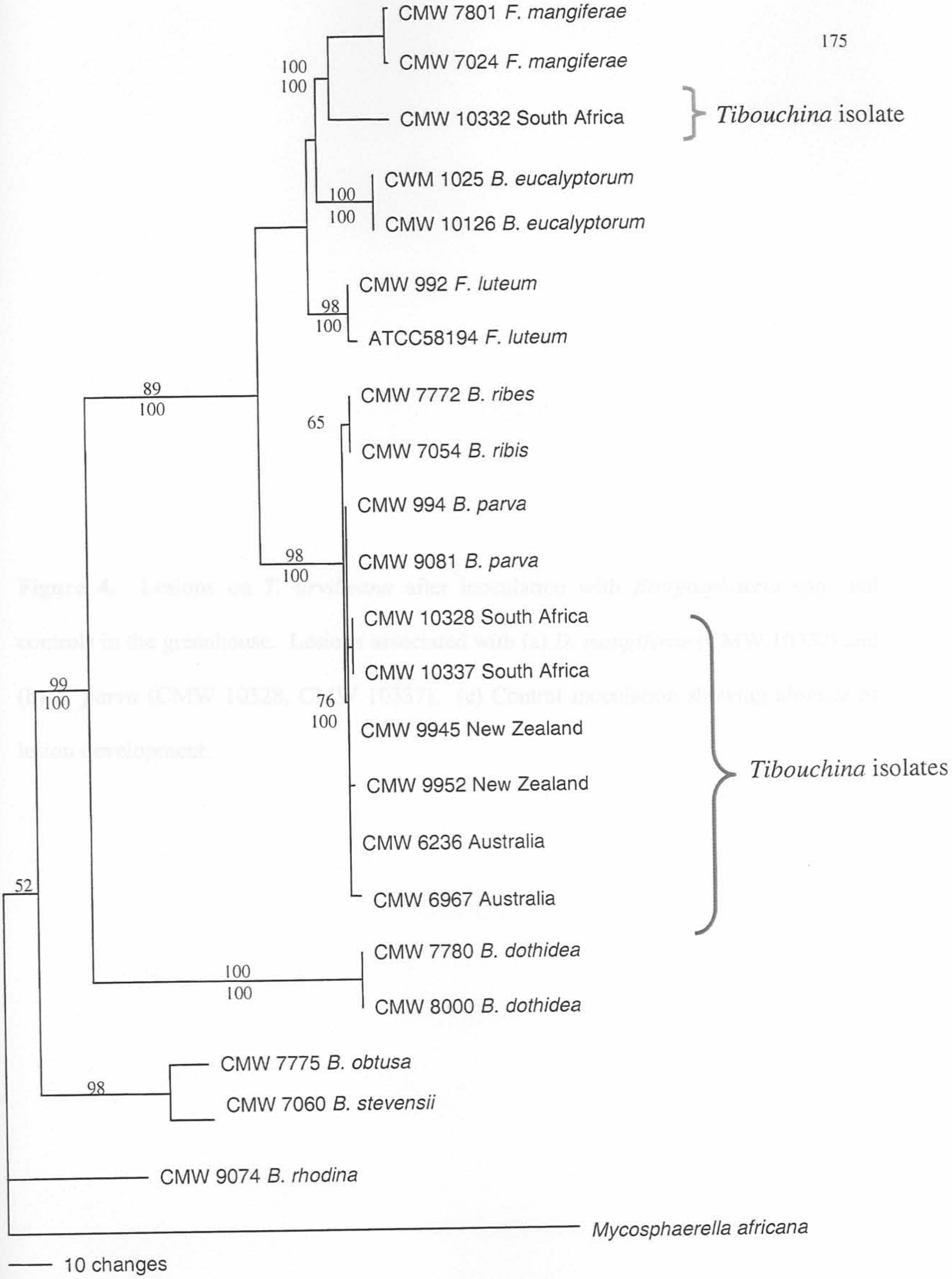

CMW 7801 *F. mangiferae*
CMW 7024 *F. mangiferae*
CMW 10332 South Africa
Tibouchina isolate
CWM 1025 *B. eucalyptorum*
CMW 10126 *B. eucalyptorum*
CMW 992 *F. luteum*
ATCC58194 *F. luteum*
CMW 7772 *B. ribes*
CMW 7054 *B. ribis*
CMW 994 *B. parva*
CMW 9081 *B. parva*
CMW 10328 South Africa
CMW 10337 South Africa
CMW 9945 New Zealand
CMW 9952 New Zealand
CMW 6236 Australia
CMW 6967 Australia
Tibouchina isolates
CMW 7780 *B. dothidea*
CMW 8000 *B. dothidea*
CMW 7775 *B. obtusa*
CMW 7060 *B. stevensii*
CMW 9074 *B. rhodina*
Mycosphaerella africana
100
100
100
100
98
100
89
100
65
98
100
76
100
99
100
52
100
100
98
10 changes

Figure 4. Lesions on *T. urvilleana* after inoculation with *Botryosphaeria* spp. and controls in the greenhouse. Lesions associated with (a) *D. mangiferae* (CMW 10332) and (b) *B. parva* (CMW 10328, CMW 10337). (c) Control inoculation showing absence of lesion development.

