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Botryosphaeria species on native South African *Syzygium cordatum* and their potential threat to *Eucalyptus*

by

Draginja Pavlic

A thesis submitted in partial fulfillment of the requirements for the degree

MAGISTER SCIENTIAE

In the Faculty of Natural and Agricultural Science, Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa

December 2004

Supervisor: Prof. Teresa A. Coutinho
Co-supervisors: Prof. Michael J. Wingfield
Dr. Bernard Slippers

I dedicate this thesis to my parents and my brother Ivan

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Declaration

I, the undersigned, hereby declare that the thesis submitted herewith for the degree *Magister Scientiae* to the University of Pretoria contains my own independent work. This work has hitherto not been submitted for any degree at any other University.

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Draginja Pavlic

December 2004

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ACKNOWLEDGEMENTS

I would like to express my sincere thanks and appreciation to the following:

Prof. Teresa Coutinho and Prof. Mike Wingfield who gave me opportunity to undertake this study at FABI. I am grateful to you and Dr. Bernard Slippers for all your support and guidance to complete this thesis.

Dr. Jolanda Roux for the knowledge, patience and understanding during many days we have spent in field.

Dr. Treena Burgess, Dr. H.F. Glen, Mr. Wilhelm Z. de Beer, Mrs. Marieka Gryzenhout and Mrs. Julia Kreiss for their assistance in completing Chapter 2. I also thank Dr. B.E. Eisenberg who provided the statistical analyses for Chapter 4.

My colleagues and friends in FABI, especially Riana, Karen, Izette and Bernice, who have helped to make my stay in South Africa enjoyable and fruitful.

My friends all around the world for their support and love. Special thanks to Gordana and Enver who have always given me courage especially during difficult times.

The University of Pretoria (UP), National Research Foundation (NRF), members of Tree Protection Co-operative Programme (TPCP) and the THRIP initiative of the Department of Trade and Industry, South Africa for financial support that allowed me to conduct and complete this study.

PREFACE

Among the disease causal agents in woody plants, the fungi appear to present the greatest potential threat. The most numerous, of more than 70 000 described species, are Ascomycetes fungi. One of the most widely distributed and economically important groups of ascomycetous fungi belongs to the genus *Botryosphaeria*.

The genus *Botryosphaeria* was established by Cesati & de Notaris in 1863. To date more than 220 species, including varieties, subspecies and *formae speciales* have been described. *Botryosphaeria* spp. have a cosmopolitan distribution and wide host range including gymnosperms and angiosperms. These fungi are common endophytes and latent opportunistic pathogens, causing disease under unfavorable environmental conditions. *Botryosphaeria* spp. are associated with various disease symptoms, but are best known as canker and die-back pathogens on woody plants and including commercial forestry species such as *Eucalyptus* (*Myrtaceae*).

Eucalyptus spp. form an important component of exotic forest plantations in South Africa, covering more than 500 000 hectares. *Botryosphaeria* spp. are considered as important pathogens on *Eucalyptus* spp. in the country. Some *Botryosphaeria* spp. also occur on native South African myrtaceous tree species, but their role and influence are unknown. These subjects are important when considering examples of the global movement of *Botryosphaeria* pathogens, and their potential to cross-infect closely related exotic and native *Myrtacea* in South Africa. This study was, therefore, undertaken to expand our knowledge on *Botryosphaeria* spp. that occur on native *Myrtaceae* in South Africa.

In the literature review I summarise what is known about *Botryosphaeria* spp. on *Eucalyptus* in its native range and in exotic plantations, particularly in South Africa. Some background regarding *Botryosphaeria* and its taxonomy is included. Furthermore, the importance, occurrence and diversity of various *Botryosphaeria* spp. on *Eucalyptus* spp. are discussed. Measures for preventing the spread of this group of fungi and management strategies are also considered.

The first aim of the experimental work was to identify *Botryosphaeria* spp. that occur on *Syzygium cordatum*, which is the most common and widely distributed native myrtaceous tree species in South Africa. To achieve this, I sampled trees from different geographical regions in the country. All isolates obtained from asymptomatic and dying branches and leaves, that resembled *Botryosphaeria* spp., were identified to the species

level. To distinguish the species and determine relationships between them, both morphological and DNA based molecular data were used.

Botryosphaeria species are separated into two groups based on their anamorphs, namely groups with *Diplodia*-like and *Fusicoccum*-like anamorphs. The majority of isolates obtained in this study belong to the *Fusicoccum* group. Isolates of two *Botryosphaeria* spp. obtained in this study, which have *Lasiodiplodia* anamorphs and fall within the *Diplodia*-like group, appear to be undescribed. One of the undescribed species was represented by only one isolate and it was not named. The other undescribed *Botryosphaeria* species obtained in this study was described as a new *Botryosphaeria* anamorph, namely *Lasiodiplodia gonubinesis*. These data are presented and discussed in Chapter 2. Identification and characterisation of the other *Botryosphaeria* spp. obtained in this study are presented in Chapter 3.

Botryosphaeria spp. obtained in this study, such as *B. dothidea*, *B. ribis* and *B. rhodina*, are pathogens on *Eucalyptus*. However, the pathogenicity of the other *Botryosphaeria* species obtained in this study were not known. To test potential threat of different *Botryosphaeria* spp. isolated from *S. cordatum* to *Eucalyptus*, a pathogenicity trial was conducted under greenhouse conditions. Tests were run on two-year old trees of *Eucalyptus camaldulensis* clone GC-540. Simultaneously, *S. cordatum* seedlings were inoculated with the same *Botryosphaeria* isolates to test their pathogenicity on this host. Data gathered from these trials are summarised and discussed in Chapter 4.

Finally, a summary is included to review the data obtained during this study and to suggest directions for future studies to better understand these fungi, their origin, movement, role and influence.

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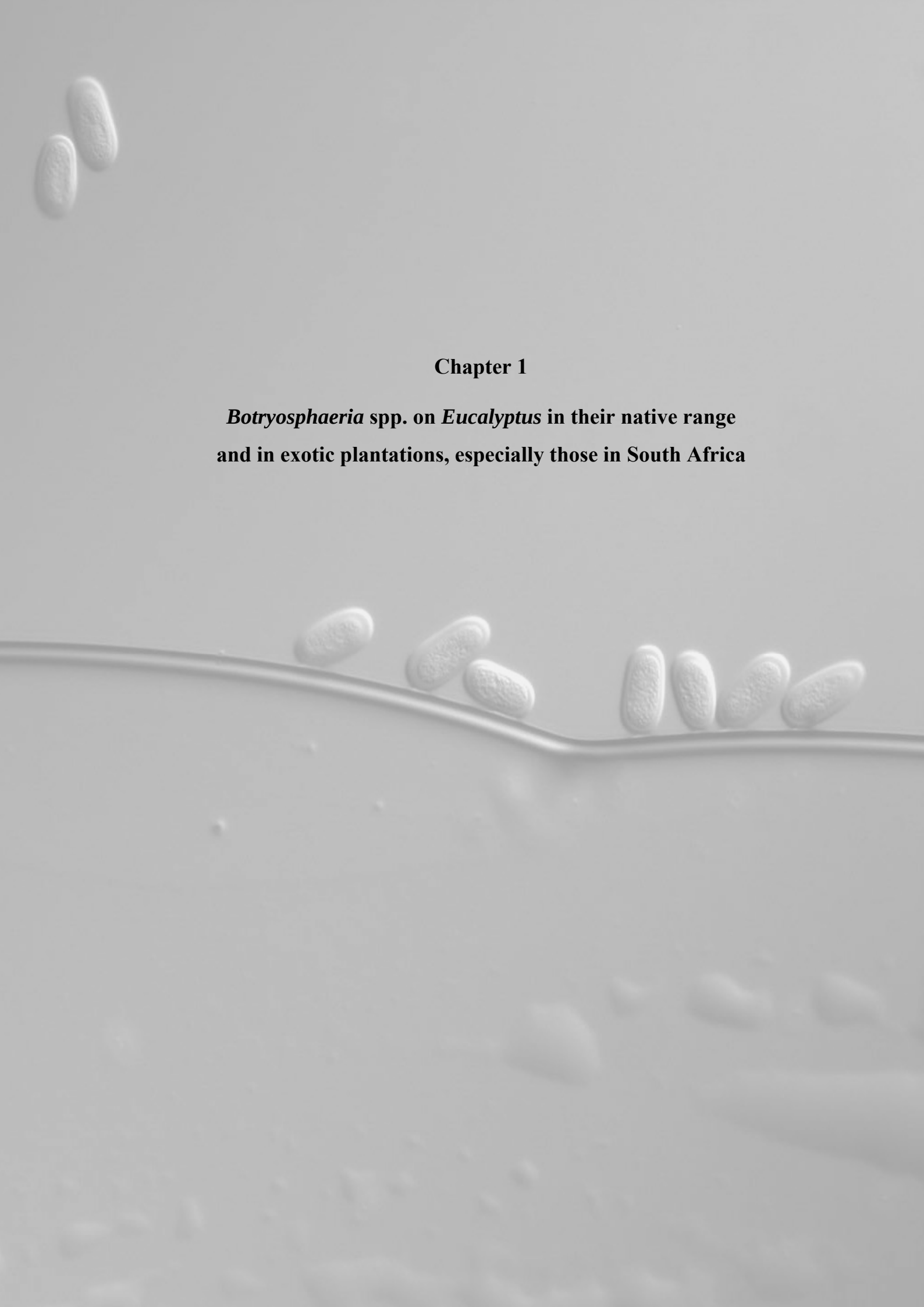


Chapter 1

Botryosphaeria spp. on *Eucalyptus* in their native range and in exotic plantations, especially those in South Africa

ABSTRACT

Botryosphaeria spp. cause diseases on many woody plants worldwide, including *Eucalyptus* spp. Most species of *Eucalyptus* are native to Australia but many species are widely planted as exotics, mostly in the Southern Hemisphere and Tropics. *Botryosphaeria* spp. have been reported from *Eucalyptus* in their native range as well as where they are grown as exotics. For a number of years it was thought that *B. dothidea* is the most wide spread *Botryosphaeria* sp. on *Eucalyptus*. Botryosphaeriaceous fungi have, however, often been misidentified, particularly due to overlapping morphological characteristics. In recent years, the identification and understanding of the ecology of *Botryosphaeria* spp. on *Eucalyptus* and other hosts, has been aided substantially through the application of DNA based techniques. Data emerging from these studies have given rise to revisions and the description of many new species. For example, it is now believed that *B. parva* is the most common species on *Eucalyptus* in exotic plantations. Previously unknown species such as *B. eucalyptorum* and *B. eucalypticola*, rather than *B. dothidea* have also been shown to be important. The aim of this literature review is to compile recent data and evaluate the importance, diversity and occurrence of *Botryosphaeria* spp. on *Eucalyptus* spp.

1.0. Introduction

Botryosphaeria spp. cause diseases on many *Eucalyptus* spp. in their native range in Australasia, as well as in exotic plantations worldwide. These fungi are Ascomycetes that commonly give rise to both anamorph and teleomorph states during their life cycle. Identification and description of species has, in the past, been based on morphological characteristics and the host on which various species occur. Because of overlapping of ascospore and conidial morphologies, similarity in culture morphology, extensive host ranges of some species, and the occurrence of more than one species on the same host, much confusion has surrounded the identification of species in this genus through its 140-year-long history (Arx & Müller1954, Pennycook & Samuels 1985, Jacobs & Rehner 1998, Smith & Stanosz 2001, Denman *et al.* 2000).

In recent years, DNA based techniques have been employed in identification and classification of fungal species. A few revisions have been made on *Botryosphaeria* spp., combining morphological and molecular data to distinguish and identify these fungi (Denman *et al.* 1999, Denman *et al.* 2003, Zhou & Stanosz 2001a, Zhou *et al.* 2001, De Wet *et al.* 2003, Slippers *et al.* 2004a). New data have shown that the distribution of species is different than was previously thought. For example, *B. dothidea* was considered to be the most widespread and common *Botryosphaeria* sp. on *Eucalyptus* (Smith *et al.* 1996b). However, combining morphological and DNA-based comparisons Slippers *et al.* (2004a) showed that *B. parva,* rather than *B. dothidea,* is the most common species on *Eucalyptus*, in many areas.

The aim of this review is to summarise data concerning *Botryosphaeria* spp. on *Eucalyptus* spp. in their native range and in exotic plantations, particularly in South Africa. Due to the fact that one of the main considerations for control of *Botryosphaeria* lies in the identification of species, some background regarding the genus and its taxonomy, with a focus on identification of species from the first descriptions to those using molecular techniques, is included. Importance, occurrence and diversity of various *Botryosphaeria* spp. will also be discussed. Measures for preventing the spread of this group of fungi and management strategies are also considered.

2.0. Taxonomy of *Botryosphaeria*

The genus *Botryosphaeria* Ces. & De Not. was established by Cesati and de Notaris in 1863 (Cesati & De Notaris 1863). These authors treated 12 species, including *B. dothidea* (Moug. : Fr.) Ces & De Not. previously described by Mougeot as *Sphaeria dothidea* Moug. : Fr. (Fries 1823). *Botryosphaeria dothidea* is the lectotype species of the genus (Barr 1972). To date 220 species, including varieties, subspecies and *formae speciales,* have been described (www.indexfungorum.org/Names/NAMES.ASP), but some of them have been reduced to synonymy with others, while the status of many species is unknown.

Botryosphaeria spp. are Ascomycetes and their taxonomy is complex and problematic at different levels of classification. The position of the genus in the higher classification of Ascomycetes is still unresolved (Denman *et al.* 2000). Since the genus was established, its taxonomic position has been changed many times due to the different morphological characteristics that have been used for taxonomic delimitation and classification within Ascomycetes (Luttrell 1955, Arx & Müller 1975, Barr 1979, Sivanesan 1984). Currently, the classification of *Botryosphaeria* by Hawksworth *et al.* (1995) is widely accepted (Denman *et al.* 2000). In this classification *Botryosphaeria* is accommodated in the family *Botryosphaeriaceae*, order *Dothideales*, phylum *Ascomycota.*

Identification of *Botryosphaeria* spp. at species level has been controversial and confused for a number of reasons. Firstly, species treated in the genus have not always accompanied by detailed morphological descriptions. Initially, some *Botryosphaeria* spp. were described primarily based on the hosts on which they were found (Cesati & De Notaris 1863, Saccardo 1877, 1882, Grossenbacher & Duggar 1911, Putterill 1919). In 1954, Arx & Müller reduced many of these species to synonymy, mainly under the names *B. quercuum* (Schwein.) Sacc. and *B. dothidea*. This was based almost exclusively on teleomorph characteristics (Arx & Müller 1954), which are now known to be highly conserved. There have not always been connections made between *Botryosphaeria* species and their anamorphs, which has led to further confusion. For example, at the time when *B. dothidea* was described, its anamorph, *Fusicoccum aesculi* Corda, was known separately, but there were no connections made between these taxa (Sutton 1980). Much later, it was noted that *F. aesculi* is the conidial state of *B. dothidea* (Sutton 1980, Pennycook & Samuels 1985, Crous & Palm 1999, Slippers *et al.* 2004a).

Identification and delimitation of species based exclusively on teleomorph characters is difficult. *Botryosphaeria* spp. develop both anamorph and teleomorph

fruiting structures during their life cycle. However, teleomorph (sexual) structures have less commonly been found in nature and have rarely been induced in culture (Shoemaker 1964, Laundon 1973, Jacobs & Rehner 1998, Slippers 2003). Morphological characteristics of the teleomorph structures are also not sufficiently informative to delimitate species, because the size and shape of spores as well as other morphological characteristics overlap (Shoemaker 1964, Laundon 1973, Sivanesan 1984, Slippers 2003). For this reason species have often been identified based on morphological characteristics of associated anamorphs (Shoemaker 1964, Pennycook & Samuels 1985, Crous & Palm 1999).

Anamorphs of *Botryosphaeria* spp. have been placed in 18 genera (Denman *et al.* 2000), but mostly into *Diplodia* Fr., *Dothiorella* Sacc., *Lasiodiplodia* Ellis & Everh., *Fusicoccum* Cda., *Botryodiplodia* (Sacc.) Sacc, *Sphaeropsis* (Sivanesan 1984) and *Macrophoma* (Sacc.) Berl. & Voglino. Anamorph characters most commonly used to distinguish between *Botryosphaeria* spp. are conidial and culture morphology (Shoemaker 1964, Pennycook & Samuels 1985, Denman *et al.* 2000, Slippers *et al.* 2004a). Identification of species based on anamorph characters also raise problems caused due to overlapping of characteristics between species and changes in spore morphology that occur as cultures age (Pennycook & Samuels 1985, Jacobs & Rehner 1998, Slippers 2003). In addition, more than one species may occur on the same host and ascospores and conidia can be found in the same part of the plant, which substantially complicates identification (Arx & Müller 1954, Slippers 2003). Furthermore, morphological characteristics of the anamorphs can be strongly influenced by the substrate on which they are grown (Sutton 1980). Thus, identification of *Botryosphaeria* spp. based on either morphological characteristics of their anamorph or teleomorph is problematic.

3.0. DNA-based studies on *Botryosphaeria*

In recent years, various DNA based techniques and comparisons of sequence data from various DNA regions have been introduced to distinguish between *Botryosphaeria* spp. and to re-evaluate the placement of their anamorphs. In many cases, these studies have included both molecular data and morphological characteristics to describe new *Botryosphaeria* spp. (Denman *et al.* 1999, Zhou *et al.* 2001, Zhou & Stanosz 2001a, b, Ma & Michailides 2002, Denman *et al.* 2003, De Wet *et al.* 2003, Slippers *et al.* 2004a).

Phylogenetic re-evaluations of the *Botryosphaeria* anamorphs have shown that they can be separated into two groups. These include those with dark conidia and *Diplodia*-like anamorphs, and those with hyaline conidia and *Fusicoccum*-like anamorphs (Zhou & Stanosz 2001a, Denman *et al.* 2000). Based on anamorph morphology and comparisions for sequences of the ITS regions of the rDNA operon Denman *et al.* (2000) concluded that anamorphs that produce hyaline conidia should be included in *Fusicoccum* and those with dark conidia might best reside in *Diplodia*. These conclusions were supported by the findings of Zhou & Stanosz (2001a) who proposed placement of *Botryosphaeria* species and related anamorphic fungi into two sections, namely Hyala and Brunnea (Zhou & Stanosz (2001a). Section Hyala comprises species that have *Fusicoccum* anamorphs: *B. ribis* Grossenb. & Duggar, *B. dothidea, B. corticis* (Demaree & Wilcox) Arx & E. Müll, *B. mamane* D. E. Gardner and *B. parva* Pennycook & Samuels. Section Brunnea includes species with *Sphaeropsis, Diplodia* and *Lasiodiplodia* anamorphs: *B. stevensii* Shoemaker, *B. obtusa* (Schwein.) Shoemaker, *B. rhodina,* (Berk. & M. A. Curtis) Arx, *B. tsugae* Funk, *B. quercuum,* and *D. pinea* (= *Sphaeropsis sapinea* (Fr. : Fr.) Dyko & Sutton). These results were not supported by mitochondrial small subunit DNA sequence data (mt SSU rDNA) (Zhou & Stanosz 2001b). Based on mt SSU rDNA sequence data *B. dothidea, B. corticis* and *B. mamane*, which have *Fusicoccum* anamorphs, were grouped within the *Diplodia* clade. The mt SSU data, however, supported the separation of *Botryosphaeria* from *Guignardia*, and also *B. dothidea* from *B. ribis* (Zhou & Stanosz 2001b).

DNA sequences most commonly used to study *Botryosphaeria* spp. are those of the internal transcribed spacer ITS gene region of the rDNA operon (Jacobs & Rehner 1998, Denman *et al.* 2000, 2003, Zhou & Stanosz 2001a, Smith *et al.* 2001, Phillips *et al.* 2002). Although the phylogenetic information obtained from ITS sequence data has contributed significantly to the taxonomy of *Botryosphaeria,* it cannot be used alone to distinguish cryptic species within *Fusicoccum* or *Diplodia*. To distinguish species such as these, multiple gene sequence data have been used (De Wet *et al.* 2003, Slippers *et al.* 2004a). Slippers *et al.* (2004a) thus showed that combined sequences from three different gene regions namely ß-tubulin, translation elongation factor 1-α (EF1-α) and ITS1, 5.8S and ITS2 can effectively delimitate closely related species such as *B. dothidea, B. ribis* and *B. parva.* Multiple gene genealogies inferred from partial sequences of six protein-coding genes and six microsatellite loci have been used to distinguish between morphotypes of *S. sapinea* and have also provided evidence that B morphotype of this

fungus represent a distinct species, described as *Diplodia scrobiculata* J. de Wet, B. Slippers & M. J. Wingfield, *sp. nov.* (De Wet *et al.* 2003).

Although closely related species within *Botryosphaeria* can be separated using multiple gene sequence data, this method is impractical, when large numbers of species need to be identified. Jacobs (2002) and Slippers (2003) developed PCR Restriction Fragment Length Polymorphism (RFLP) techniques that could distinguish between commonly encountered *Botryosphaeria* spp. PCR-RFLP profiles of ITS rDNA were useful to distinguish distantly and closely related *Botryosphaeria* species, but some cryptic species such as *B. ribis* and *B. parva* could not be distinguished from each other (Slippers 2003). PCR-RFLP fingerprints of an unknown region, however, distinguished these species. This technique requires sequence information from representative isolates of different species, but once developed, can be used for rapid and effective identification of large numbers of isolates.

4.0. From latent pathogen to disease agent

The genus *Botryosphaeria* includes a suite of species that are common endophytes, as well as opportunistic, wound and stress related pathogens (Schoeneweiss 1981). These fungi occur on many woody and herbaceous hosts, including both gymnosperms and angiosperms (Arx & Müller 1954, Barr 1972). As pathogens, *Botryosphaeria* spp. are important causal agents of canker and die-back on economically important woody crops, including many forest tree species. *Botryosphaeria* spp. can also be facultative parasites or saprophytes on dead and dying wood and other plant material (Arx & Müller 1954, Sivanesan 1984). Some species, for example *B. dothidea* and *B. rhodina,* have broad host ranges and in some cases have been reported on more than 100 genera of plants (Smith 1934, Punithalingam & Holliday 1973, Punithalingam 1979). Others are known from a single host or one genus of host plants. Examples of the former group are *B. eucalyptorum* Smith, Crous & M.J. Wingf. and *B. eucalypticola* Slippers, Crous & M.J. Wingf. prov. nom. on *Eucalyptus*; *D. pinea* and *D. scrobiculata* on *Pinus* and other conifers (Smith *et al.* 2001, De Wet *et al.* 2003, Slippers 2003). In this section these different life cycles and epidemiological roles of these fungi are explored.

4.1. Mode of infection

Botryosphaeria spp. are able to infect their hosts in different ways. Many researchers have shown that wounds are the main infection sites for these pathogens (Wiehe 1952, Witcher & Clayton 1963, Schreiber 1964, Punithalingam & Holliday 1973, English *et al.* 1975, McGlohon 1982, Sutton & Boyne 1983, Maas & Uecker 1984, Creswell & Milholland 1988, Smith *et al.* 1994). In wound related infections, success of infection is influenced by the age of wounds and that susceptibility of wounds to infection decreases with age (Schreiber 1964, Creswell & Milholland 1987).

Contrary to the view that wounds are the primary modes of infection, many recent studies have shown that these fungi are able to infect unwounded plant tissues (Milholland 1972, Weaver 1974, Brown & Hendrix 1981, Pusey 1989, Michailides 1991, Smith 1995). *Botryosphaeria* infections can, for example, be established through natural wounds caused by detachment of petioles, buds or fruits or through leaf and buds scars (Michailides 1991). Non-wound infections by *Botryosphaeria* spp. occur through natural openings, lenticels and stomata, on different parts of host plant, as has been shown for different plant species (Weaver 1979, Brown & Hendrix 1981, Michailides 1991, Smith 1995). Germ tubes from conidia of *B. dothidea* infect *E. grandis* leaves directly through stomata and many infections may occur in a single leaf (Smith 1995). Infectious germ tubes from conidia of *B. dothidea* also enter through stomatal openings on leaves, rachises and shoots, and through lenticels of fruit of pistachio (Michailides 1991). Infections by *B. dothidea* can be initiated through the lenticels on young stems and branches on peach and apple trees (Weaver 1974, Brown & Hendrix 1981).

Even though wounds are not necessary for infections, wounded trees become infected more readily and developed larger lesions than unwounded ones (Brown & Hendrix 1981). For example, *B. dothidea, B. obtusa* and *B. rhodina* induce cankers more frequently and cause more severe symptoms on wounded than on non-wounded tissues of peach trees (Britton & Hendrix 1986). Although *B. obtusa* can invade branches on different *Pistacia* spp. through intact bark, wounding of branches prior to inoculation resulted in significantly larger cambial lesions (Swart & Botes 1995).

4.2. Source of inoculum

Inoculum can be either conidia or ascospores. Conidia are more abundant than ascospores and have been indicated as the primary source of inoculum (Sutton 1981). Any plant material previously infected by a *Botryosphaeria* sp. may serve as a source of inoculum in orchards or plantations (Weaver 1979, Pusey 1989). *Botryosphaeria* spp. usually mature and develop fruiting structures on dead plant tissues (Arx & Müller 1954, Sivanesan 1984). Viable spores and mycelium of *B. dothidea* have been found on up to 3−4 year old stem cankers on pistachio (Michailides 1991). Both mature conidia and ascospores can be found on dead plant material, such as branches, leaves, bark, mummified fruits (Weaver 1974, 1979, Sutton 1981, Sutton & Boyne 1983).

Ascospores and conidia play different roles in the spread of *Botryosphaeria* spp. Although ascospores are dispersed by both air and water (Sutton 1981, Pusey 1989), they are more commonly airborne and may be wind-blown. For this reason, ascospores are thought to be important in long distance spread of disease (Weaver 1979, Sutton 1981). Conidia are primarily water-borne and are mostly found in run-off water dropping from branches (Sutton 1981). They are more important in spreading disease within a tree or through plantations and orchards (Weaver 1979).

4.3. Influence of temperature and moisture on epidemiology

Spore release and germination, as well as their ability to infect the host, are strongly influenced by temperature (Weaver 1979, Arauz & Sutton 1989a, b, Sutton & Arauz 1991). Conidia of *B. dothidea* from diseased peach bark were released during spring, summer and autumn (Weaver 1979). This indicated a wide temperature range suitable for conidial release and thus made infections possible for extended periods of time. The study of Weaver (1979) showed that maximum conidial production and germination, as well as the greatest number of infections of peach branches, occurred in summer, due to the favourable temperatures in this season. The optimum temperature for germination and germ tube development was shown to be between 25−35 °C, while optimum temperature for mycelium growth was 28 °C (Weaver 1979).

Besides temperature, moisture conditions play an important role in release and dispersal of *Botryosphaeria* inoculum, either ascospores or conidia. Weaver (1979) showed that rainfall is responsible for release and dispersal of conidia of *B. dothidea* from

diseased peach bark. The amount and duration of rainfall were the most important factors in the dispersal of ascospores and conidia of *B. dothidea* in apple orchards (Sutton 1981). In another study, rainfall triggered the discharge of ascospores and conidia and was the most important mechanism for conidial dispersal (Sutton 1981). However, Pusey (1989) reported that discharge of ascospores occurred during or soon after periods of wetness, not necessarily due to rainfall but rather to dew or mist conditions. Swart and Wingfield (1987), working on *D. pinea* on *Pinus* spp. in different climatic regions of South Africa, showed that conidia are primarily dispersed during periods of rainfall. However, the number of discharged spores is not always directly correlated to number of infections, because conditions favourable for spore release may not necessarily be favourable for infection. Although large numbers of spores may be discharged during heavy rains, light rain may be more favourable for spore deposition and infection (Creswell & Milholland 1988).

Like spore dispersal, infection and disease development are strongly influenced by temperature and moisture regime. In general, warm wet conditions have been reported to be favourable for infection and disease development associated with *Botryosphaeria* spp. (Weaver 1979, Sutton & Boyne 1983, Arauz & Sutton 1989a, b, Michailides & Morgan 1992, Parker 1993). The largest number of *B. dothidea* infections on blueberry occur during the period when maximum temperatures were within the range for optimum germination and germ tube growth, which is at 25−30 °C (Creswell & Milholland 1988). Optimum temperature range for infection and disease development of pinnacle and shoot blight of pistachio caused by *B. dothidea* was shown to be 27−30 °C when adequate moisture was available (Michailides & Morgan 1992). The same study, showed that continuous wetness duration of longer than 12 h favoured incidence and severity of disease development. Optimum temperatures for apple fruit infections by *B. obtusa* have been shown to range from 20−24 °C, while optimum temperature for leaf infection was 26 °C (Arauz & Sutton 1989a). Lower temperatures are a limiting factor for infections and disease development. Thus, conidia of *B. dothidea* on pistachio did not germinate below 12 °C (Michailides & Morgan 1992) and apple fruit infections by *B. dothidea* could not be induced below 20 °C (Kohn & Hendrix 1983).

4.4. Disease severity

Disease severity and symptoms expression are influenced by temperatures and plant physiology, which change seasonally (Pusey & Bertrand 1993). More severe diseases occur in spring and summer than in autumn (Pusey & Bertrand 1993). Severe symptoms of white rot disease on apple, caused by *B. dothidea*, only developed when fruit began to ripen in late summer (Sutton & Boyne 1983). Less severe infections occur on almond branches inoculated in winter rather than in the other seasons (English *et al.* 1975). Success of artificial inoculations is also affected by season, and spring and summer inoculations are mostly more successful than autumn or winter inoculations (Weaver 1979, Pusey & Bertrand 1993).

Beside environmental conditions, severity of disease on plants infected by *Botryosphaeria* depends on factors such as susceptibility of the host and virulence of the species or strains causing infections. For example, different apple cultivars have been shown to differ in susceptibility to *B. dothidea* infections (Latorre & Toledo 1984). Severity of the disease on each of the cultivars differed depending on the *B. dothidea* isolate used for inoculations, indicating that different strains of *B. dothidea* have significantly different levels of virulence. In another example, different races were identified among strains of *B. corticis* (Demaree & Wilcox) Arx & Muller that differ in pathogenicity to blueberry (Milholland & Galletta 1969, Milholland 1984). Schreiber (1964) compared the pathogenicity of isolates of *B. ribis* from apple, American holly and *Rhododendron*. The isolates from different hosts vary significantly in pathogenicity on *Rhododendron* and an isolate from *Rhododendron* was the most pathogenic. De Wet *et al.* (2002) showed that the A, B, C morphotypes of *D. pinea* differ significantly in pathogenicity. Similarly, many other studies confirm differences in degree of susceptibility to the pathogen among cultivars as well as differences among strains of *Botryosphaeria* spp. in virulence on individual cultivars (Pusey *et al.* 1985, Creswell & Milhollands 1986, Britton *et al.* 1990).

4.5. Stress

Botryosphaeria spp. are considered to be latent pathogens that can be present in asymptomatic plant tissues and cause disease rapidly under unfavourable environmental conditions (Smith *et al.* 1996a). Disease symptoms occur when plants are exposed to

physiological stress caused by unsuitable environmental conditions such as drought, freezing, hot or cold winds, hail wounds, nutritional imbalance or damage cause by insects or by other pathogens (Wene & Schoeneweiss 1980, Schoeneweiss 1981, Smith *et al.* 1996a, Pusey 1989). The most frequently observed stresses in woody plants are water stress due to drought and freezing (Wene 1979, Wene & Schoeneweiss 1980, Schoeneweiss 1981, Madar *et al.* 1989, Cline 1994).

Plants are predisposed to freezing stress when temperatures reach threshold levels, which are mostly between –20 and –30 °C, regardless of the host species or pathogen (Schoeneweiss 1981). Symptom development and severity are dependent on the threshold level, and not on duration of plant exposure to this temperature (Wene 1979). Disease symptoms that appeared on plants stressed by freezing, often healed during the growing season, indicating that freezing predisposition is reversible or elastic, but also may become irreversible when plant injury is severe (Schoeneweiss 1981). However, freezing stress in woody plants usually occur when a period of mild weather is followed by a rapid and extensive drop in temperature to nearly zero, rather than exposure to extremely low temperature (Schoeneweiss 1981).

Drought stress was found to be the major predisposing stress factor of many woody plants to infection by *Botryosphaeria* spp. (Neely 1968, Crist & Schoeneweiss 1975, McPartland & Schoeneweiss 1984, Madar *et al.* 1989). Drought-stressed pistachio trees developed more severe symptoms of *Botryosphaeria* blight disease than non-stressed trees (Ma *et al.* 2001). Spore germination, germ tube elongation and mycelial growth of *B. dothidea* increased when the water potential decreased (Ma *et al.* 2001). Drought stress thus has a dual effect on disease development, because susceptibility to disease and growth of the pathogen increase when drought stress on the host, increases (Crist & Schoeneweiss 1975, Ma *et al.* 2001). In contrast, Old *et al.* (1990) found that drought stress had no significant effect on disease symptom development on seedlings of *E. delegatensis* and *E. maculata* inoculated with *B. ribis.* This may be explained as a greater adaptation of these species to water stress (Old *et al.* 1990).

4.6. Symptoms

As pathogens of woody plants *Botryosphaeria* spp. can cause a symptoms on all parts of a tree. The most commonly reported symptoms on woody plants are cankers and die-back of stems and twigs (Davison & Tay 1983, Webb 1983, Shearer *et al.* 1987, Smith

et al. 1994). On *Eucalyptus* these symptoms are followed by extensive production of kino. Other symptoms include coppice failure, bleeding necrosis, root cankers, seed capsule abortion, leaf lesions, and in severe cases tree death (Neely 1968, Webb 1983, Barnard *et al.* 1987, Crous *et al.* 1989, Smith *et al.* 1994, Old & Davison 2000, Roux *et al.* 2000, 2001).

5.0. *Eucalyptus*

The genus *Eucalyptus* belongs to one of the oldest plant families, namely the *Myrtaceae* (Johnson & Briggs 1981). It is a largely southern hemisphere family with a three thousand species and is particularly well represented in the tropical and temperate regions of Australasia and Central and South America (Johnson & Briggs 1981). Species are either trees or shrubs. One of the most numerous genera in the *Myrtaceae* is *Eucalyptus*, comprising more than 700 species.