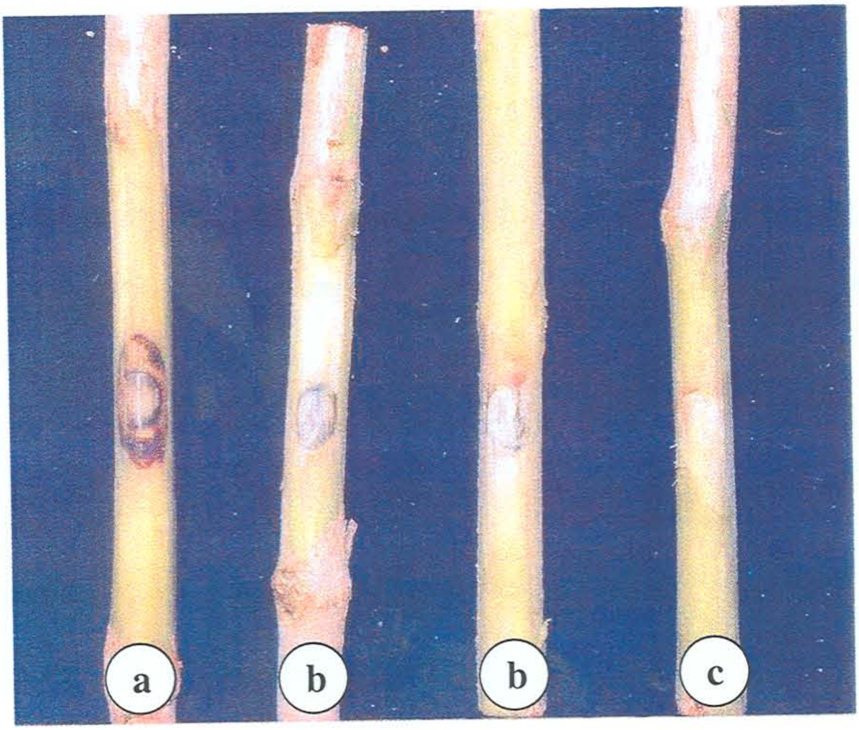
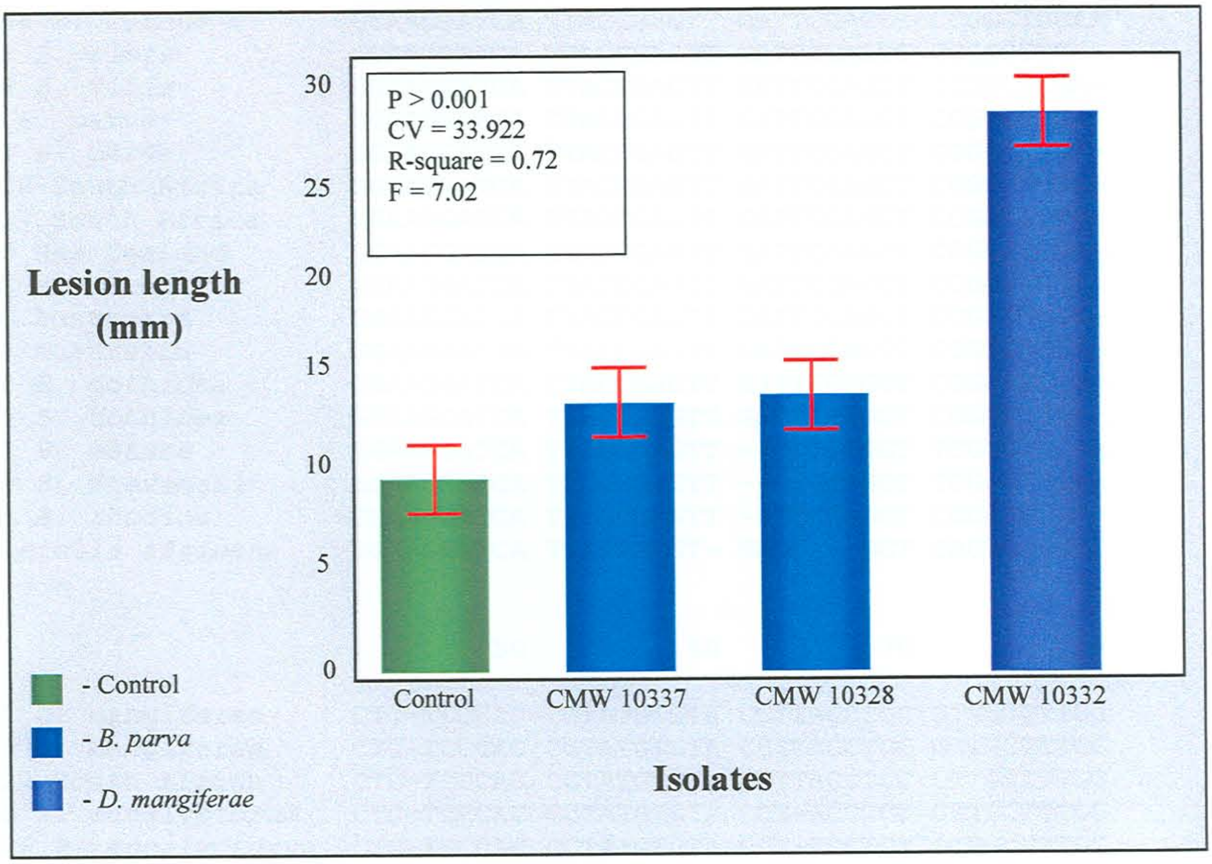

a
b
b
c

Fig. 5. Bar chart indicating the average lesion lengths in millimetres resulting from inoculation trials of *B. parva* and *D. mangiferae* on *Tibouchina urvilleana*. Error bars indicate standard deviations of the data.


P > 0.001
CV = 33.922
R-square = 0.72
F = 7.02
Lesion length (mm)
30
25
20
15
10
5
0
Control
CMW 10337
CMW 10328
CMW 10332
Isolates
🟩 - Control
🟦 - *B. parva*
🟦 - *D. mangiferae*