Eucalyptus spp., commonly known as gum trees, are endemic in mainland Australia and Tasmania occurring mostly south of the Tropic of Capricorn (Poynton 1979). Only a few species are indigenous in the forests of Indonesia, Papua New Guinea and the Philippines (Eldridge *et al.* 1997, Turnbull 2000). *Eucalyptus* dominates 124 million hectares (Mha) of Australia's natural forests and woodlands (Turnbull, 2000). However, production of wood for industrial purposes is limited to about 13 Mha of these natural forests. Increasing demands for wood and pulp products and lack of availability of natural forests for exploitation, has led to the rapid expansion of areas under *Eucalyptus* plantations in Australia. Between 1994 and 1998, eucalypt plantations in Australia increased from 125 000 ha to 287 000 ha (Turnbull, 2000).

Introduction of *Eucalyptus* into new areas, outside Australia, started at the beginning of the 19th century. The tree was first introduced into Europe as a curiosity in botanical gardens (Turnbull 2000). Subsequently, Europe became a second centre of eucalypt distribution, mostly to Latin America and Africa (Turnbull 2000). Apart from Australia, numerous *Eucalyptus* spp. have been introduced into more than 100 countries on all continents (Evans *et al.* 2000). In many of these countries, *Eucalyptus* spp. are of immense economic importance (Poynton 1979, Evans 1992, Turnbull 2000).

Eucalyptus spp. are cultivated to meet a wide variety of needs. *Eucalyptus* wood is used for paper pulp, charcoal, fuel wood, poles, mining timber, sawlogs, railway sleepers, fiberboard, viscose/rayon, furniture and building construction (Poynton 1979, Eldridge *et*

al. 1997). The leaves of all eucalypts contain essential oils that are used for medicinal and perfumery purposes, while bark of some species is used for tannin production. Beside these commercial applications, *Eucalyptus* may also be planted to prevent soil erosion, to reduce water levels in damp areas, as windbreaks, to provide shelter and shade as well as ornamental trees (Poynton 1979).

Rapid growth, wide adaptability and great variety of end uses has made *Eucalyptus* spp. the most widely grown trees in exotic plantations worldwide (Ciesla *et al.* 1996). Exotic *Eucalyptus* plantations have been established on more than 14 Mha, mostly in the Southern Hemisphere and Tropics, with increasing planting ongoing in temperate regions. The largest plantations have been established in Brazil, comprising more than 3 Mha, followed by India, China, South Africa, Portugal, Spain and Chile. It is estimated that there will be 20 Mha of *Eucalyptus* plantations outside Australia by the year 2010 (Turnbull 2000).

Exotic *Eucalyptus* plantations have been established in many African countries. *Eucalyptus* was introduced into South Africa in 1828. Nine seedlings of *E. globulus*, which originally grew in Mauritius from Australian seeds, were planted in the Governmental garden in Cape Town. The other *Eucalyptus* spp. reached the Cape Colony during the nineteenth century and from this area were spread throughout Natal, Free State and Transvaal provinces (Poynton 1979). The first trials were established during 1930s with seed stock received from Australia (Poynton 1979). Today *Eucalyptus* spp. make up almost half of 1.35 Mha of exotic plantations in South Africa (Anonymous 2002). *Eucalyptus* plantations are established mainly in the eastern and partly the southern part of the country. Two species dominate South African commercial plantations. These are *E. grandis* covering 80 % of the total area planted to *Eucalyptus,* and *E. nitens. E. grandis* has been planted in the warmer, more humid parts of the summer rainfall area, while *E. nitens*, as a cold tolerant species, is restricted to forest areas with cold winter conditions. Areas planted to various clones, mostly *E. grandis*-based, have increased significantly in recent years (Anonymous 2002).

5.1. *Botryosphaeria* diseases

Growing in diverse geographical regions and different climate conditions worldwide, *Eucalyptus* is exposed to many potential pests and pathogens (Burgess & Wingfield 2001). Often, when trees are planted in new environments, they are exposed to stress conditions,

and thus latent or opportunistic pathogens can cause disease. Some of the most important of these pathogens on *Eucalyptus* are *Botryosphaeria* spp. (Old & Davison 2000, Burgess & Wingfield 2001).*Botryosphaeria* spp. cause serious diseases on *Eucalyptus* spp. in their native range in Australia as well as in exotic plantations worldwide (Davison & Tay 1983, Webb 1983, Sharma *et al.* 1984, Shearer *et al.* 1987, Smith *et al.* 1994, Sankaran *et al.* 1995, Roux *et al.* 2000, 2001, Slippers *et al.* 2004b). Disease symptoms can develop on all parts of the tree and also on trees of all ages, from seedlings to mature trees (Webb 1983, Crous *et al.* 1989, Old *et al.* 1990, Smith *et al.* 1994, Roux *et al.* 2000, 2001). These fungi are mostly associated with canker and die-back symptoms followed by extensive production of kino (Sharma *et al*. 1984, Shearer *et al.* 1987, Smith *et al.* 1994, Roux *et al.* 2000, 2001).

Botryosphaeria species may also occur on *Eucalyptus* endophytically within healthy plant tissue (Fisher *et al.* 1993, Smith *et al.* 1996a, b). *Botryosphaeria dothidea* was shown to be an endophyte in the bark, leaves and xylem of asymptomatic *E. nitens* in England (Fisher *et al.* 1993). The same fungus was found to be common in asymptomatic leaves of different *Eucalyptus* spp. in South Africa (Smith *et al.* 1996a, b). As endophytes and opportunistic latent pathogens *Botryosphaeria* spp. pose a threat to trees throughout the whole of their life in response to any stress (Burgess & Wingfield 2001). They are also easily moved between regions, within healthy plant tissue. Thus, it is important to characterize the occurrence and distribution of *Botryosphaeria* spp. on *Eucalyptus* in its native range, as well in exotic plantations.

5.2. *Botryosphaeria* in Australia

Fungal pathogens are integral part of forest communities around the world (Hansen 1999). In native forest communities trees differ in susceptibility to pathogens due to genetic differences and age diversity. Thus, disease symptoms can develop on individual trees or groups of trees, but not reach epidemic levels (Hansen 1999, Burgess & Wingfield 2001). Trees and their pathogens have co-evolved and thus plants may have developed defence mechanisms against the native pathogen. When forests are undisturbed they are generally protected from severe disease outbreaks caused by native pathogens (Burgess & Wingfield 2001).

The fungus known as *Botryosphaeria ribis* is commonly found in native *Eucalyptus* forests in Australia (Davison & Tay 1983, Shearer 1994, Old & Davison

2000). The first record of *Botryosphaeria* causing disease in *Eucalyptus* was in Western Australia, associated with stem canker, twig and branch die-back on native *E. marginata* (Davison & Tay 1983). Although *B. "ribis"* is wide spread throughout Australia there are no reports regarding the significance of the disease caused by this pathogen in undisturbed forests (Burgess & Wingfield 2001).

Other *Botryosphaeria* spp. have recently been reported from native *Eucalyptus* in eastern Australia (Slippers *et al*. 2004b). *Botryosphaeria eucalyptorum* was first reported on native *Eucalyptus* and represented almost 50 % of isolates from this host and area (Slippers *et al*. 2004b). The second most common species was closely related to *B. eucalyptorum* and described as a new species *B. eucalypticola* Slippers, Crous & M. J. Wingf., prov nom. (Slippers *et al*. 2004b). Wide distribution of these fungi, unique host associations and dominance in this endemic niche indicates that *B. eucalyptorum* and *B. eucalypticola* prov nom. are native to Australia (Slippers *et al*. 2004b). *Botryosphaeria dothidea, B. australis*, *B. lutea* and isolates belonging to the *B. parva-B. ribis* complex have also been found, but they occur only sporadically (Slippers *et al*. 2004b).

Most *Eucalyptus* plantations are established in the western parts of Australia. Although *Eucalyptus* spp. are native to Australia, plantations comprise species not endemic to the specific regions, such as Tasmanian blue gums (*E. globulus*) in Western Australia. Shearer *et al.* (1987) found that *B. ribis* was associated with canker symptoms and death of *E. radiata* introduced into Western Australia, but only in species selections trials. Thus far, there have not been reports of severe disease caused by *Botryosphaeria* spp. in *Eucalyptus* plantations in Australia (Old 2000, Burgess & Wingfield 2001). However, it is expected that these pathogen could become a problem in the future, especially where *Eucalyptus* are planted outside their natural range and in marginal areas (Old 2000, Burgess & Wingfield 2001).

5.3. Exotic *Eucalyptus* plantations

Trees in exotic plantations can be threatened by two categories of pathogens, those that are introduced and those that are native to the areas where the exotic plantations have been established (Wingfield 1999, Wingfield *et al.* 2001a, Burgess & Wingfield 2001, 2002). Exotic *Eucalyptus* spp. are often planted in close association with native plants closely related to *Eucalyptus*. Thus, native pathogens from related species can be moved to *Eucalyptus* and cause serious disease (Crous & Swart 1995, Wingfield 1999, Burgess &

Wingfield 2001). An example is *Eucalyptus* rust caused by *Puccinia psidii* G. Winter that has moved from native *Myrtaceae* to *Eucalyptus* in South America (Coutinho *et al.* 1998).

Due to their occurrence as endophytes, *Botryosphaeria* spp. can be easily overlooked and introduced into new regions with germplasm or vegetative material (Denman *et al.* 2003, Burgess & Wingfield 2002, Slippers 2003, Slippers *et al*. 2004b). *Botryosphaeria* spp. that infect *Eucalyptus* in exotic plantations in South Africa, Hawaii and Uruguay were compared with those found on native *Eucalyptus* in eastern Australia (Slippers *et al*. 2004b). *Botryosphaeria eucalyptorum*, which is the dominant *Botryosphaeria* species in Australia, was also commonly isolated from exotic *Eucalyptus* in South Africa and Uruguay. This species appears to have been introduced into these areas with planting stock or with seed from Australia (Slippers *et al*. 2004b). This would be the first report of transfer of *Botryosphaeria* on *Eucalyptus* between continents. Thus, more attention should be paid on suitable measures to be applied to avoid transfer of these fungi into new areas.

Eucalyptus trees in exotic plantations are more vulnerable to attack by pests and pathogens than trees in native forest (Burgess & Wingfield 2001). This is due to risks associated with genetically uniform blocks of trees (monoculture) and site conditions that are often not suitable to the species being planted (Wingfield 1987, Potts & Pederic 2000, Burgess & Wingfield 2001). Growing in unfavourable environmental conditions, trees can be predisposed to disease caused by opportunistic pathogens such as *Botryopshaeria* spp. The most important of these pathogens is *B. rhodina* in the tropics and *B. ribis* in sub-tropics and temperate regions (Burgess & Wingfield 2001). Geographical distribution and some of diseases caused by *Botryosphaeria* spp. on *Eucalyptus* in exotic plantations reported in literature are summarised in Table 1.

5.4. Exotic *Eucalyptus* plantations in South Africa

The first record of a *Botryosphaeria* sp. infecting *Eucalyptus* in plantations in South Africa was in 1989 (Crous *et al.* 1989). *Botryosphaeria dothidea* was found associated with leaf lesions and tip blight of *Eucalyptus* spp. in the western Cape (Crous *et al.* 1989). Smith *et al.* (1994) found that *B. dothidea* was a common and wide spread on various *Eucalyptus* spp. (*Eucalyptus grandis* Hill: Maid., *Eucalyptus nitens* Deane et Maid. Maid., *Eucalyptus macarthurii* Deane et Maid. and *Eucalyptus smithii* R.T. Bak.) in South Africa, causing twig die-back, cankers and mortality of diseased trees. *B. dothidea* was considered

to be one of the most important pathogens of *Eucalyptus* in South Africa (Smith *et al.* 1994). In a recent study on *Botryosphaeria* spp. associated with *Eucalyptus,* the identity of isolates reported as *B. dothidea* was evaluated (Smith *et al.* 1994) and confirmed that *B. parva* rather than *B. dothidea* was commonly found on *Eucalyptus* in South Africa (Slippers *et al*. 2004b). Because these two species are morphologically similar, further research should be conducted to confirm the identity of *Botryosphaeria* species previously reported as *B. ribis* or *B. dothidea* on *Eucalyptus.*

Another *Botryosphaeria* species recently isolated from stem canker of *Eucalyptus* spp. in South Africa, and described as a new species is *B. eucalyptorum* (Smith *et al.* 2001). This species was commonly encountered with *B. dothidea* and appears to occur within the same niche (Smith *et al.* 2001). Pathogenicity tests showed that isolates of *B. eucalyptorum* are less virulent than those of *B. dothidea* and are associated only with cankers on the main stems of *E. grandis* and *E. nitens* (Smith *et al.* 2001).

Due to the movement of pathogens around the world and potential threat of native pathogens to exotic *Eucalyptus* plantations, many recent studies have considered the occurrence of fungal pathogens on native hosts in areas where *Eucalyptus* are intensively planted (Rodas 2003, Heath 2003, Slippers 2003). These studies have shown that pathogens, which can cause severe diseases on *Eucalyptus,* also occur on native plants and thus pose a threat to *Eucalyptus*. However, there have not been any detailed studies on *Botryosphaeria* spp. on native hosts closely related to *Eucalyptus* in South Africa. Due to the widespread occurrence of many *Botryosphaeria* spp. on exotic and indigenous hosts in South Africa (Crous *et al.* 2000), which are either economically important or in need of conservation, a study of this group of pathogens on native South African trees would be valuable.

6.0. Management strategies

Management of diseases caused by latent, opportunistic and stress related pathogens, such as *Botryosphaeria* spp., is difficult. Various fungicides have been tested for their ability to suppress disease caused by *Botryosphaeria* spp. (Milholland 1976, McGlohon 1982, Brown & Britton 1986, Cline & Milholland 1992, Parker 1993, Brown-Rytlewski & McManus 2000). Although application of fungicides reduces infection and disease symptoms, they might not provide long term control of these pathogens (Brown & Britton 1986, Cline & Milholland 1992, Brown-Rytlewski & McManus 2000). Apart from the

efficacy of some fungicides against *Botryosphaeria* diseases, chemical control measures are not in general use in *Eucalyptus* plantations. This is because chemical application would be impractical over large areas and it would also be environmentally undesirable (Gadgil *et al.* 2000, Wingfield *et al.* 2001b).

Quarantine measures could play a very important role in restricting the introduction of pathogens into new areas (Gadgil *et al.* 2000, Wingfield *et al.* 2001a, b, Burgess & Wingfield 2002). These measures are especially important for pathogens that cause diseases on *Eucalyptus* trees, which are widely planted as exotics. Although more attention has been given to quarantine measures during the last century, new pathogens continue to appear in exotic plantations in the areas where they were previously unknown (Wingfield *et al.* 2001a, b). For example, the new species *B. eucalyptorum,* recently reported on *Eucalyptus* in South Africa (Smith *et al.* 2001) appears to have been introduced from Australia (Slippers *et al.* 2004b). Plant quarantine measures regularly fail to exclude tree pathogens due to lack of strict control at the borders and also due to the lack of information on pathogens that might already be present in a country (Wingfield *et al.* 2001a, b).

The most effective strategy for management of disease in *Eucalyptus* plantations is screening species, clones and hybrids for tolerance of the disease and breeding of resistant genotypes (Wingfield 1999, Gadgil *et al.* 2000, Simpson & Podger 2000, Evans *et al.* 2000). Selection of disease-resistant clones and hybrids is being implemented to avoid losses caused by *Botryosphaeria* spp. on *Eucalyptus* in South Africa (Smith *et al.* 1994). However, this is a long term strategy and its disadvantage is that clones resistant to one species can be highly susceptible to another (Gadgil *et al.* 2000). The introduction of new exotic pathogens or pathogens from surrounding native flora, to which a specific clone is not resistant, would present a serious threat to clonal forestry.

Appropriate silviculture plays an important role in the management of disease, particularly those caused by opportunistic and stress related pathogens such as *Botryosphaeria* spp. Silvicultural practices that are known to reduce susceptibility and disease incidence include careful site selection, matching of tree species and genotypes to sites, fertilization and other ground preparations, control of insects and weeds, correct pruning and thinning and removal of dead plant material. All of these measures contribute to a high level of tree vigour and decrease conditions favouring disease development (Gadgil *et al.* 2000, Simpson & Podger 2000).

Knowledge of which fungi exist in an area and also an understanding of their ecology, epidemiology, pathology, evolution and other characteristics are fundamental to making appropriate decisions about silvicultural practices, resistance programmes and quarantine measures (Palm 1999, Wingfield *et al.* 2001a). Other strategies suggested to minimize the impact of these pathogens in exotic plantation forestry include increasing diversity of planting stock, rapid screening techniques to identify resistant genotypes, biological control and potential, the use of transgenic trees (Wingfield *et al.* 2001a). None of these strategies alone can provide successful control of diseases in exotic plantations. Thus, integration of different strategies in a management system should provide the best solutions to minimizing risks and avoiding damage due to pathogens in exotic plantations.

7.0. Conclusions

- *Botryosphaeria* spp. are common endophytes and latent, opportunistic pathogens on many economically important plants including *Eucalyptus* in their native range as well as in exotic plantations worldwide.
- The genus *Botryosphaeria* comprises closely related species and species complexes. Therefore, identification and delimitation of the species has been problematic.
- DNA based comparisons combined with morphological data provide effective tools for correct identification of species and should be applied in future identification of species that occur on *Eucalyptus.* They will also be valuable in re-evaluating the identity of *Botryosphaeria* spp. that have already been recorded from *Eucalyptus.*
- Because *Botryosphaeria* spp. can cause latent infections of *Eucalyptus* spp., in the absence of symptoms, they can be easily overlooked and introduced into new areas. The potential threat of these fungi to other agricultural crops as well as to indigenous flora in new environments must be studied and appropriate quarantine measures applied.
- Future research should investigate disease etiology and epidemiology, and also determine factors that influence disease development. Such data will facilitate proper control strategies.
- In South Africa, *Botryosphaeria* spp. have been recorded on many host plants. Their presence on native hosts closely related to *Eucalyptus* could pose a threat to *Eucalyptus.* Therefore, future studies should also include investigations on

Botryosphaeria spp. on native hosts closely related to *Eucalyptus.* The studies in this dissertation will partially address this question.