APPENDIX 1

	10	20	30	40
CMW 7801 *D.mangiferae*	GGAAGGATCA	TTACCGAGTT	GATTCGAGCT	CCGGCTCGA-
CMW 7024 *D. mangiferae*	GGAAGGATCA	TTACCGAGTT	GATTCGAGCT	CCGGCTCGA-
CMW 10322 South Africa	GGAAGGATCA	TTACCGAGTT	GATTCGAGCT	CCGGCTCGA-
CWM 1025 *B. eucalyptorum*	GGAAGGATCA	TTACCGAGTT	GACTCGAGCT	CCGGCTCGA-
CMW 10126 *B. eucalyptorum*	GGAAGGATCA	TTACCGAGTT	GACTCGAGCT	CCGGCTCGA-
CMW 992 *F. luteum*	GGAAGGATCA	TTACCGAGTT	GATTCGAGCT	CCGGCTCGA-
ATCC58194 *F. luteum*	GGAAGGATCA	TTACCGAGTT	GATTCGAGCT	CCGGCTCGAA
CMW 7772 *B. ribis*	GGAAGGATCA	TTACCGAGTT	GATTCGAGCT	CCGGCTCGA-
CMW 7054 *B. ribis*	GGAAGGATCA	TTACCGAGTT	GATTCGAGCT	CCGGCTCGA-
CMW 994 *B. parva*	GGAAGGATCA	TTACCGAGTT	GATTCGAGCT	CCGGCTCGA-
CMW 9081 *B. parva*	GGAAGGATCA	TTACCGAGTT	GATTCGAGCT	CCGGCTCGA-
CMW 10328 South Africa	GGAAGGATCA	TTACCGAGTT	GATTCGAGCT	CCGGCTCTA-
CMW 10337 South Africa	GGAAGGATCA	TTACCGAGTT	GATTCGAGCT	CCGGCTCTA-
CMW 9945 New Zealand	GGAAGGATCA	TTACCGAGTT	GATTCGAGCT	CCGGCTCGA-
CMW 9952 New Zealand	GGAAGGATCA	TTACCGAGTT	GATTCGAGCT	CCGGCTCGA-
CMW 6236 Australia	GGAAGGATCA	TTACCGAGTT	GATTCGAGCT	CCGGCTCGA-
CMW 6967 Australia	GGAAGGATCA	TTACCGAGTT	GATTCGAGCT	CCGGCTCGA-
CMW 7780 *B. dothidea*	GGAAGGATCA	TTACCGAGTT	GATTCGGGCT	CCGGCCCGA-
CMW 8000 *B. dothidea*	GGAAGGATCA	TTACCGAGTT	GATTCGGGCT	CCGGCCCGA-
CMW 7775 *B. obtusa*	GGAAGGATCA	TTACCGAGTT	--CTCGGGCT	TCGGCTCGAA
CMW 7060 *B. stevensii*	GGAAGGATCA	TTACCGAGTT	--CTCGAGCT	TCGGCTCGAA
CMW 9074 *B. rhodina*	GGAAGGATCA	TTACCGAGTT	--TTCGAGCT	CCGGCTCGA-
Mycosphaerella africana	GGAAGGATCA	TTACTGAGT-	GAGG---GCT	CACGCCCGAC