8.0. References

Ahumada R (2002). Diseases in commercial *Eucalyptus* plantations in Chile, with special reference to *Mycosphaerella* and *Botryosphaeria* species. M.Sc. thesis. Department of Microbiology and Plant Pathology, University of Pretoria, South Africa.

Anonymous (2002). *Commercial timber resources and roundwood processing in South Africa 2000 / 2001*. Forestry Economics Services, South Africa.

Arauz LF, Sutton TB (1989a). Temperature and wetness duration requirements for apple infection by *Botryosphaeria obtusa*. *Phytopathology* **79**: 440−444.

Arauz LF, Sutton TB (1989b). Influence of temperature and moisture on germination of ascospores and conidia of *Botryosphaeria obtusa*. *Phytopathology* **79**: 667−674.

Arx JA von, Müller E (1954). Die Gattungen der amerosporen Pyrenomyceten. *Beiträge zur kryptogamenflora der Schweiz* **11**: 1−434.

Arx JA von, Müller E (1975). A re-evaluation of the bitunicate *Ascomycetes* with keys to families and genera. *Studies in Mycology* **9**: 1−159.

Barnard EL, Geary T, English JT, Gilly SP (1987) Basal cankers and coppice failure of *Eucalyptus grandis* in Florida. *Plant Disease* **71**: 358−361.

Barr ME (1972). Preliminary studies on the *Dothideales* in temperate North America. *Contributions from the University of Michigan Herbarium* **9**: 523−638.

Barr ME (1979). A classification of the *Loculoascomycetes*. *Mycologia* **71**: 935−954.

Britton KO, Hendrix FF (1986). Effects of branch age and wounding on invasion of peach trees by *Botryosphaeria* spp.. *Phytopathology* **76**: 1065 (abstract).

Britton KO, Hendrix FF, Pusey PL, Okie WR, Reilly CC, Daniell JW (1990). Evaluating the reaction of peach cultivars to infection by three *Botryosphaeria* spp. *HortScience* **25**: 468−470.

Brown EA, Britton KO (1986). *Botryosphaeria* diseases of apple and peach in the Southeastern United States. *Plant Disease* **70**: 480−484.

Brown EA, Hendrix FF (1981). Pathogenicity and histopathology of *Botryosphaeria dothidea* on apple stems. *Phytopathology* **71**: 375−379.

Brown-Rytlewski DE, McManus PS (2000). Virulence of *Botryosphaeria dothidea* and *Botryospaheria obtusa* on apple and management of stem canker with fungicides. *Plant Disease* **84**: 1031−1037.

Burgess T, Wingfield MJ (2001). Impact of fungal pathogens in natural forests ecosystems: A focus on *Eucalyptus*. In: *Microorganisms in plant conservation and biodiversity* (Sivasithamparam K, Dixon KW, eds). Kluwer Academic press, The Netherlands: 285−306.

Burgess T, Wingfield MJ (2002). Quarantine is important in restricting the spread of exotic seed-borne tree pathogens in the southern hemisphere. *International Forestry Review* **4**: 56−65.

Cesati V, De Notaris G (1863). Schema di classificazione degle sferiacei italici aschigeri piu' o meno appartenenti al genere *Sphaeria* nell'antico significato attribuitoglide Persoon. Comment Soc Crittog Ital **1**, **4**: 177−240.

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

Cline WO (1994) Infection of cold-injured blueberry stems by *Botryosphaeria dothidea. Plant Disease* **78**: 1010 (abstract).

Cline WO, Milholland RD (1992). Root dip treatments for controlling blueberry stem blight caused by *Botryosphaeria dothidea* in container-grown nursery plants. *Plant Disease* **76**: 136−138.

Coutinho TA, Wingfield MJ, Alfenas AC, Crous PW (1998). *Eucalyptus* rust: A disease with the potential for serious international implications. *Plant Disease* **82**: 819−825.

Creswell TC, Milholland RD (1988). Spore release and infection periods of *Botryosphaeria dothidea* on blueberry in North Carolina. *Plant Disease* **72**: 342−346.

Creswell TC, Milholland RD (1986). Factors affecting development of Blueberry stem blight caused by *Botryosphaeria dothidea*. *Phytopathology* **76**: 1081 (abstract).

Creswell TC, Milholland RD (1987). Response of blueberry genotypes to infection by *Botryosphaeria dothidea*. *Plant Disease* **71**: 710−713.

Crist CR, Schoeneweiss DF (1975). The influence of controlled stresses on susceptibility of European white birch stems to attack by *Botryosphaeria dothidea. Phytopathology* **65**: 369−373.

Crous PW, Knox-Devies PS, Wingfield MJ (1989). Newly recorded foliage fungi of *Eucalyptus* spp. in South Africa. *Phytophylactica* **21**: 85−88.

Crous PW, Phillips AJL, Baxter AP (2000). *Phytopathogenic Fungi from South Africa*. University of Stellenbosch, Department of Plant Pathology Press, South Africa.

Crous PW, Palm ME (1999). Reassessment of the anamorph genera *Botryodiplodia, Dothiorella* and *Fusicoccum*. *Sydowia* **52**: 167−175.

Crous PW, Swart WJ (1995). Foliicolous fungi of *Eucalyptus* spp. from Eastern Madagascar: implications for South Africa. *South African Forestry Journal* **172**: 1−6.

Davison EM, Tay FCS (1983). Twig branch and upper trunk cankers of *Eucalyptus marginata*. *Plant Disease* **67**: 1285−1287.

Denman S, Crous PW, Wingfield MJ (1999). A taxonomic reassessment of *Phyllachora proteae*, a leaf pathogen of *Proteaceae*. *Mycologia* **91**: 510−516.

Denman S, Crous PW, Taylor JE, Kang JC, Pascoe I, Wingfield MJ (2000). An overview of the taxonomic history of *Botryosphaeria* and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. *Studies in Mycologia* **45**: 129−140.

Denman S, Crous PW, Groenewald JZ, Slippers B, Wingfield BD, Wingfield MJ (2003). Circumscription of *Botryosphaeria* species associated with *Proteaceae* based on morphology and DNA sequence data. *Mycologia* **95**: 294−307.

De Wet J, Burgess T, Slippers B, Preisig O, Wingfield BD, Wingfield MJ (2003). Multiple gene genealogies and microsatellite markers reflect relationships between morphotypes of *Sphaeropsis sapinea* and distinguish a new species of *Diplodia*. *Mycological Research* **107**: 557−566.

De wet J, Wingfield MJ, Coutinho T, Wingfield BD (2002). Characterisation of the 'C' morphotype of the pine pathogen *Sphaeropsis sapinea*. *Forest Ecology and Management* **161**: 181−188.

Eldridge K, Davidson J, Harwood C, Van Wyk G (1997). *Eucalypt domestication and breeding*. Oxford, Science Publications, UK.

English H, Davis JR, De Vay JE (1975). Relationship of *Botryosphaeria dothidea* and *Hendersonula toruloidea* to a canker disease of almond. *Phytopathology* **65**: 114−122.

Evans G, Dempsey S, McLachlan K, Coleman C, McRae C (2000). Quarantine risks associated with importation of *Eucalyptus* seed. Australian Quarantine and Inspection Service (AQIS), Canbera, Australia.

Evans J (1992). *Plantation Forestry in the Tropics*. 2nd edition. Oxford University Press, USA.

Farr DF, Bills GF, Chamuris GP, Rossman AY (1989). *Fungi on Plant and Plant Products in the United States*. The American Phytopathological Spciety Press, Minnesota, USA.

Fisher PJ, Petrini O, Sutton BC (1993). A comparative study of fungal endophytes in leaves, xylem and bark of *Eucalyptus nitens* in Australia and England. *Sydowia* **45**: 1−14.

Fries E (1823) Systema Mycologicum **2**(2): 423−424.

Gadgil PD, Wardlaw TJ, Ferreira FA, Sharma JK, Dick MA, Wingfield MJ, Crous PW (2000) Management of disease in Eucalypt plantations. In: *Diseases and Pathogens of Eucalypts* (Keane PJ, Kile GA, Podger FD, Brown BN, eds). CSIRO Publishing, Australia: 519−529.

Gezahgne A (2003). Diseases of exotic plantation forestry trees in Ethioipia. Ph. D. dissertation. Department of Microbiology and Plant Pathology, University of Pretoria, South Africa.

Grossenbacher JG, Duggar BM (1911). A contribution to the life history, parasitism and biology of *Botryosphaeria ribis*. *New York State Agricultural Experiment Station, Technical Bulletin* **18**: 113−190.

Hansen EM (1999). Disease and diversity in forest ecosystems. *Australasian Plant Pathology* **28**: 313−319.

Hawksworth DL, Kirk PM, Sutton BC, Pegler DN (1995). Ainsworth & Bisby's Dictionary of the fungi. 8th edition. CAB International.

Heath RN (2003). Studies to consider the possible origin of three canker pathogens of *Eucalyptus* in South Africa. M.Sc. thesis. Department of Microbiology and Plant Pathology, University of Pretoria, South Africa.

Jacobs KA, Rehner SA (1998). Comparison of cultural and morphological characters and ITS sequences in anamorphs of *Botryosphaeria* and related taxa. *Mycologia* **90**: 601−610.

Jacobs R (2002). Characterisation of *Botryosphaeria* species from mango in South Africa. M.Sc. thesis. Department of Microbiology and Plant Pathology, University of Pretoria, South Africa.

Johnson LAS, Briggs BG (1981). Three old southern families - *Myrtaceae*, *Proteaceae* and *Restionaceae*. In: *Ecological Biogeography of Australia*. (Keast A. eds). W Junk, The Hague: 427−464.

Kohn FC Jr., Hendrix FF (1983). Influence of sugar content and pH on development of white rot on apples. *Plant Disease* **67**: 410−412.

Latorre BA, Toledo MV (1984). Occurrence and relative susceptibility of apple cultivars to *Botryosphaeria* canker in Chile. *Plant Disease* **68**: 36−39.

Laundon GF (1973). *Botryosphaeria obtusa, B. stevensii,* and *Otthia spiraeae* in New Zealand. *Transactions of the British Mycological Society* **61**: 369−374.

Luttrell ES (1955) The ascostromatic Ascomycetes. *Mycologia* **47**: 511−532.

Ma Z, Michailides TJ (2002). Characterization of *Botryosphaeria dothidea* isolates collected from Pistachio and other plant hosts in California. *Phytopathology* **92**: 519−526.

Ma Z, Morgan DP, Michailides TJ (2001). Effects of water stress on Botryosphaeria blight of Pistachio caused by *Botryosphaeria dothidea*. *Plant Disease* **85**: 745−749.

Maas JL, Uecker FA (1984). *Botryosphaeria dothidea* cane canker of thornless blackberry. *Plant Disease* **68**: 720−726.

Madar Z, Solel Z, Kimchi M (1989). Effect of water stress in cypress on the development of cankers caused by *Diplodia pinea* f. sp. *cupressi* and *Seiridium cardinale*. *Plant Disease* **73**: 484−486.

Masuka A (1990). A new canker of *Eucalyptus grandis* Hill ex Maiden in Zimbabwe. *Commonwealth Forestry Review* **69**: 195−200.

McGlohon NE (1982). *Botryosphaeria dothidea* - Where will it stop? *Plant Disease* **66**: 1202−1203.

McPartland JM, Schoeneweiss DF (1984). Hyphal Morphology of *Botryosphaeria dothidea* in vessels of unstressed and drought-stressed stems of *Betula alba*. *Phytopathology* **74**: 358−362.

Michailides TJ (1991). Pathogenicity, distribution, sources of inoculum and infection courts of *Botryosphaeria dothidea* on pistachio. *Phytopathology* **81**: 566−573.

Michailides TJ, Morgan DP (1992). Effect of temperature and wetness duration on infection of pistachio by *Botryosphaeria dothidea* and management of disease by reducing duration of irrigation. *Phytopathology* **82**: 1399−1406.

Milholland RD (1972). Histopathology and pathogenicity of *Botryosphaeria dothidea* on bluebbery stems. *Phytopathology* **62**: 654−660.

Milholland RD (1976). Translocation of Benomyl in highbush blueberry and its effect on *Botryosphaeria corticis* and *Gloeosporium minus*. *Plant Disease Reporter* **60**: 126−130.

Milholland RD (1984). Occurance of a new race of *Botryosphaeria corticis* on highbush and rabbiteye bluberry. *Plant Disease* **68**: 522−523.

Milholland RD, Galletta GJ (1969). Pathogenic variation in among isolates of *Botryosphaeria corticis* on Blueberry. *Phytopathology* **59**: 1540−1543.

Neely D (1968) Bleeding necrosis of sweet gum in Illinois and Indiana. *Plant Disease Reporter* **52**: 223−225.

Nakabonge G (2002). Diseases associated with plantation forestry in Uganda. M.Sc. thesis. Department of Microbiology and Plant Pathology, University of Pretoria, South Africa.

Old KM (2000). Eucalypt diseases of complex etiology. In: *Diseases and Pathogens of Eucalypts* (Keane PJ, Kile GA, Podger FD, Brown BN, eds). SCIRO Publishing, Australia: 411−425.

Old KM, Davison EM (2000). Canker diseases of Eucalypts. In: *Diseases and Pathogens of Eucalypts* (Keane PJ, Kile GA, Podger FD, Brown BN, eds). SCIRO Publishing, Australia: 241−257.

Old KM, Gibbs R, Craig I, Myers BJ, Yuan ZQ (1990). Effect of drought and defoliation on the susceptibility of *Eucalypts* to canker caused by *Endothia gyrosa* and *Botryosphaeria ribis*. *Australian Journal of Botany* **38**: 571−581.

Old KM, Qing YZ (1994). Foliar and stem diseases of *Eucalyptus* in Vietnam and Thailand. CSIRO Division of Forestry, Australian Centre for International Agricultural Research.

Palm ME (1999). Mycology and world trade: a view from the front line. *Mycologia* **91**: 1−12.

Parker KC (1993). Effect of temperature and wetness duration on apple fruit infection and eradicant activity of fungicides against *Botryosphaeria dothidea*. *Plant Disease* **77**: 181−185.

Pennycook SR, Samuels GJ (1985). *Botryosphaeria* and *Fusicoccum* species associated with ripe fruit rot of *Actinidia deliciosa* (Kiwifruit) in New Zealand. *Mycotaxon* **24**: 445−458.

Phillips AJL, Fonseca F, Povoa V, Castilho R, Nolasco G (2002). A reassessment of the anamorphic fungus *Fusicoccum luteum* and description of its teleomorph *Botryosphaeria lutea* sp. nov. *Sydowia* **54**: 59−77.

Potts BM, Pederick LA (2000). Morphology, phylogeny, origin, distribution and genetic diversity of the *Eucalyptus*. In: *Diseases and Pathogens of Eucalypts* (Keane PJ, Kile GA, Podger FD, Brown BN, eds). SCIRO Publishing, Australia: 11−34.

Poynton RJ (1979) *Tree planting in Southern Africa, Vol. 2. The Eucalyptus.* Department of Forestry. South Africa.

Punithalingam E (1979). *Plant diseases attributed to Botryodiplodia theobromae Pat.* J. Cramer, Germany.

Punithalingam E, Holliday P (1973). *Botryosphaeria ribis.* CMI descriptions of pathogenic fungi and bacteria. No. 395. Commonwealth Mycological Institute, Kew, Surrey, England.

Pusey PL (1989). Availability and dispersal of ascospores and conidia of *Botryosphaeria* in Peach orchards. *Phytopathology* **79**: 635−639.

Pusey PL, Bertrand PF (1993) Seasonal infection of non-wounded peach bark by *Botryosphaeria dothidea*. *Phytopathology* **83**: 825−829.

Pusey PL, Reilly CC, Okie WR (1985). Unique strain of *Botryosphaeria dothidea* involved in peach tree fungal gummosis. *Phytopathology* **75**: 1374−1375.

Putterill VA (1919). A new apple tree canker. *South African Journal of Science* **16**: 254−271.

Rodas PCA (2003). Diseases of *Eucalyptus* in Colombia. M.Sc. thesis. Department of Microbiology and Plant Pathology, University of Pretoria, South Africa.

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

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

Saccardo PA (1877). Fungi Veneti novi vel critici. *Michelia* **1**: 1−72.

Saccardo PA (1882). Sylloge fungorumomnium hucusque cognitorum **1**: 456−466.

Sankaran KV, Sutton BC, Minter DW (1995). A checklist of fungi recorded on *Eucalyptus*. *Mycological Papers* 170. CAB International, UK.

Schoeneweiss DF (1981). The role of environmental stress in diseases of woody plants. *Plant Disease* **65**: 308−314.

Schreiber LR (1964). Stem canker and die-back of *Rhododendron* caused by *Botryosphaeria ribis* Gross. & Dug. *Plant Disease Reporter* **48**: 207−210.

Sharma JK (1986). Potential threat of native pathogens on exotic *Eucalypts* in Kerala. *Eucalypts in India Past, Present and Future* **72**: 367−376.

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

Shearer BL (1994). The major plant pathogens occurring in native ecosystems of south-western Australia. *Journal of the Royal Society of Western Australia* **77**: 113−122.

Shearer BL, Tippett JT, Bartle JR (1987). *Botryosphaeria ribis* infection associated with death of *Eucalyptus radiata* in species selection trials. *Plant Disease* **71**: 140−145.

Shoemaker RA (1964). Conidial states of some *Botryosphaeria* species on *Vitis* and *Quercus*. *Canadian Journal of Botany* **42**: 1297−1301.

Simpson JA, Podger FD (2000). Management of *Eucalypt* diseases-options and constraints. In: *Diseases and Pathogens of Eucalypts* (Keane PJ, Kile GA, Podger FD, Brown BN, eds). SCIRO Publishing, Australia: 427−444.

Sivanesan A (1984). The bitunicate ascomycetes and their anamorphs. J.C. Cramer: Vaduz, Germany.

Slippers B (2003). Taxonomy, phylogeny- and ecology of botryosphaeriaceous fungi occurring on various woody hosts. Ph.D. dissertation. Department of Microbiology and Plant Pathology, University of Pretoria, South Africa.

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

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

Smith CO (1934). Inoculations showing the wide host range of *Botryosphaeria ribis*. *Journal of Agricultural Research* **49**: 467−476.

Smith H (1995). *Botryosphaeria dothidea* as pathogen and endophyte of *Eucalyptus* in South Africa. M.Sc. thesis. Department of Microbiology and Biochemistry, University of the Orange Free State, South Africa.

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

Smith H, Wingfield MJ, Crous PW, Coutinho TA (1996a). *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. *South African Journal of Botany* **62**: 86−88.

Smith H, Wingfield MJ, Petrini O (1996b). *Botryosphaeria dothidea* endophytic in *Eucalyptus grandis* and *Eucalyptus nitens* in South Africa. *Forest Ecology and management* **89**: 189−195.

Smith H, Crous PW, Wingfield MJ, Coutinho TA, Wingfield BD (2001). *Botryosphaeria eucalyptorum* sp. nov., a new species in the *B. dothidea*-complex on *Eucalyptus* in South Africa. *Mycologia* **93**: 277−285.

Smith DR, Stanosz GR (2001). Molecular and morphological differentiation of *Botryosphaeria dothidea* (anamorph *Fusicoccum aesculi*) from some other fungi with *Fusicoccum* anamorphs. *Mycologia* **93**: 505−515.

Sutton BC (1980). The coelomycetes fungi imperfecti with pycnidia, acervuli and stromata. Commonwelth Mycological Institute: Kew, Surrey, England.

Sutton TB (1981). Production and dispersal of ascospores and conidia by *Physalospora obtuse* and *Botryosphaeria dothidea* in apple orchards. *Phytopathology* **71**: 584−589.

Sutton TB, Arauz LF (1991). Influence of temperature and moisture on germination of ascospores and conidia of *Botryosphaeria dothidea*. *Plant Disease* **75**: 1146−1149.

Sutton TB, Boyne JV (1983). Inoculum availability and pathogenic variation in *Botryosphaeria dothidea* in apple production areas of North Carolina. *Plant Disease* **67**: 503−506.

Swart WJ, Botes W-M (1995). First report of stem canker caused by *Botryosphaeria obtusa* on pistachio. *Plant Disease* **79**: 1036−1038.

Swart WJ, Wingfield MJ (1987). Conidial dispersal of *Sphaeropsis sapinea* in three climatic regions of South Africa. *Plant Disease* **71**: 1038−1040.

Turnbull JW (2000). Economic and social importance of Eucalypts. In: *Diseases and Pathogens of Eucalypts* (Keane PJ, Kile GA, Podger FD, Brown BN, eds). SCIRO Publishing, Australia: 1−9.

Weaver DJ (1974). A gummosis disease of peach trees caused by *Botryosphaeria dothidea*. *Phytopathology* **64**: 1429−432.

Weaver DJ (1979). Role of conidia of *Botryosphaeria dothidea* in the natural spread of peach tree gummosis. *Phytopathology* **69**: 330−334.

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

Wene EG (1979). Stress predisposition of woody plants to stem canker cause by *Botryosphaeria dothidea*. Ph.D. dissertation, University of Illinois, Illinois.

Wene EG, Schoeneweiss DF (1980). Localized freezing predisposition to *Botryosphaeria* canker in differentially frozen woody stems. *Canadian Journal of Botany* **58**: 1455−1458.

Wiehe PO (1952). Life cycle of *Botryosphaeria ribis* on *Aleurites montana*. *Phytopathology* **42**: 521−526.

Wingfield MJ (1987). Diseases in South African forest plantations. *Forestry Handbook* **2**: 153−164.

Wingfield MJ (1999). Pathogens in exotic plantations forestry. *International Forestry Review* **1**(3): 163−168.

Wingfield MJ, Slippers B, Roux J, Wingfield BD (2001a). World wide movement of exotic forest fungi, especially in the Tropics and the Southern hemisphere. *Bioscience* **51**: 134−140.

Wingfield MJ, Roux J, Coutinho T, Govender P, Wingfield BD (2001b). Plantation disease and pest management in the next century. *Southern African Forestry Journal* **190**: 67−71.

Witcher W, Clayton CN (1963). Blueberry stem blight caused by *Botryosphaeria dothidea* (*B. ribis*). *Phytopathology* **53**: 705−712.

Zhou S, Smith DR, Stanosz GR (2001). Differentiation of *Botryosphaeria* species and related anamorphic fungi using inter simple or short sequence repeat (ISSR) fingerprinting. *Mycological Research* **105**: 919−926.

Zhou S, Stanosz GR (2001a). Relationships among *Botryosphaeria* species and associated anamorphic fungi inferred from the analyses of ITS and 5.8S rDNA sequences. *Mycologia* **93**: 516−527.

Zhou S, Stanosz GR (2001b). Primers for amplification of mt SSU rDNA, and a phylogenetic study of *Botryosphaeria* and associated anamorphic fungi. *Mycological Research* **105**: 1033−1044.

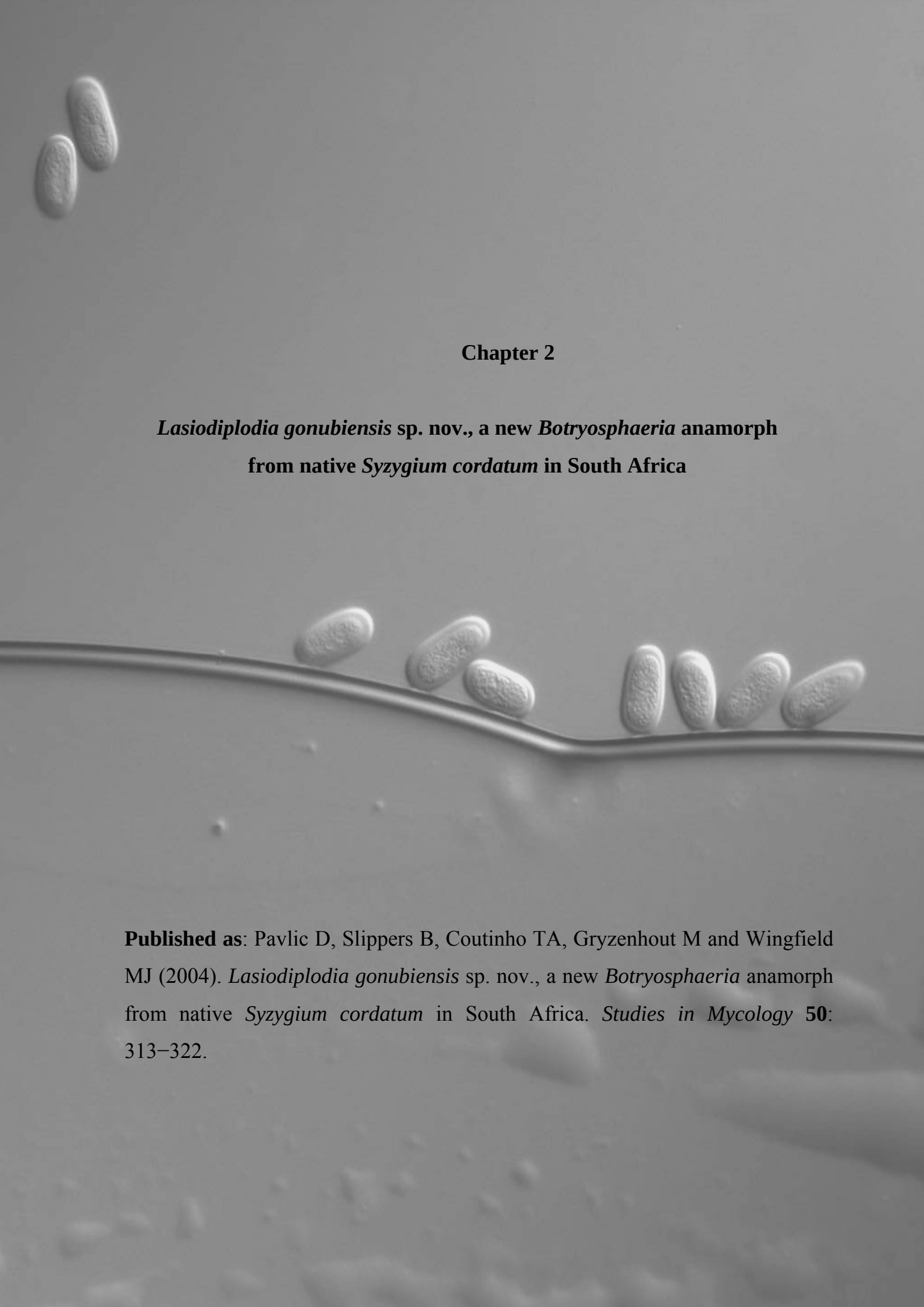
Table 1. Geographical distribution of *Botryosphaeria* spp. in exotic *Eucalyptus* plantations and some of diseases symptoms reported in literature.

***Botryosphaeria* spp.**	***Eucalyptus* hosts**	**Symptoms**	**Distribution**	**References**
B. dothidea	*E. grandis*	Basal cankers, coppice failure	USA	Barnard *et al.* 1987
	Eucalyptus spp.	Basal canker, twig die-back	USA	Farr *et al.* 1989
	Eucalyptus spp.	Leaf lesions, tip blight	South Africa	Crous *et al.* 1989
	E. grandis	Stem cankers	Zimbabwe	Masuka 1990
*B. dothidea**	*E. grandis, E. nitens, E. macarthurii, E. smithii,*	Twig die-back, cankers, trees mortality	South Africa	Smith *et al.* 1994
	E. grandis (clones), *E. grandis* × *E. camaldulensis,*			
	E. grandis × *E. urophylla*			
B. parva	*E. globules, E. nitens*	Canker and die-back, death of young trees	Chile	Ahumada 2002
		branch cankers		
	Eucalyptus spp.	Stem canker	Uganda	Nakabonge 2002
	E. globulus, E. grandis, E. saligna, E. citriodora	Canker and die-back	Ethiopia	Gezahgne 2003
	Eucalyptus spp.		Uruguay	Slippers 2003
	Eucalyptus spp.		Hawaii	Slippers 2003
B. ribis	*E. camaldulensis*	Seed capsule abortion, twig die-back	USA	Webb 1983
	E. grandis	Dieback of shoots and twigs, stem and	Colombia	Rodas 2003
		branch cankers		

Table 1. Continued.

***Botryosphaeria* spp.**	***Eucalyptus* hosts**	**Symptoms**	**Distribution**	**References**
B. rhodina	*E. grandis, E tereticornis*	Root collar canker, stem canker	India	Sharma *et al.* 1984, 1986
	E. grandis, E. urophylla, E. urophylla × *E. pellita,*	Root and root collar canker, stem canker	Republic of Congo	Roux *et al.* 2000
	E. urophila × *E. grandis*, *E. territicornis* × *E. grandis*			
	E. grandis	Stem canker	Uganda	Roux *et al.* 2001
B. eucalyptorum	*E. grandis, E. nitens*	Cankers on the main stem	S. Africa	Smith *et al.* 2001, Slippers 2003
	E. globulus, E. nitens	Canker and die-back, death of young trees	Chile	Ahumada 2002
	Eucalyptus spp.		Uruguay	Slippers 2003
B. eucalypticola	*Eucalyptus* spp.		Hawaii	Slippers 2003
	Eucalyptus spp.		S. Africa	Slippers 2003
Botryosphaeria spp.	*E. camaldulensis*	Canker and stem death	Vietnam	Old & Qing 1994

* Isolates identified as *B. dothidea* (Smith *et al.*1994) were re-evaluated as *B. parva* (Slippers *et al.* 2004)



Chapter 2

Lasiodiplodia gonubiensis sp. nov., a new *Botryosphaeria* anamorph from native *Syzygium cordatum* in South Africa

Published as: Pavlic D, Slippers B, Coutinho TA, Gryzenhout M and Wingfield MJ (2004). *Lasiodiplodia gonubiensis* sp. nov., a new *Botryosphaeria* anamorph from native *Syzygium cordatum* in South Africa. *Studies in Mycology* **50**: 313−322.

ABSTRACT

Botryosphaeria spp. are common and widely distributed pathogens on many economically important crops, including forest tree species. These fungi cause a wide variety of symptoms on trees of all ages, but are mostly associated with canker and die-back of branches and main stems. As disease agents, *Botryosphaeria* spp. are often encountered in their anamorph state, namely species of *Fusicoccum, Diplodia* or *Lasiodiplodia*. During a recent survey of botryosphaeriaceous fungi from native *Syzygium cordatum* in South Africa, an unfamiliar *Lasiodiplodia* sp. was isolated. The aim of this study was to compare this apparently undescribed species with other species of *Botryosphaeria* using morphological characteristics and DNA sequence data of the rDNA internal transcribed spacers, ITS1 and ITS2. Based on sequence data, the isolates from *S. cordatum* were more closely related to *B. rhodina* (anamorph *Lasiodiplodia theobromae*) than other *Botryosphaeria* spp., but also phylogenetically distinct from this species. Conidia of the species from *S. cordatum* were also different to those of *L. theobromae*. We conclude that the isolates from *S. cordatum* represent an undescribed *Lasiodiplodia* sp. and provide the name *Lasiodiplodia gonubiensis* for it.

INTRODUCTION

Botryosphaeria Ces. & De Not. (*Dothideales*) contains species that have a cosmopolitan distribution and wide host range, including gymnosperms and angiosperms (von Arx & Müller 1954, Barr 1972). These fungi are common endophytes and latent, opportunistic pathogens on many woody plants such as *Eucalyptus* spp. (Fisher *et al*. 1993, Smith *et al*. 1996a, b). Typical disease symptoms associated with *Botryosphaeria* spp. are canker and die-back, followed by kino exudation, and in severe cases tree death (Davison & Tay 1983, Webb 1983, Sharma *et al*. 1984, Shearer *et al*. 1987, Smith *et al*. 1994, Old & Davison 2000, Roux *et al*. 2000, 2001, Smith *et al*. 2001a).

Eucalyptus belongs to one of the oldest plant families, namely the *Myrtaceae* (Johnson & Briggs 1981). It is largely a Southern Hemisphere family with more than 3000 species and is particularly well represented in the tropical and temperate regions of Australasia and Central and South America (Johnson & Briggs 1981). Myrtaceous species are also an integral part of Southern African indigenous flora (Palgrave 1977). The most common and widely distributed myrtaceous tree in South Africa is *Syzygium cordatum* Hochst. (Palgrave 1977).

Most *Eucalyptus* spp. are native to Australia (Poynton 1979), but are the most widely grown trees in exotic plantations in other parts of the world (Ciesla *et al*. 1996). These exotic plantations are often planted in close association with native myrtaceous trees that are closely related to *Eucalyptus* (Burgess & Wingfield 2001). A danger in such cases is that pathogens from either of these related native and introduced hosts could cross-infect the other host group and cause serious diseases (Crous & Swart 1995, Wingfield 1999, Burgess & Wingfield 2001). An example of this is the rust fungus *Puccinia psidii* G. Winter that occurs on native *Myrtaceae* in South America, and has become one of the most important pathogens on exotic *Eucalyptus* in this region (Coutinho *et al*. 1998).

Because of its wide distribution, and the fact that this tree often grows alongside plantations of *Eucalyptus*, we conducted a survey of botryosphaeriaceous fungi occurring on native *Syzygium cordatum* in South Africa. This survey resulted in isolates of a *Lasiodiplodia* sp. *Lasiodiplodia* spp. are anamorphs of *Botryosphaeria* and a very common species, particularly in tropical areas, is *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. (von Arx 1974, Punithalingam 1976, 1979), teleomorph *B. rhodina* (Berk. & M. A. Curtis) Arx (von Arx 1974). The fungus from *S. cordatum* is similar to *L.*

theobromae but has distinctly larger conidia and no teleomorph state has been found. The aim of this study was to identify the unknown *Lasiodiplodia* sp. using both morphological characteristics and comparisons of DNA sequence data of the Internal Transcribed Spacer regions (ITS) of the rDNA operon.