	50	60	70	80
CMW 7801 *D. mangiferae*	CTC-TCCCAC	CCTATGTGTA	CCTTACCTCC	GTTGCTTTGG
CMW 7024 *D. mangiferae*	CTC-TCCCAC	CCTATGTGTA	CCTTACCTCC	GTTGCTTTGG
CMW 10322 South Africa	CTC-TCCCAC	CCTATGTGTA	CCTTACCTCC	GTTGCTTTGG
CWM 1025 *B. eucalyptorum*	CTC-TCCCAC	CCTATGTGTA	CCT-ACCTCT	GTTGCTTTGG
CMW 10126 *B. eucalyptorum*	CTC-TCCCAC	CCTATGTGTA	CCT-ACCTCT	GTTGCTTTGG
CMW 992 *F. luteum*	CTC-TCCCAC	CCCATGTGTA	CCT-ACCTCT	GTTGCTTTGG
ATCC58194 *F. luteum*	CTC-TCCCAC	CCCATGTGTA	CCT-ACCTCT	GTTGCTTTGG
CMW 7772 *B. ribis*	CTC-TCCCAC	CCAATGTGTA	CCT-ACCTCT	GTTGCTTTGG
CMW 7054 *B. ribis*	CTC-TCCCAC	CCAATGTGTA	CCT-ACCTCT	GTTGCTTTGG
CMW 994 *B. parva*	CTC-TCCCAC	CCAATGTGTA	CCT-ACCTCT	GTTGCTTTGG
CMW 9081 *B. parva*	CTC-TCCCAC	CCAATGTGTA	CCT-ACCTCT	GTTGCTTTGG
CMW 10328 South Africa	CTC-TCCCAC	CCAATGTGTA	CCT-ACCTCT	GTTGCTTTGG
CMW 10337 South Africa	CTC-TCCCAC	CCAATGTGTA	CCT-ACCTCT	GTTGCTTTGG
CMW 9945 New Zealand	CTC-TCCCAC	CCAATGTGTA	CCT-ACCTCT	GTTGCTTTGG
CMW 9952 New Zealand	CTC-TCCCAC	CCAATGTGTA	CCT-ACCTCT	GTTGCTTTGG
CMW 6236 Australia	CTC-TCCCAC	CCAATGTGTA	CCT-ACCTCT	GTTGCTTTGG
CMW 6967 Australia	CTC-TCCCAC	CCAATGTGTA	CCT-ACCTCT	GTTGCTTTGG
CMW 7780 *B. dothidea*	-TCCTCCCAC	CCTTTGTGTA	CCT-ACCTCT	GTTGCTTTGG
CMW 8000 *B. dothidea*	-TCCTCCCAC	CCTTTGTGTA	CCT-ACCTCT	GTTGCTTTGG
CMW 7775 *B. obtusa*	-TC-TCCCAC	CCTTTGTGAA	CAT-ACCTCT	GTTGCTTTGG
CMW 7060 *B. stevensii*	-TC-TCCCAC	CCTTTGTGAA	CAT-ACCTCT	GTTGCTTTGG
CMW 9074 *B. rhodina*	CTC-TCCCAC	CCTTTGTGAA	CGT-ACCTCT	GTTGCTTTGG
Mycosphaerella africana	C---TCCAAC	CCTTTGTGAA	CCA-A-CTCT	GTTGCTTCGG

```
                                     90        100        110        120

CMW 7801 D. mangiferae       CGGGCCGCGG TCCTCCGCA- CCGGCTCCCC -TCGAGGGGG
CMW 7024 D. mangiferae       CGGGCCGCGG TCCTCCGCA- CCGGCTCCCC -TCGAGGGGG
CMW 10322 South Africa       CGGGCCGCGG TCCTCCGCA- CCGGCTCCCC -TCGAGGGGG
CWM 1025 B. eucalyptorum     CGGGCCGCGG TCCTCCGCA- CCGGCTCCCT TT---GGGGG
CMW 10126 B. eucalyptorum    CGGGCCGCGG TCCTCCGCA- CCGGCTCCCT TT---GGGGG
CMW 992 F. luteum            CGGGCCGCGG TCCTCCGCA- CCGACCCCCG TTCG-GGGGG
ATCC58194 F. luteum          CGGGCCGCGG TCCTCCGCA- CCGACCCCCG TTCG-GGGGG
CMW 7772 B. ribis            CGGGCCGCGG TCCTCCGCA- CCGGCGCCC- TTCGGGGGGG
CMW 7054 B. ribis            CGGGCCGCGG TCCTCCGCA- CCGGCGCCC- TTCGGGGGGG
CMW 994 B. parva             CGGGCCGCGG TCCTCCGCA- CCGGCGCCC- TTCG-AGGGG
CMW 9081 B. parva            CGGGCCGCGG TCCTCCGCA- CCGGCGCCC- TTCG-AGGGG
CMW 10328 South Africa       CGGGCCGCGG TCCTCCGCA- CCGGCGCCC- TTCG-AGGGG
CMW 10337 South Africa       CGGGCCGCGG TCCTCCGCA- CCGGCGCCC- TTCG-AGGGG
CMW 9945 New Zealand         CGGGCCGCGG TCCTCCGCA- CCGGCGCCC- TTCG-AGGGG
CMW 9952 New Zealand         CGGGCCGCGG TCCTCCGCA- CCGGCGCCC- TTCG-AGGGG
CMW 6236 Australia           CGGGCCGCGG TCCTCCGCA- CCGGCGCCC- TTCG-AGGGG
CMW 6967 Australia           CGGGCCGCGG TCCTCCGCA- CCGGCGCCC- TTCG-AGGGG
CMW 7780 B. dothidea         CGGGCCGCGG TCCTCCGCGG CCGGCCCCCC TCCCCGGGGG
CMW 8000 B. dothidea         CGGGCCGCGG TCCTCCGCGG CCGGCCCCCC TCCCCGGGGG
CMW 7775 B. obtusa           CGGCTC---- TTTGCCGCG- AGGAGGCCC- TCGC-GGGCC
CMW 7060 B. stevensii        CGGCTC---- -TTGCCGCG- TGGAGGCCC- TCAA-AAAGC
CMW 9074 B. rhodina          CGGCTC---- ---------- ---------- ----------
Mycosphaerella africana      GGG-CGACC- ------CCGC CG------TT TCGGCGACGG


                                    130        140        150        160

CMW 7801 D. mangiferae       CTGG--CCA- GCGC---CCG CCAGAGGACC ACAAAACTCC
CMW 7024 D. mangiferae       CTGG--CCA- GCGC---CCG CCAGAGGACC ACAAAACTCC
CMW 10322 South Africa       CTGG--CCA- GCGC---CCG CCAGAGGACC ACAAAACTCC
CWM 1025 B. eucalyptorum     CTGG--CCA- GCGT---CCG CCAGAGGACC ACAAAACTCC
CMW 10126 B. eucalyptorum    CTGG--CCA- GCGT---CCG CCAGAGGACC ACAAAACTCC
CMW 992 F. luteum            CCGG--CCA- GCGC---CCG CCAGAGGACC ACAAAACTCC
ATCC58194 F. luteum          CCGG--CCA- GCGC---CCG CCAGAGGACC ACAAAACTCC
CMW 7772 B. ribis            CTGG--CCA- GCGC---CCG CCAGAGGACC ATAAAACTCC
CMW 7054 B. ribis            CTGG--CCA- GCGC---CCG CCAGAGGACC ATAAAACTCC
CMW 994 B. parva             CTGG--CCA- GCGC---CCG CCAGAGGACC ATAAAACTCC
CMW 9081 B. parva            CTGG--CCA- GCGC---CCG CCAGAGGACC ATAAAACTCC
CMW 10328 South Africa       CTGG--CCA- GCGC---CCG CCAGAGGACC ATAAAACTCC
CMW 10337 South Africa       CTGG--CCA- GCGC---CCG CCAGAGGACC ATAAAACTCC
CMW 9945 New Zealand         CTGG--CCA- GCGC---CCG CCAGAGGACC ATAAAACTCC
CMW 9952 New Zealand         CTGG--CCA- GCGC---CCG CCAGAGGACC ATAAAACTCC
CMW 6236 Australia           CTGG--CCA- GCGC---CCG CCAGAGGACC ATAAAACTCC
CMW 6967 Australia           CTGG--CCA- GCGC---CCG CCAGAGGACC ATAAAACTCC
CMW 7780 B. dothidea         GTGG--CCA- GCGC---CCG CCAGAGGACC ATCAAACTCC
CMW 8000 B. dothidea         GTGG--CCA- GCGC---CCG CCAGAGGACC ATCAAACTCC
CMW 7775 B. obtusa           CCCC--CGC- GCGCTTTCCG CCAGAGGACC TTCAAACTCC
CMW 7060 B. stevensii        CCCC--CCGT GCGCTT-CCG CCAGAGGACC TTCAAACTCC
CMW 9074 B. rhodina          ---------- -CGG---CCG CCAAAGGACC TTCAAACTCC
Mycosphaerella africana      CGGCCC---- -------CCG ---GAGGT-C ATCAAA-CAC
```

	170	180	190	200
CMW 7801 *D. mangiferae*	AGTCAGTGAA	CGTTGCAGCC	TGAAAAAC-A	AGTTAATAAA
CMW 7024 *D. mangiferae*	AGTCAGTGAA	CGTTGCAGCC	TGAAAAAC-A	AGTTAATAAA
CMW 10322 South Africa	AGTCAGTAAA	CGTTGCAGCC	TGAAAAAC-A	AGTTAATAAA
CWM 1025 *B. eucalyptorum*	AGTCAGTAAA	CGTTGCAGTC	TGAAAAAC-A	AGTTAATAAA
CMW 10126 *B. eucalyptorum*	AGTCAGTAAA	CGTTGCAGTC	TGAAAAAC-A	AGTTAATAAA
CMW 992 *F. luteum*	AGTCAGTAAA	CGTCGCAGTC	TGAGAAAC-A	AGTTAATAAA
ATCC58194 *F. luteum*	AGTCAGTAAA	CGTCGCAGTC	TGAGAAAC-A	AGTTAATAAA
CMW 7772 *B. ribis*	AGTCAGTGAA	CTTCGCAGTC	TGAAAAAC-A	AGTTAATAAA
CMW 7054 *B. ribis*	AGTCAGTGAA	CTTCGCAGTC	TGAAAAAC-A	AGTTAATAAA
CMW 994 *B. parva*	AGTCAGTGAA	CTTCGCAGTC	TGAAAAAC-A	AGTTAATAAA
CMW 9081 *B. parva*	AGTCAGTGAA	CTTCGCAGTC	TGAAAAAC-A	AGTTAATAAA
CMW 10328 South Africa	AGTCAGTGAA	CTTCGCAGTC	TGAAAAAC-A	AGTTAATAAA
CMW 10337 South Africa	AGTCAGTGAA	CTTCGCAGTC	TGAAAAAC-A	AGTTAATAAA
CMW 9945 New Zealand	AGTCAGTGAA	CTTCGCAGTC	TGAAAAAC-A	AGTTAATAAA
CMW 9952 New Zealand	AGTCAGTGAA	CTTCGCAGTC	TGAAAAAC-A	AGTTAATAAA
CMW 6236 Australia	AGTCAGTGAA	CTTCGCAGTC	TGAAAAAC-A	AGTTAATAAA
CMW 6967 Australia	AGTCAGTGAA	CTTCGCAGTC	TGGAAAAC-A	AGTTAATAAA
CMW 7780 *B. dothidea*	AGTCAGTAAA	CGATGCAGTC	TGAAAAAC-A	T-TTAATAAA
CMW 8000 *B. dothidea*	AGTCAGTAAA	CGATGCAGTC	TGAAAAAC-A	T-TTAATAAA
CMW 7775 *B. obtusa*	AGTCAGTAAA	CGTCGACGTC	TGATAAAC-A	AGTTAATAAA
CMW 7060 *B. stevensii*	AGTCAGTAAA	CGTCGACGTC	TGAAAAAC-A	AGTTAATAAA
CMW 9074 *B. rhodina*	AGTCAGTAAA	CGCAGACGTC	TGATAAAC-A	AGTTAATAAA
Mycosphaerella africana	TGCATCTTTG	CGTCGGAGTC	T--TAAAGTA	AATTAA---A

	210	220	230	240
CMW 7801 *D. mangiferae*	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
CMW 7024 *D. mangiferae*	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
CMW 10322 South Africa	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
CWM 1025 *B. eucalyptorum*	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
CMW 10126 *B. eucalyptorum*	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
CMW 992 *F. luteum*	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
ATCC58194 *F. luteum*	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
CMW 7772 *B. ribis*	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
CMW 7054 *B. ribis*	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
CMW 994 *B. parva*	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
CMW 9081 *B. parva*	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
CMW 10328 South Africa	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
CMW 10337 South Africa	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
CMW 9945 New Zealand	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
CMW 9952 New Zealand	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
CMW 6236 Australia	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
CMW 6967 Australia	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
CMW 7780 *B. dothidea*	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
CMW 8000 *B. dothidea*	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
CMW 7775 *B. obtusa*	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
CMW 7060 *B. stevensii*	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
CMW 9074 *B. rhodina*	CTAAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA
Mycosphaerella Africana	C-AAAACTTT	CAACAACGGA	TCTCTTGGTT	CTGGCATCGA

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                                       250        260        270        280

CMW 7801 D. mangiferae        TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 7024 D. mangiferae        TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 10322 South Africa        TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CWM 1025 B. eucalyptorum      TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 10126 B. eucalyptorum     TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 992 F. luteum             TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
ATCC58194 F. luteum           TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 7772 B. ribis             TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 7054 B. ribis             TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 994 B. parva              TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 9081 B. parva             TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 10328 South Africa        TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 10337 South Africa        TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 9945 New Zealand          TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 9952 New Zealand          TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 6236 Australia            TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 6967 Australia            TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 7780 B. dothidea          TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 8000 B. dothidea          TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 7775 B. obtusa            TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 7060 B. stevensii         TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 9074 B. rhodina           TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
Mycosphaerella africana       TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA


                                       290        300        310        320

CMW 7801 D. mangiferae        GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 7024 D. mangiferae        GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 10322 South Africa        GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CWM 1025 B. eucalyptorum      GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 10126 B. eucalyptorum     GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 992 F. luteum             GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
ATCC58194 F. luteum           GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 7772 B. ribis             GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 7054 B. ribis             GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 994 B. parva              GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 9081 B. parva             GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 10328 South Africa        GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 10337 South Africa        GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 9945 New Zealand          GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 9952 New Zealand          GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 6236 Australia            GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 6967 Australia            GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 7780 B. dothidea          GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 8000 B. dothidea          GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 7775 B. obtusa            GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 7060 B. stevensii         GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 9074 B. rhodina           GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
Mycosphaerella africana       GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
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                                      330        340        350        360