MATERIALS AND METHODS

Isolates

Isolates of an unknown *Lasiodiplodia* sp. were collected in the Eastern Cape Province, South Africa in July 2002 (Table 1). Isolations were made from asymptomatic twigs and leaves of naturally growing *S. cordatum*. Leaf and twig portions (5 cm in length) were washed in running tap water, surface disinfected by submerging them for 1 min sequentially in 96 % ethanol, undiluted bleach (3.5–5 % available chlorine) and 70 % ethanol, and rinsed in sterile water. The disinfected twig portions were halved and pieces from the pith tissue (2 mm²) and segments of the leaves (3 mm²) were placed on 2 % malt extract agar (MEA) (2 % malt extract, 1.5 % agar; Biolab, Midrand, Johannesburg, S.A.). Plates were incubated at 20 °C under continuous near fluorescent light for two weeks and colonies resembling *Botryosphaeria* spp. were selected. These colonies were maintained on 2 % MEA at 25 °C and stored at 5 °C. Isolates are maintained in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa at 5 ºC on MEA. A duplicate set of isolates has also been deposited in the collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

Morphology and cultural characteristics

To induce sporulation, isolates were grown on 2 % water agar (WA) (Biolab, S.A.) with sterilized pine needles placed onto the medium, at 25 °C under near-UV light. Herbarium specimens were also sought for *L. theobromae* to compare with the fungus from *S. cordatum*. In the original descriptions of the species (Patouillard & De Lagerheim 1892) and genus (Clendinin 1896), no reference is made to type material. CBS, ATCC and IMI do not have cultures from the original host and location (*Theobroma cacao* Linné in

Ecuador), and no herbarium material from the same origin could be located in BPI. Until the original material can be located or an epitype specimen assigned, it is necessary to rely on descriptions from the literature. For comparative purposes, we thus compiled a table from previous descriptions, to provide conidial dimensions for this species, as well as many species that have been reduced to synonymy with *L. theobromae* (Table 2).

Released conidia and squash mounts of pycnidia formed on the pine needles, were mounted in lactophenol on microscope slides and examined microscopically. Sections of pycnidia were made by hand and mounted in lactophenol to observe conidiophore morphology. Fifty measurements were taken of pycnidia, conidia, conidiogenous cells and paraphyses for each isolate, and the ranges and averages were computed. Measurements and digital photographs were made using a HRc Axiocam digital camera and accompanying Axiovision 3.1 software (Carl Zeiss Ltd., München, Germany).

Colony growth rate for isolates CMW 14077 and CMW 14078 was studied at temperatures ranging from 5 to 35 °C, at 5 ° intervals in the dark. Mycelial plugs, 6 mm in diam, were transferred to 2 % MEA in 90 mm diam Petri dishes from the edges of 7-day-old, single conidial cultures. Four plates were used for each isolate at each temperature. Two perpendicular measurements were taken of the colony diameter daily until the mycelium of the fastest growing isolates had covered the plates. Average colony diameter of each isolate was calculated from the eight readings per isolate. Colony morphology and colour were determined from cultures grown on 2 % MEA at 25 ºC in the dark. Colony colours (upper surface and reverse) were obtained by comparison with the colour charts of Rayner (1970).

DNA extraction and ITS rDNA amplification

For DNA extraction, single conidial cultures were grown on 2 % MEA for 7 days at 25 °C in the dark. The mycelium was scraped directly from the medium and transferred to Eppendorf tubes (1.5 mL). DNA was extracted using a modified phenol:chlorophorm DNA extraction method of Raeder & Broda (1985). The resulting DNA pellets were re-suspended in 50 µL sterile SABAX water. RNAse (1 mg/ml) was added to DNA samples and incubated overnight at 37 °C to degrade residual protein or RNA. DNA was separated by electrophoresis on a 1.5 % agarose gel, stained with ethidium bromide and visualized under ultra-violet light. DNA concentrations were estimated against λ standard size markers.

Using the primer pair ITS1 and ITS4 (White *et al*. 1990), the ITS1 and ITS2 regions, and 5.8S gene of the ribosomal RNA (rRNA) operon were amplified using the PCR protocol of Slippers *et al*. (2004). PCR products were separated as described above and sizes of PCR products were estimated against a 100 bp molecular weight marker XIV (Roche Diagnostics, Johannesburg, S.A.). The PCR products were purified using a High Pure PCR Product Purification Kit (Roche Diagnostics, Mannheim, Germany).

DNA sequencing and analysis

The purified PCR products were sequenced in both directions using the same primers used for the PCR reactions. The ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Warrington, U.K.) was used for sequencing reactions as specified by the manufacturers. Sequence reactions were run on an ABI PRISM 3100™ automated DNA sequencer (Perkin-Elmer, Warrington, U.K.). The nucleotide sequences were analyzed using Sequence Navigator version 1.0.1. (Perkin-Elmer Applied BioSystems, Inc., Foster City, California) software and manually aligned by inserting gaps. Sequence data for isolates of the unknown species have been deposited in GenBank (Table 1). The DNA sequences of the isolates of the unknown species were compared with those of other *Botryosphaeria* spp. These included twenty-one ITS rDNA sequences of *B. parva* Pennycook & Samuels, *B. dothidea* (Moug. : Fr.) Ces. & De Not., *B. eucalyptorum* Crous, H. Smith & M.J. Wingf., *B. lutea* A.J.L. Phillips, *B. obtusa* (Schwein.) Shoemaker, *B. stevensii* Shoemaker, *B. tsugae* Funk, *Diplodia pinea* (Desm.) J. Kickx (= *Sphaeropsis sapinea* (Fr. : Fr.) Dyko & Sutton), *B. corticola* A.J.L. Phillips, Alves & Luque and *B. rhodina* obtained from GenBank (Table 1), arising from previous studies (Jacobs & Rehner 1998, Smith *et al*. 2001a, Zhou & Stanosz 2001, Alves *et al.* 2004, Slippers *et al*. 2004). The trees were rooted using the GenBank sequence of *Guignardia philoprina* (Berk. & M.A. Curtis) Aa and *Mycosphaerella africana* Crous & M.J. Wingf.

The DNA sequence data were manually aligned in PAUP version 4.0b10 (Swofford 1999) by the insertion of gaps. Gaps were treated as missing data and all characters included in the analyses were unordered and of equal weight. Most parsimonious trees were found using the heuristic search function with 1000 random addition replicates and tree bisection and reconstruction (TBR) selected as branch swapping algorithm. Branches of zero length were collapsed and all multiple, equally

parsimonious trees were saved. Branch support was determined using 1000 bootstrap replicates (Felsenstein 1985). The data set was also analysed by distance analyses using the Kimura-2 parameter (Kimura 1980). The sequence alignment and phylogenetic tree have been deposited in TreeBASE as S1133, M1944.

RESULTS

Morphology and cultural characteristics

The isolates from *S. cordatum* produced anamorph structures on the pine needles on WA within two to three weeks (Figs 1–8). No sexual (teleomorph) structures were observed during this study. The conidia (Figs 3, 4) were similar to those described for *L. theobromae* in shape, colour and striation (Clendinin 1896, Punithalingam 1976, Sivanesan 1984). These isolates, however, differed from *L. theobromae* in having markedly longer and wider conidia (28−)32−36(−39) × (14−)16−18.5(−21) µm, while those of *L. theobromae* are mostly 18−30 × 10−15 µm (Table 2). Furthermore, aging conidia of the strains from *S. cordatum* become one to three septate, (Figs 4, 5, 11), which is different to the single septate conidia that are typical of *L. theobromae* (Table 2).

DNA sequence comparisons

PCR products of approximately 560 base pairs (bp) were amplified. Unreliable sequence data from the ends of sequences were excluded. Alignment of the sequences resulted in a total of 534 characters, of which 386 uninformative characters were excluded, and 148 parsimony informative characters were used in the analyses. The parsimony analysis (using heuristic searches) produced six most parsimonious trees of 318 steps (CI = 0.758, RI = 0.869) that only differed in the length of the internal branches, and one of these trees was chosen for presentation (Fig. 12). A bootstrap search of 1000 replicates (Fig. 12) and distance analyses produced a tree of the same topology as the most parsimonious trees.

The species included in this comparison formed eleven terminal groupings, designated as groups I to XI (Fig. 12). Groups I to IV include *Botryosphaeria* spp. with *Fusicoccum*-like anamorphs: *B. parva*, *B. lutea*, *B. eucalyptorum* and *B. dothidea*. Groups V to IX (Fig. 12) include *Botryosphaeria* spp. with *Diplodia*-like anamorphs: *B. obtusa, Diplodia pinea, B. stevensii, B. tsugae* and *B. corticola.* Isolates of the unnamed species

from *S. cordatum* grouped most closely to *B. rhodina* (anamorph *L. theobromae*) (group X), but also resided in a clearly distinct group (group XI) with 95 % bootstrap support (Fig. 12). These two groups were more closely related to isolates that have diplodia-like anamorphs (groups V to IX), but also clearly separated from them with 78 % bootstrap value.

Taxonomy

Based on morphological characteristics and DNA sequence comparisons, we conclude that the fungus isolated from native *S. cordatum* in South Africa is distinct from *L. theobromae* and other *Botryosphaeria* anamorph spp. examined in our study. Our data also indicate that this fungus should reside in *Lasiodiplodia* as a new taxon. We provide the following description for this new species.

Lasiodiplodia gonubiensis Pavlic, Slippers & M.J. Wingf., **sp. nov.** Figs 1–11.
Pycnidia subimmersa, solitaria, globosa, papillata, atroplumbea, mycelio tecta, usque ad 460 µm diametro. Paraphyses cylindricae, non septatae, hyalinae. Cellulae conidiogenae holoblasticae, cylindricae, hyalinae. Conidia primo hyalina, unicellulares, ellipsoidea vel obovoidea, parietibus crassis, contentu granulari, apice rotundata, interdum basi truncata. Conidia senecta cinnamomescentes vel sepiacescentes, longitudinaliter striata, unum ad tria septa formantes.

Typus: PREM 58127, fruiting structures induced on needles of *Pinus* sp. on WA, South Africa, Eastern Cape Province, Gonubie, *Syzygium cordatum*, Jul. 2002, D. Pavlic (cultura viva CMW 14077 = CBS 115812).

Pycnidia (formed on WA on sterilized pine needles within 7−21 d) semi-immersed, solitary, globose, papillate, leaden black, covered by mycelium, up to 460 µm diam (Fig. 1). *Paraphyses* cylindrical, aseptate, hyaline, (14−)26.5−47(−65) × (1.5−)2–2.5(−3) µm (Figs 2, 7, 9). *Conidiogenous cells* holoblastic, cylindrical, hyaline, (6.5−)10−15(−18) × (1−)2−4(−4.5) µm (Figs 7–9). *Conidia* initially hyaline, unicellular, ellipsoid to obovoid, thick-walled with granular content, rounded at apex, occasionally truncate at base (Figs 3, 6, 7, 9, 10). Aging conidia become cinnamon to sepia with longitudinal striations,

forming one to three septa, (28−)32−36(−39) × (14−)16−18.5(−21) µm (av. 33.8 × 17.3 µm, n = 100, l/w 1.9) (Figs 4, 5, 11).

Etymology: Referring to the town Gonubie, South Africa from where the fungus was collected.

Cultural characteristics: Cultures initially white to smoke grey with fluffy, aerial mycelium, becoming olivaceous grey on the surface after 3−4 d, with thick aerial mycelium, margins slightly irregular; reverse side of the colonies dark slate blue. Optimum temperature for colony growth 25 °C, covering the medium surface (90 mm diam Petri dishes) after 5 d in the dark. The isolates that grew at 35 °C produced a coral red pigment within 4 d.

Substrate: Symptomless leaves and branches of *S. cordatum*.

Distribution: Eastern Cape Province (Gonubie), South Africa.

Specimens examined: **South Africa**, Eastern Cape Province, Gonubie, *Syzygium cordatum,* Jul. 2002, D. Pavlic, **holotype** PREM 58127, fruiting structures induced on needles of *Pinus* sp. on WA, culture ex-type CMW 14077 = CBS 115812; Eastern Cape Province, Gonubie, *Syzygium cordatum,* Jul. 2002, D. Pavlic, **paratype** PREM 58128, fruiting structures induced on needles of *Pinus* sp. on WA, culture ex-type CMW 14078 = CBS 116355.

DISCUSSION

In this study we have identified and described the new species of *Lasiodiplodia, L. gonubiensis*, that grows endophytically on native *S. cordatum* in South Africa. Based on its phylogenetic relationships, we expect that the teleomorph of this fungus will be a species of *Botryosphaeria*. Despite careful examination of dead branches and twigs of *S. cordatum*, we have not been able to find a sexual state for this fungus. Ideally, we would provide a name in *Botryosphaeria* for it, but this is not recommended by the ICBN (Art. 59.2, Greuter *et al*. 2000).

Lasiodiplodia gonubiensis was identified as a species of *Lasiodiplodia* based on conidial shape and striation, which are characters typical for this genus (von Arx 1974). Conidia of *L. gonubiensis* are similar in appearance to those of *L. theobromae* (Clendinin 1896, Griffon & Maublanc 1909, Goos 1961, Punithalingam 1976, 1979, Sivanesan 1984). However, *L. gonubiensis* can be distinguished from *L. theobromae* by its substantially larger and multiseptate conidia. These conidial characters have also been

useful to distinguish other closely related *Botryosphaeria* anamorphs, such as *B. ribis* and *B. parva* (Slippers *et al.* 2004).

Lasiodiplodia gonubiensis grouped separately from other *Botryosphaeria* spp. based on comparison of partial nrDNA ITS sequence data. The results of the phylogenetic study further showed that *L. gonubiensis* was closely related, but clearly distinct from isolates of *L. theobromae*. This is another example where ITS rDNA sequence data were useful to distinguish a new botryosphaeriaceous species. Recent studies have used this region extensively, combined with morphological data, to describe new *Botryosphaeria* spp. and to re-evaluate the placement of their anamorphs (Jacobs & Rehner 1998, Denman *et al*. 1999, Smith *et al*. 2001a, Smith & Stanosz 2001, Zhou & Stanosz 2001, Denman *et al.* 2003, Slippers 2003, Alves *et al.* 2004). Despite the general phylogenetic usefulness of this region of the genome, there are cryptic species that cannot be separated based solely on ITS rDNA sequence data (De Wet *et al*. 2003, Slippers *et al*. 2004). In these cases sequence data of multiple gene regions have revealed the cryptic species.

The teleomorph of *L. gonubiensis* was not observed in this study. *Lasiodiplodia* spp. are, however, well-known as anamorphs of *Botryosphaeria*. This is confirmed in this study, because *L. gonubiensis* groups significantly more closely to other *Botryosphaeria* spp. than even the closely related genus *Guignardia.* Due to the rarity of *Botryosphaeria* teleomorphs and their overlapping morphological features, species are often identified based on morphological characteristics of associated anamorphs (Shoemaker 1964, Laundon 1973, Sivanesan 1984, Jacobs & Rehner 1998, Slippers 2003). This has been true for *L. gonubiensis,* which could easily be distinguished from other closely related species based on conidial morphology.

In this study, *L. gonubiensis* and *L. theobromae* (teleomorph *B. rhodina*) grouped together as a sub-clade, within a greater clade that contains *Botryosphaeria* spp. with anamorphs in *Diplodia.* Previous phylogenetic re-evaluations have shown that *Botryosphaeria* anamorphs can be separated into two groups, namely *Diplodia*-like anamorphs with ellipsoid, thick-walled dark conidia, and *Fusicoccum*-like anamorphs with hyaline conidia (Denman *et al.* 2000, Zhou & Stanosz 2001). *Lasiodiplodia* has, however, always grouped separately within the *Diplodia* clade (Denman *et al.* 2000, Zhou & Stanosz 2001, Slippers 2003, Slippers *et al.* 2004), as was the case in our study. It has been proposed that all *Botryosphaeria* anamorphs might either be placed in *Fusicoccum* or *Diplodia*, with *Lasiodiplodia* residing in *Diplodia* (Denman *et al.* 2000). Because it is morphologically distinct, especially based on its obvious and unique conidial striations

(von Arx 1974), there seemed little reason from our data to change the name of this important tree pathogen. *Lasiodiplodia* has also not formally been reduced to synonymy with *Diplodia* and we have thus chosen to assign the new species from *S. cordatum* to *Lasiodiplodia* rather than *Diplodia*.

Lasiodiplodia gonubiensis is the first species in this genus to be found on native trees in South Africa. The closely related *L. theobromae* is an important opportunistic pathogen recorded from more than 500 host plants, mostly in tropical and subtropical regions (Punithalingam 1976). *Lasiodiplodia theobromae* has not been reported from native trees in South Africa, but it occurs on exotic *Acacia*, *Eucalyptus* and *Pinus* spp. in South Africa (Cilliers 1993, Crous *et al*. 2000, Burgess *et al*. 2003).

Lasiodiplodia gonubiensis was discovered as an endophyte in asymptomatic twigs and leaves of *S. cordatum*. Other *Botryosphaeria* spp. are common endophytes and latent, opportunistic pathogens on *Eucalyptus* (Fisher *et al*. 1993, Smith *et al*. 1996a, b). For these fungi, disease symptoms typically develop when trees are exposed to unfavourable environmental conditions. *Lasiodiplodia gonubiensis* might thus also be a latent pathogen although we have not found it in association with disease symptoms.

Lasiodiplodia gonubiensis could become a pathogen of commercial *Eucalyptus* spp. in South Africa. Both *S. cordatum* and *Eucalyptus* reside in the *Myrtaceae* and they are sufficiently related that they could share pathogens. This would be consistent with the fact that *B. parva* has been shown to infect both hosts (Smith *et al.* 2001a, Slippers *et al*. 2004). Although *B. parva* has been found as a pathogen on *Eucalyptus,* its pathogenicity on *S. cordatum* is not known. Future studies will consider the pathogenicity and potential threat of *L. gonubiensis* and other *Botryosphaeria* spp. to both *Syzygium* and *Eucalyptus* spp.