CMW 7801 D. mangiferae        CCCTTGGTAT TCCGAGGGGC ATGCCTGTTC GAGCGTCATT
CMW 7024 D. mangiferae        CCCTTGGTAT TCCGAGGGGC ATGCCTGTTC GAGCGTCATT
CMW 10322 South Africa        CCCTTGGTAT TCCGAGGGGC ATGCCTGTTC GAGCGTCATT
CWM 1025 B. eucalyptorum      CCCTTGGTAT TCCGAGGGGC ATGCCTGTTC GAGCGTCATT
CMW 10126 B. eucalyptorum     CCCTTGGTAT TCCGAGGGGC ATGCCTGTTC GAGCGTCATT
CMW 992 F. luteum             CCCTTGGTAT TCCGAGGGGC ATGCCTGTTC GAGCGTCATT
ATCC58194 F. luteum           CCCTTGGTAT TCCGAGGGGC ATGCCTGTTC GAGCGTCATT
CMW 7772 B. ribis             CCCTTGGTAT TCCGAGGGGC ATGCCTGTTC GAGCGTCATT
CMW 7054 B. ribis             CCCTTGGTAT TCCGAGGGGC ATGCCTGTTC GAGCGTCATT
CMW 994 B. parva              CCCTTGGTAT TCCGAGGGGC ATGCCTGTTC GAGCGTCATT
CMW 9081 B. parva             CCCTTGGTAT TCCGAGGGGC ATGCCTGTTC GAGCGTCATT
CMW 10328 South Africa        CCCTTGGTAT TCCGAGGGGC ATGCCTGTTC GAGCGTCATT
CMW 10337 South Africa        CCCTTGGTAT TCCGAGGGGC ATGCCTGTTC GAGCGTCATT
CMW 9945 New Zealand          CCCTTGGTAT TCCGAGGGGC ATGCCTGTTC GAGCGTCATT
CMW 9952 New Zealand          CCCTTGGTAT TCCGAGGGGC ATGCCTGTTC GAGCGTCATT
CMW 6236 Australia            CCCTTGGTAT TCCGAGGGGC ATGCCTGTTC GAGCGTCATT
CMW 6967 Australia            CCCTTGGTAT TCCGAGGGGC ATGCCTGTTC TAGCGTCATT
CMW 7780 B. dothidea          CCTTTGGTAT TCCGAAGGGC ATGCCTGTTC GAGCGTCATT
CMW 8000 B. dothidea          CCTTTGGTAT TCCGAAGGGC ATGCCTGTTC GAGCGTCATT
CMW 7775 B. obtusa            CCCCTGGCAT TCCGGGGGGC ATGCCTGTTC GAGCGTCATT
CMW 7060 B. stevensii         CCCTTGGCAT TCCGAGGGGC ATGCCTGTTC GAGCGTCATT
CMW 9074 B. rhodina           CCCTTGGTAT TCCGGGGGGC ATGCCTGTTC GAGCGTCATT
Mycosphaerella africana       CCCGTGGTAT TCCGCGGGGC ATGCCTGTTC GAGCGTCATT


                                      370        380        390        400

CMW 7801 D. mangiferae        TCAACCCTCA AGCTCTGCTT GGTATTGGGC TCCG----TC
CMW 7024 D. mangiferae        TCAACCCTCA AGCTCTGCTT GGTATTGGGC TCCG----TC
CMW 10322 South Africa        TCAACCCTCA AGCTCTGCTT GGTATTGGGC TCCG----TC
CWM 1025 B. eucalyptorum      TCAACCCTCA AGCTTTGCTT GGTATTGGGC CCCG----TC
CMW 10126 B. eucalyptorum     TCAACCCTCA AGCTTTGCTT GGTATTGGGC CCCG----TC
CMW 992 F. luteum             TCAACCCTCA AGCTCTGCTT GGTATTGGGC TCCG----TC
ATCC58194 F. luteum           TCAACCCTCA AGCTCTGCTT GGTATTGGGC TCCG----TC
CMW 7772 B. ribis             TCAACCCTCA AGCTCTGCTT GGTATTGGGC TCCG----TC
CMW 7054 B. ribis             TCAACCCTCA AGCTCTGCTT GGTATTGGGC TCCG----TC
CMW 994 B. parva              TCAACCCTCA AGCTCTGCTT GGTATTGGGC CCCG----TC
CMW 9081 B. parva             TCAACCCTCA AGCTCTGCTT GGTATTGGGC CCCG----TC
CMW 10328 South Africa        TCAACCCTCA AGCTCTGCTT GGTATTGGGC CCCG----TC
CMW 10337 South Africa        TCAACCCTCA AGCTCTGCTT GGTATTGGGC CCCG----TC
CMW 9945 New Zealand          TCAACCCTCA AGCTCTGCTT GGTATTGGGC CCCG----TC
CMW 9952 New Zealand          TCAACCCTCA AGCTCTGCTT GGTATTGGGC CCCG----TC
CMW 6236 Australia            TCAACCCTCA AGCTCTGCTT GGTATTGGGC CCCG----TC
CMW 6967 Australia            TCAACCCTCA AGCTCTGCTT GGTATTGGGC CCCG----TC
CMW 7780 B. dothidea          ACAACCCTCA AGCTCTGCTT GGTATTGGGC ACCG----TC
CMW 8000 B. dothidea          ACAACCCTCA AGCTCTGCTT GGTATTGGGC ACCG----TC
CMW 7775 B. obtusa            ACAACCCTCA AGCTCTGCTT GGTATTGGGC GCCG----TC
CMW 7060 B. stevensii         ACAACCCTCA AGCTCTGCTT GGTATTGGGC GACG----TC
CMW 9074 B. rhodina           ACAACCCTCA AGCTCTGCTT GGAATTGGGC ACCG----TC
Mycosphaerella Africana       TCACCACTCA AGCCTAGCTT GGTATTGGGC GTCGCGGTTC
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                                     410        420        430        440