REFERENCES

Appel O, Laubert R (1907). Bemerkenswerte Pilze I. 1. *Lasiodiplodia nigra* Appel und Laubert. *Arbeiten aus der Kaiserlichen Biologischen Anstalt für Land-und Forstwirtschaft* **5**: 147−148.

Alves A, Correia A, Luque J, Phillips A (2004). *Botryosphaeria corticola,* sp. nov. on *Quercus* species, with notes and description of *Botryosphaeria stevensii* and its anamorph, *Diplodia mutila*. *Mycologia* **96**: 598–613.

Arx JA von (1974). *The genera of fungi sporulating in pure culture*. J. Cramer: Vaduz, Germany.

Arx JA von, Müller E (1954). Die Gattungen der amerosporen Pyrenomyceten. *Beiträge zur Kryptogamenflora der Schweiz* **11(1)**: 1−434.

Barr ME (1972). Preliminary studies on the *Dothideales* in temperate North America. *Contributions from the University of Michigan Herbarium* **9**: 523−638.

Burgess T, Wingfield MJ (2001). Impact of fungal pathogens in natural forests ecosystems: A focus on *Eucalyptus*. In *Microorganisms in plant conservation and biodiversity* (Sivasithamparam K, Dixon KW, eds). Kluwer Academic press, The Netherlands: 285−306.

Burgess T, Wingfield MJ, Wingfield BD (2003). Development and characterization of microsatellite loci for tropical tree pathogen *Botryosphaeria rhodina*. *Molecular Ecology Notes* **3**: 91−94.

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

Cilliers A (1993). A review of *Lasiodiplodia theobromae* with particular reference to its occurrence on coniferous seeds. *South African Forestry Journal* **166**: 47−52.

Clendinin L (1896). *Lasiodiplodia* Ellis. & Everh. *n. gen*. *The Botanical Gazette* **21**: 92−93.

Cooke MC (1897). Some exotic fungi. *Grevillea* **7**: 94−96.

Coutinho TA, Wingfield MJ, Alfenas AC, Crous PW (1998). *Eucalyptus* rust: A disease with the potential for serious international implications. *Plant Disease* **82**: 819−825.

Crous PW, Phillips AJL, Baxter AP (2000). *Phytopathogenic Fungi from South Africa*. University of Stellenbosch, Department of Plant Pathology Press, University of Stellenbosch Printers, Stellenbosch, South Africa.

Crous PW, Swart WJ (1995). Foliicolous fungi of *Eucalyptus* spp. from Eastern Madagascar: implications for South Africa. *South African Forestry Journal* **172**: 1−6.

Davison EM, Tay FCS (1983). Twig branch and upper trunk cankers of *Eucalyptus marginata*. *Plant Disease* **67**: 1285−1287.

Denman S, Crous PW, Groenewald JZ, Slippers B, Wingfield BD, Wingfield MJ (2003). Circumscription of *Botryosphaeria* species associated with *Proteaceae* based on morphology and DNA sequence data. *Mycologia* **95**: 294−307.

Denman S, Crous PW, Taylor JE, Kang JC, Pascoe I, Wingfield MJ (2000). An overview of the taxonomic history of *Botryosphaeria* and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. *Studies in Mycology* **45**: 129−140.

Denman S, Crous PW, Wingfield MJ (1999). A taxonomic reassessment of *Phyllachora proteae*, a leaf pathogen of *Proteaceae*. *Mycologia* **91**: 510−516.

De Wet J, Burgess T, Slippers B, Preisig O, Wingfield BD, Wingfield MJ (2003). Multiple gene genealogies and microsatellite markers reflect relationships between morphotypes of *Sphaeropsis sapinea* and distinguish a new species of *Diplodia*. *Mycological Research* **107**: 557−566.

Ellis JB, Barth J (1902). *Botryodiplodia gossypii* E. & B. *Journal of Mycology* **8**: 175−176.

Felsenstein J (1985). Confidence intervals on phylogenetics: an approach using bootstrap. *Evolution* **39**: 783−791.

Fisher PJ, Petrini O, Sutton BC (1993). A comparative study of fungal endophytes in leaves, xylem and bark of *Eucalyptus nitens* in Australia and England. *Sydowia* **45**: 1−14.

Goos RD, Cox EA, Stotzky G (1961). *Botryodiplodia theobromae* and its association with *Musa* species. *Mycologia* **53**: 262−277.

Greuter W, McNeill J, Barrie FR, Burdet HM, Demoulin V, Filgueiras TS, Nicolson DH, Silva PC, Skog JE, Trehane P, Turland NJ, Hawksworth DL (2000). International Code of Botanical Nomenclature (Saint Louis Code). XVI International Botanical Congress, St. Louis, Missouri, July-August 2000. Koeltz Scientific Books Königstein, Germany.

Griffon WM, Maublanc A (1909). Sur une maladie du cacaoyer. *Bulletin de la Société Mycologique de France* **25**: 51−58.

Hennings P (1897). Fungi camerunenses. I. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Planzengeographie* **22**: 72−111.

Higgins BB (1916). Plum wilt and its nature. *Georgia Experiment Station Bulletin* **118**: 1−29.

Jacobs KA, Rehner SA (1998). Comparison of cultural and morphological characters and ITS sequences in anamorphs of *Botryosphaeria* and related taxa. *Mycologia* **90**: 601−610.

Johnson LAS, Briggs BG (1981). Three old southern families - *Myrtaceae*, *Proteaceae* and *Restionaceae*. In *Ecological Biogeography of Australia*. (Keast A. eds). W Junk, The Hague: 427−464.

Kimura M (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**: 111−120.

Laundon GF (1973). *Botryosphaeria obtusa*, *B. stevensii* and *Otthia spiraeae* in New Zealand. *Transactions of the British Mycological Society* **61**: 369−374.

Massee G (1910). Fungi exotici: X. *Bulletin of Miscellaneous Information* **1**: 1−9.

Nowell W (1923). *Diseases of crop-plants in the Lesser Antilles*. Imperial Department of Agriculture, The West India Committee, London.

Old KM, Davison EM (2000). Canker diseases of Eucalypts. In *Diseases and Pathogens of* Eucalypts (Keane PJ, Kile GA, Podger FD, Brown BN, eds). CSIRO Publishing, Australia: 241−257.

Palgrave KC (1977). *Trees of Southern Africa*. 1st edn. C. Struik publishers, South Africa.

Patouillard N, De Lagerheim G (1892). Champignons de l'equateur (Pegillus II). *Bulletin de la Societé Mycologique de France* **8**: 113−140.

Pole Evans IB (1910). On the structure and life-history of *Diplodia natalensis* n. sp. *Science Bulletin of the Department of Agriculture Union of South Africa* **4**: 1−24.

Poynton RJ (1979). *Tree planting in Southern Africa, Vol. 2. The Eucalypts.* Department of Forestry, South Africa.

Prillieux N, Delacroix G (1894). Sur quelques champignons nouveaux ou peu connus parasites sur les plantes cultivées. *Bulletin trimestriel de la Sociétié Mycologique de France* **10**: 161−169.

Punithalingam E (1976). *Botryodiplodia theobromae.* CMI descriptions of pathogenic fungi and bacteria. No. 519. Commonwealth Mycological Institute, Kew, Surrey, England.

Punithalingam E (1979). Plant diseases attributed to *Botryodiplodia theobromae* Pat. J. Cramer, Germany.

Raeder U, Broda P (1985). Rapid preparation of DNA from filamentous fungi. *Letters in Applied Microbiology* **1**: 17−20.

Rayner RW (1970). *A mycological colour chart*. CMI and British Mycological Society, Kew, Surrey, UK.

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

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

Saccardo PA (1917). *Diplodia ananassae* Sacc. *sp. n. Atti della Accademia scientifica veneto-trentino-istriana* Ser. III **10**: 75−76.

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

Shearer BL, Tippett JT, Bartle JR (1987). *Botryosphaeria ribis* infection associated with death of *Eucalyptus radiata* in species selection trials. *Plant Disease* **71**: 140−145.

Shoemaker RA (1964). Conidial states of some *Botryosphaeria* species on *Vitis* and *Quercus*. *Canadian Journal of Botany* **42**: 1297−1301.

Sivanesan A (1984). *The bitunicate ascomycetes and their anamorphs*. J. Cramer, Vaduz, Germany.

Slippers B (2003). Taxonomy, phylogeny- and ecology of botryosphaeriaceous fungi occurring on various woody hosts. Ph.D. dissertation. Department of Microbiology and Plant Pathology, University of Pretoria, South Africa.

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

Smith DR, Michailides TJ, Stanosz GR (2001b). Differentiation of a *Fusicoccum* sp. causing pinacle and shoot blight on California pistachio trees from *Botryosphaeria dothidea*. *Plant Disease* **85**: 1235−1240.

Smith DR, Stanosz GR (2001). Molecular and morphological differentiation of *Botryosphaeria dothidea* (anamorph *Fusicoccum aesculi*) from some other fungi with *Fusicoccum* anamorphs. *Mycologia* **93**: 505−515.

Smith H, Crous PW, Wingfield MJ, Coutinho TA, Wingfield BD (2001a). *Botryosphaeria eucalyptorum sp. nov.*, a new species in the *B. dothidea*-complex on *Eucalyptus* in South Africa. *Mycologia* **93**: 277−285.

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

Smith H, Wingfield MJ, Crous PW, Coutinho TA (1996a). *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. *South African Journal of Botany* **62**: 86−88.

Smith H, Wingfield MJ, Petrini O (1996b). *Botryosphaeria dothidea* endophytic in *Eucalyptus grandis* and *Eucalyptus nitens* in South Africa. *Forest Ecology and Management* **89**: 189−195.

Swofford DL (1999). PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland, Massachusetts: Sinauer Associates.

Sydow VH, Sydow P, Butler EJ (1916). Fungi Indiae orientalis. *Annales Mycologici* **14**: 196−220.

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

White TJ, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols: a guide to methods and applications.* (Innis MA, Gelfand DH, Snisky JJ, White TJ, eds) Academic Press, San Diego: 315−322.

Wingfield MJ (1999). Pathogens in exotic plantations forestry. *International Forestry Review* **1**: 163−168.

Zhou S, Stanosz GR (2001). Relationships among *Botryosphaeria* species and associated anamorphic fungi inferred from the analyses of ITS and 5.8S rDNA sequences. *Mycologia* **93**: 516−527.

Table 1. Isolates of *Botryosphaeria* , *Guignardia* and *Mycosphaerella* species considered in the phylogenetic study.

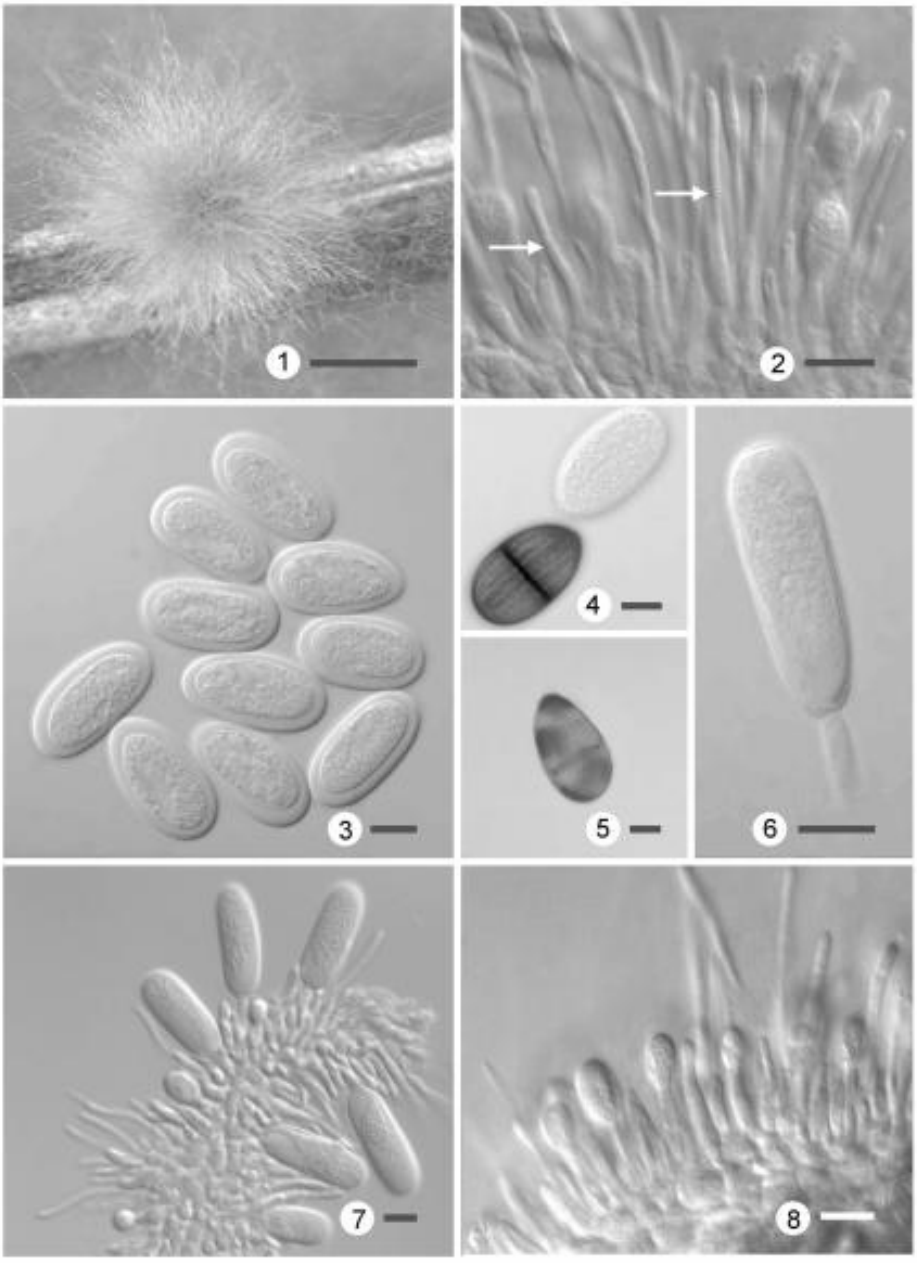
Culture no.[1]	Other no.[1]	Identity	Host	Location	Isolator	GenBank no.
CMW 9081	ICMP 8003	*Botryosphaeria parva*	*Populus nigra*	New Zealand	G.J. Samuels	AY236943
CMW 10124	BOT 681	*B. parva*	*Heteropyxis natalensis*	KwaZulu-Natal, S. Africa	H. Smith	AF283676
CMW 9075	ICMP 8019	*Botryosphaeria dothidea*	*P. nigra*	New Zealand	G.J. Samuels	AY236950
CMW 8000		*B. dothidea*	*Prunus* sp.	Crocifisso, Switzerland	B. Slippers	AY236949
CMW 10125	BOT 24	*Botryosphaeria eucalyptorum*	*Eucalyptus grandis*	Mpumalanga, S. Africa	H. Smith	AF283686
CMW 10126	BOT 16	*B. eucalyptorum*	*E. grandis*	Mpumalanga, S. Africa	H. Smith	AF283687
CMW 992	KJ 93.52	*Botryosphaeria lutea*	*Actinidia deliciosa*	New Zealand	G.J. Samuels	AF027745
CMW 9076	ICMP 7818	*B. lutea*	*Malus domestica*	New Zealand	S.R. Pennycook	AY236946
CMW 7774		*Botryosphaeria obtusa*	*Ribes* sp.	New York, U.S.A.	B. Slippers/G. Hudler	AY236953
	KJ 93.56	*B. obtusa*	Hardwood shrub	New York, U.S.A.	G.J. Samuels	AF027759
	KJ 93.27	*Botryosphaeria rhodina*	*Quercus* sp.	California, U.S.A.	E. Hecht-Poinar	AF027761
	ZS 96-112	*B. rhodina*	*Pinus radiata*	S. Africa	W. Swart	AF243401
	ZS 96-172	*B. rhodina*	*Theobroma cacao*	Sri Lanka	E. Muller	AF243400
CMW 10130	BOT 977	*B. rhodina*	*Vitex donniana*	Uganda	J. Roux	AY236951
CMW 9074		*B. rhodina*	*Pinus* sp.	Mexico	T. Burgess	AY236952
CMW 7060	CBS 431	*Botryosphaeria stevensii*	*Fraxinus excelsior*	Netherlands	H.A. van der Aa	AY236955
	ZS 94-6	*B. stevensii*	*Malus pumila*	New Zealand	N. Tisserat	AF243407
	CBS 112545	*Botryosphaeria corticola*	*Quercus ilex*	Spain	M.A. Sanchez/A. Trapero	AY259089
	CBS 112551	*B. corticola*	*Quercus suber*	Portugal	A. Alves	AY259101
	CBS 418.64	*Botryosphaeria tsugae*	*Tsuga heterophylla*	Canada	A. Funk	AF243405
	KJ 94.07	*Diplodia pinea*	*Pinus resinosa*	Wisconsin, U.S.A.	D.R. Smith	AF027758
CMW 14077	BOT 2779	*Lasiodiplodia gonubiensis*	*Syzygium cordatum*	Eastern Cape, S. Africa	D. Pavlic	AY639595
CMW 14078	BOT 2780	*L. gonubiensis*	*S. cordatum*	Eastern Cape, S. Africa	D. Pavlic	AY639594
CMW 3025		*Mycosphaerella africana*	*Eucalyptus viminales*	Stellenbosch, S. Africa	P.W. Crous	AF 283690
CMW 7063	CBS 447	*Guignardia philoprina*	*Taxus baccata*	Netherlands	H.A. van der Aa	AY236956

[1]Culture collections: BOT and CMW = Tree Pathology Co-operative Programme, Forestry and Agricultural Biotechnology Institute, University of Pretoria; KJ = Jacobs and Rehner (1998); CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; ICMP = International Collection of Microorganisms from Plants, Auckland, New Zealand; ZS = Zhou and Stanosz (2001).