CMW 7801 D. mangiferae         CTC--CGCGG ACGCGCCTCA AAGACCT-CG GCGGTGGCGT
CMW 7024 D. mangiferae         CTC--CGCGG ACGCGCCTCA AAGACCT-CG GCGGTGGCGT
CMW 10322 South Africa         CTC--CGCGG ACGCGCCTCA AAGACCT-CG GCGGTGGCGT
CWM 1025 B. eucalyptorum       CTC--TGTGG ACGCGCCTCA AAGACCT-CG GCGGTGGCGT
CMW 10126 B. eucalyptorum      CTC--TGTGG ACGCGCCTCA AAGACCT-CG GCGGTGGCGT
CMW 992 F. luteum              CTCT--GTGG ACGCGCCTCG AAGACCT-CG GCGGTGGCGT
ATCC58194 F. luteum            CTCT--GTGG ACGCGCCTCG AAGACCT-CG GCGGTGGCGT
CMW 7772 B. ribis              CTC--CACGG ACGCGCCTTA AAGACCT-CG GCGGTGGCGT
CMW 7054 B. ribis              CTC--CACGG ACGCGCCTTA AAGACCT-CG GCGGTGGCGT
CMW 994 B. parva               CTCC--ACGG ACGCGCCTTA AAGACCT-CG GCGGTGGCGT
CMW 9081 B. parva              CTCC--ACGG ACGCGCCTTA AAGACCT-CG GCGGTGGCGT
CMW 10328 South Africa         CTCC--ACGG ACGCGCCTTA AAGACCT-CG GCGGTGGCGT
CMW 10337 South Africa         CTCC--ACGG ACGCGCCTTA AAGACCT-CG GCGGTGGCGT
CMW 9945 New Zealand           CTCC--ACGG ACGCGCCTTA AAGACCT-CG GCGGTGGCGT
CMW 9952 New Zealand           CTCC--ACGG ACGCGCCTTA AAGACCT-CG GCGGTGGCGT
CMW 6236 Australia             CTCC--ACGG ACGCGCCTTA AAGACCT-CG GCGGTGGCGT
CMW 6967 Australia             CTCC--ACGG ACGCGCCTTA AAGACCT-CG GCGGTGGCGT
CMW 7780 B. dothidea           CTTT--GCGG GCGCGCCTCA AAGACCT-CG GCGGTGGCGT
CMW 8000 B. dothidea           CTTT--GCGG GCGCGCCTCA AAGACCT-CG GCGGTGGCGT
CMW 7775 B. obtusa             CTCTCTGCGG ACGCGCCTTA AAGACCT-CG GCGGTG-GCT
CMW 7060 B. stevensii          CTCTCTGCGG ACGCGCCTCA AAGACCT-CG GCGGTG-GCT
CMW 9074 B. rhodina            CTCACTGCGG ACGCGCCTCA AAGACCT-CG GCGGTG-GCT
Mycosphaerella africana        CG-------- -CGCGCCTTA AAGT-CTCCG GC--TGAGCA


                                     450        460        470        480

CMW 7801 D. mangiferae         CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 7024 D. mangiferae         CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 10322 South Africa         CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CWM 1025 B. eucalyptorum       CTTG---CCT CAAGCGTAGT AGAAAT-CAC --CTCGCTTT
CMW 10126 B. eucalyptorum      CTTG---CCT CAAGCGTAGT AGAAAT-CAC --CTCGCTTT
CMW 992 F. luteum              CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
ATCC58194 F. luteum            CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 7772 B. ribis              CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 7054 B. ribis              CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 994 B. parva               CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 9081 B. parva              CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 10328 South Africa         CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 10337 South Africa         CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 9945 New Zealand           CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 9952 New Zealand           CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 6236 Australia             CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 6967 Australia             CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 7780 B. dothidea           CTTG---CCT CAAGCGTAGT AGAACA-TAC ATCTCGCTTC
CMW 8000 B. dothidea           CTTG---CCT CAAGCGTAGT AGAACA-TAC ATCTCGCTTC
CMW 7775 B. obtusa             GTTCAGCCCT CAAGCGTAGT AGAATA-CAC --CTCGCTTT
CMW 7060 B. stevensii          GTTCAGCCCT CAAGCGTAAT AGAATA-CAC --CTCGCTTT
CMW 9074 B. rhodina            GTTCAGCCCT CAAGCGTAGT AGAATA-CAC --CTCGCTTT
Mycosphaerella africana        GTTCGTCTC- TAAGCGTTGT GGCATATATT ---TCGCT--
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                                    490        500        510        520

CMW 7801 D. mangiferae        GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTTT-GAATA
CMW 7024 D. mangiferae        GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTTT-GAATA
CMW 10322 South Africa        GGAGCGCACG GAGTCACGCG GCGGACGAAC CTTT-GAATA
CWM 1025 B. eucalyptorum      GGAGCGCATG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 10126 B. eucalyptorum     GGAGCGCATG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 992 F. luteum             GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
ATCC58194 F. luteum           GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 7772 B. ribis             GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 7054 B. ribis             GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 994 B. parva              GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 9081 B. parva             GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 10328 South Africa        GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 10337 South Africa        GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 9945 New Zealand          GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 9952 New Zealand          GGAGCGCACG GCGTCGCCCG CCGGACGAAA CTT-TGAAT-
CMW 6236 Australia            GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 6967 Australia            GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 7780 B. dothidea          GGAGCGCAGG GCGTCGCCCG CCGGACGAAC CTTCTGAAC-
CMW 8000 B. dothidea          GGAGCGCAGG GCGTCGCCCG CCGGACGAAC CTTCTGAAC-
CMW 7775 B. obtusa            GGAGCGGTTG GCGTCGCCCG CCGGACGAAC CTTCTGAAC-
CMW 7060 B. stevensii         GGAGCGGTTG GCGTCGCCCG CCGGACGAAC CTTCTGAAC-
CMW 9074 B. rhodina           GGAGCGGTTG GCGTCGCCCG CCGGACGAAC CTTCTGAAC-
Mycosphaerella africana       GAAAGAGTTC GGGACGGCTT TTGGCCG--- -TT-AAATC-


                                   530        540

CMW 7801 D. mangiferae        TTTTTTCTCA A-GGTTGACC
CMW 7024 D. mangiferae        TTTTTTCTCA A-GGTTGACC
CMW 10322 South Africa        TTTTTTCTCA A-CGTTGACC
CWM 1025 B. eucalyptorum      -T-TTTCTCA A-GGTTGACC
CMW 10126 B. eucalyptorum     -T-TTTCTCA A-GGTTGACC
CMW 992 F. luteum             -T-TTTCTCA A-GGTTGACC
ATCC58194 F. luteum           -TATTTCTCA A-GGTTGACC
CMW 7772 B. ribis             -TATTTCTCA A-GGTTGACC
CMW 7054 B. ribis             -TATTTCTCA A-GGTTGACC
CMW 994 B. parva              -TATTTCTCA A-GGTTGACC
CMW 9081 B. parva             -TATTTCTCA A-GGTTGACC
CMW 10328 South Africa        -TATTTCTCA A-GGTTGACC
CMW 10337 South Africa        -TATTTCTCA A-GGTTGACC
CMW 9945 New Zealand          -TATTTCTCA A-GGTTGACC
CMW 9952 New Zealand          -TATTTCTCA A-GGTTGACC
CMW 6236 Australia            -TATTTCTCA A-GGTTGACC
CMW 6967 Australia            -TATTTCTCA A-GGTTGACC
CMW 7780 B. dothidea          -T-TTTCTCA A-GGTTGACC
CMW 8000 B. dothidea          -T-TTTCTCA A-GGTTGACC
CMW 7775 B. obtusa            -T-TTTCTCA A-GGTTGACC
CMW 7060 B. stevensii         -T-TTTCTCA A-GGTTGACC
CMW 9074 B. rhodina           -T-TTTCTCA A-GGTTGACC
Mycosphaerella africana       ---TTTCTTA AAGGTTGACC
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SUMMARY

Studies presented in this dissertation highlight the importance of alternative hosts in the biology and spread of *Eucalyptus* canker pathogens. Trees related to *Eucalyptus* such as *Syzygium cordatum* (Myrtaceae) and *Tibouchina* spp. (Melastomataceae) are shown to be hosts of some of the most important *Eucalyptus* canker pathogens in South Africa. Furthermore, these trees are clearly shown as sources of inoculum, in many cases not previously recognised.

Chapter 1 presents a review of literature dealing with the worldwide movement of plant pathogens, especially those important to forestry. It is clear from this review that with increasing global trade, new introductions of plant pathogens are likely to occur, and most probably to increase. Furthermore, an increased movement of pathogens from native to exotic hosts and *vice versa* appears to be likely. In order to reduce the impact of these pathogens, increased knowledge of their occurrence and biology is crucial. What is specifically needed to reduce this threat is increased knowledge on the means of spread; rapid and accurate techniques for identification and detection of the pathogens; and determination of areas of origin of high-risk pathogens. As the problems of invasive species are cosmopolitan, it is suggested that co-operative ventures between countries will be needed to reduce the threats relating to tree pathogens.

The remaining chapters of this dissertation focus on three tree genera belonging to the Myrtales. These include the commercially important *Eucalyptus* spp., ornamental *Tibouchina* spp. and native African *Syzygium* spp. In the past, it has been suggested that the canker pathogen *Cryphonectria cubensis* originated in Indonesia. Recent studies based on morphology and DNA sequence data have, however, shown that *C. cubensis* represents three distinct taxonomic groups, including isolates from South America and central Africa; south east Asia and South Africa. Results of studies presented in Chapter 2 supply data identifying a possible native host for South African *C. cubensis*. They also show that *C. cubensis* is common on the native *S. cordatum* and relatively wide-spread on this host in forestry areas of South Africa.

Chapter 3 of the dissertation supports the hypothesis that the South African form of *C. cubensis* is native to the country. The genetic diversity of *C. cubensis* in South Africa, determined using SSR markers, clearly shows that the fungus has a high diversity. Results show that the fungus has been present on native *Syzygium cordatum* for a longer time period than it has on the two exotic hosts (*Eucalyptus* and *Tibouchina* spp.). They also indicate that a limited number of genotypes originating from the *Syzygium* population have infected the two exotic hosts in South Africa.

During a survey of *Tibouchina* spp. for *C. cubensis*, an undescribed *Endothelia* sp. was discovered. Morphology and DNA sequence data have revealed that the undescribed species is closely related to *C. eucalypti*, which is a well-known canker pathogen of *Eucalyptus*. Pathogenicity trials showed that the undescribed species is more virulent than *C. eucalypti* on *Tibouchina* spp. Pathogenicity of this fungus was not tested on *Eucalyptus* spp., but results on *Tibouchina* indicate a possible threat to this economically important tree. This discovery clearly indicates how easy it would be for a pathogen to spread on an inconspicuous host such as *Tibouchina* spp. They are widely planted as ornamentals, and planting stock has certainly not been carefully screened for *Eucalyptus* pathogens.

In the last chapter of this dissertation, I report on the discovery of another important pathogen of *Eucalyptus* spp. from *Tibouchina* spp. A survey of *T. urvilleana* in South Africa, New Zealand and Australia revealed the presence of two *Botryosphaeria* spp. The most common of the two was identified as *B. parva*, a known pathogen of *Eucalyptus* spp. The other species, *D. mangiferae* is an important pathogen of mango in Australia. The discovery of *D. mangiferae* on *T. urvilleana* in South Africa represents the first report of this important pathogen in the country. This has serious implications for the significant South African mango industry. Both *B. parva* and *D. mangiferae* were shown to be pathogenic to *T. urvilleana* with *D. mangiferae* the most virulent.

This dissertation reports on two new hosts for important *Eucalyptus* pathogens, a new species of *Endothelia*, as well as presenting a first report of an important agricultural pathogen in South Africa. In a relatively limited study, I hope to have highlighted the enormous threat that the unchecked movement of any plant material has to commercial forestry and agricultural operations. This is especially clear from the discovery of an important mango pathogen on a host in the Myrtales. Clearly many pathogens do not spread only on plants closely related to their known hosts. In order to ensure the sustainability of commercial forestry and agricultural crops, it is crucially important to drastically improve quarantine procedures in many countries. To effectively achieve this goal, fungal identification techniques will need to be improved. Furthermore, education of people regarding the threat of uncontrolled plant movement will also need to substantially augmented.