Table 2. Conidial measurements and septation for *Lasiodiplodia theobromae* described under different synonyms.

Species	Host	Origin	Conidia size	No. of septa	Reference
Diplodia gossypina Cooke	*Gossypium* sp.	India	22 ×12 µm	–	Cooke 1879
Botryodiplodia theobromae Pat.	*Theobroma cacao*	Ecuador	25–35 × 12–15 µm	1	Patouillard & DeLagerheim 1892
Macrophoma vestita Prill. & Delacr.	*T. cacao*	Equatorial America	25–28 × 13 µm	1	Prillieux & Delacroix 1894
Lasiodiplodia tubericola Ellis & Everh.	*Ipomoea batatas*	Java	18–22 ×11–14 µm	1	Clendinin 1896
Diplodia cacaoicola P. Henn.	*T. cacao*	Kamerun	22–28 × 12–14 µm	1	Hennings 1897
Botryodiplodia gossypii Ellis & Barthol.	*Gossypium herbaceum*	U.S.A.	15–22 × 12 µm	1	Ellis & Barth 1902
Lasiodiplodia nigra K.R. Appel & Laubert	*T. cacao, Carica papaya*	Samoa	28–32 × 18–21 µm	1	Appel & Laubert 1907
Lasiodiplodia theobromae (Pat.) Griffon & Maubl.	*T. cacao*	Equatorial America	20–30 × 11–15 µm	1	Griffon & Maublanc 1909
Diplodia rapax Massee	*Hevea brasiliensis*	Singapore, Ghana	32–35 × 15–16 µm	1	Massee 1910
Diplodia natalensis Pole-Evans	*Citrus* sp.	South Africa	24–15 µm	1	Pole Evans 1910
Lasiodiplodia triflorae B.B. Higgins	*Prunus* sp.	U.S.A.	22–25 × 13–16.5 µm	1	Higgins 1916
Diplodia maniothi Sacc.	*Manihot utilissimae*	–	16–22 × 10–12 µm	1	Sydow *et al.* 1916
Diplodia musae Died.	*Musae sapientium*	–	17–20 ×10–13 µm	1	Sydow *et al.* 1916
Diplodia ananassae Sacc.	*Ananas sativus*	Philippines	23–25 ×11–12 µm	1	Saccardo 1917
Diplodia theobromae (Pat.) W. Nowell	*T. cacao*	–	25–30 × 12–15 µm	1	Nowell 1923

Figs 1–8. Micrographs of fruiting structures of *Lasiodiplodia gonubiensis.* 1. Pycnidium formed in culture on pine needles, covered with mycelium. 2. Paraphyses (arrows). 3. Conidia. 4. Brown conidium with one septum. 5. Brown conidium with two septa. 6. Conidium with attached conidiogeneous cell. 7, 8. Conidia, conidiogenous cells and paraphyses. Bars 1 = 500 µm; 2–8 = 10 µm.


1
2
3
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5
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7
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Figs 9–11. Line drawings of *Lasiodiplodia gonubiensis*. 9. Conidia, conidiogeneous cells and paraphyses. 10. Aseptate conidia. 11. 1–3 septate conidia. Bar = 10 µm.

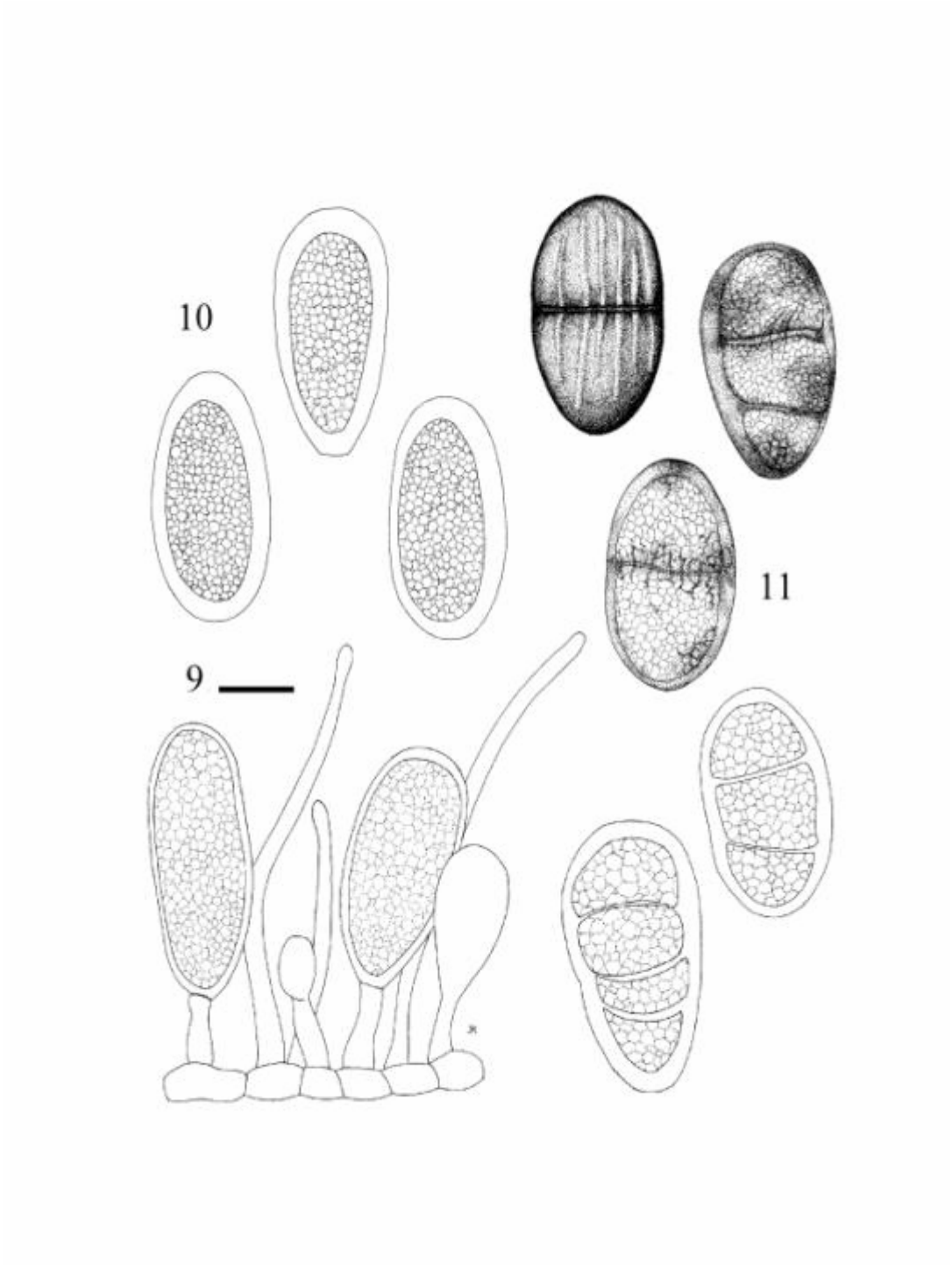
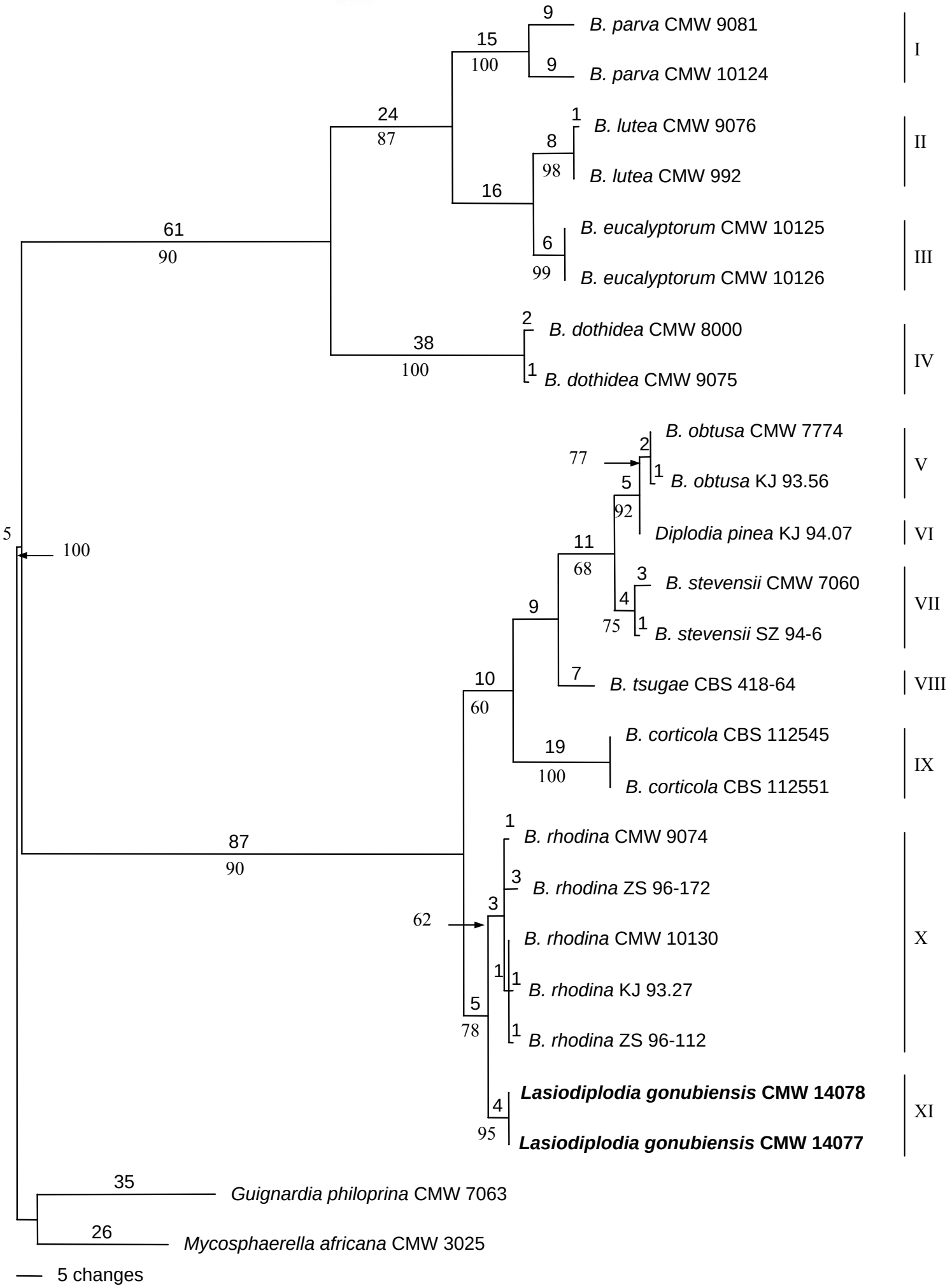
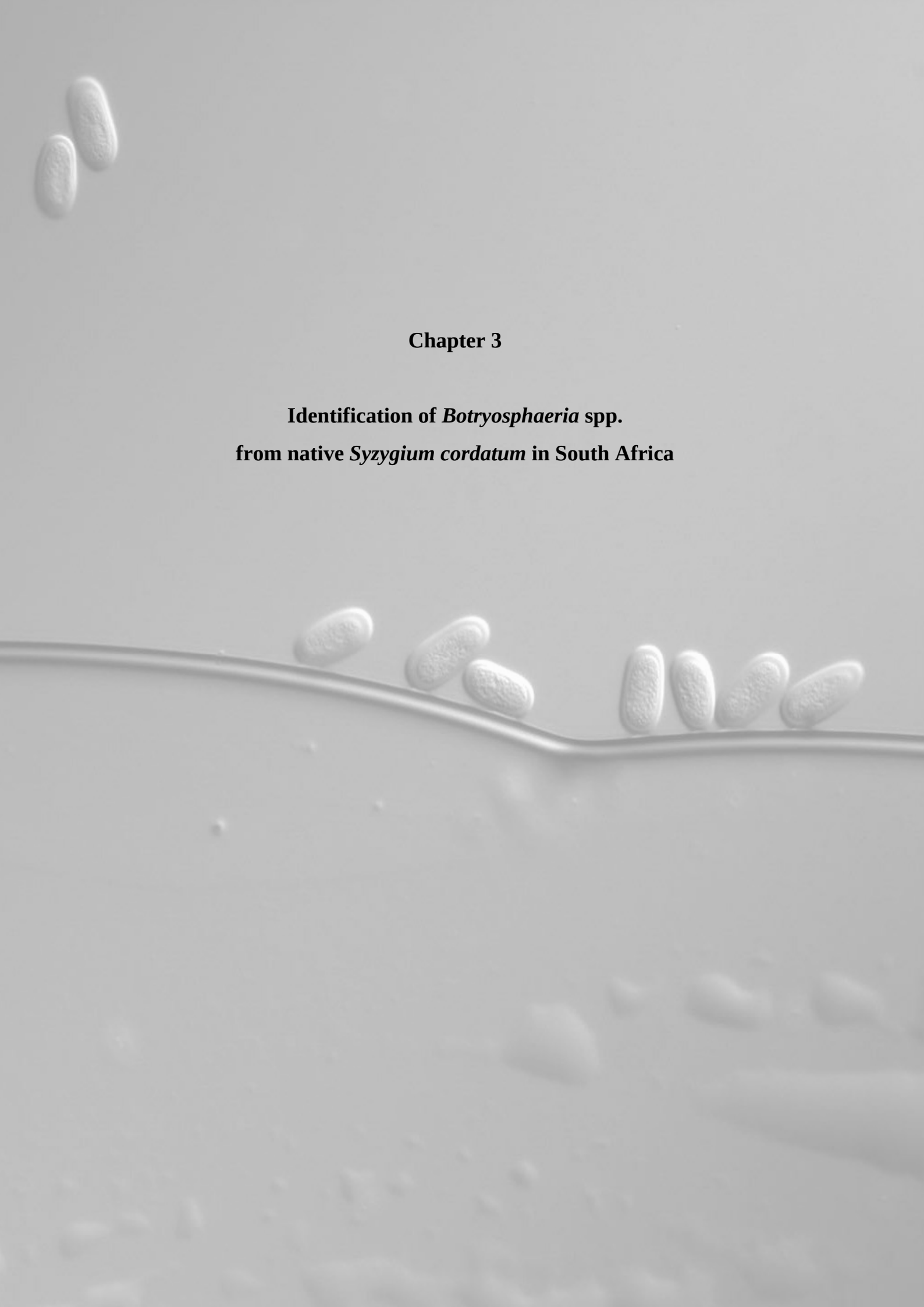

10
11
9

Fig. 12. Phylogram showing relationships amongst *Botryosphaeria* spp. based on parsimony analysis of the ITS1, 5.8 S and ITS2 rDNA sequence data (tree length = 318 steps, CI = 0.758, RI = 0.869). The tree is rooted to the outgroups *Guignardia philoprina* and *Mycosphaerella africana*. Bootstrap values (1000 replicates) are indicated below the internodes, and branch lengths, proportional to the number of steps, are indicated above the internodes.


9
B. parva CMW 9081
15
100
9
B. parva CMW 10124
I
24
87
1
B. lutea CMW 9076
8
98
B. lutea CMW 992
II
16
61
90
B. eucalyptorum CMW 10125
6
99
B. eucalyptorum CMW 10126
III
2
B. dothidea CMW 8000
38
100
1
B. dothidea CMW 9075
IV
B. obtusa CMW 7774
77
2
1
B. obtusa KJ 93.56
V
5
92
Diplodia pinea KJ 94.07
VI
5
100
11
68
3
B. stevensii CMW 7060
4
75
1
B. stevensii SZ 94-6
VII
9
7
B. tsugae CBS 418-64
VIII
10
60
B. corticola CBS 112545
19
100
B. corticola CBS 112551
IX
1
B. rhodina CMW 9074
87
90
3
B. rhodina ZS 96-172
3
62
B. rhodina CMW 10130
1
1
B. rhodina KJ 93.27
5
78
1
B. rhodina ZS 96-112
X
Lasiodiplodia gonubiensis CMW 14078
4
95
Lasiodiplodia gonubiensis CMW 14077
XI
35
Guignardia philoprina CMW 7063
26
Mycosphaerella africana CMW 3025
5 changes



Chapter 3

Identification of *Botryosphaeria* spp. from native *Syzygium cordatum* in South Africa

ABSTRACT

Botryosphaeria spp. are common endophytes and opportunistic, latent pathogens on many forest trees, including *Eucalyptus* spp. (*Myrtaceae*). In South Africa, botryosphaeriaceous fungi are amongst the most important canker pathogens in exotic *Eucalyptus* plantations. These fungi have recently been reported from native South African trees closely related to *Eucalyptus*, such as *Syzygium cordatum* and *Heteropyxis natalensis*. This finding initiated a survey of botryosphaeriaceous fungi from native *S. cordatum* (*Myrtaceae*) in South Africa. *Botryosphaeria* strains were isolated from dying and asymptomatic twigs and leaves of *S. cordatum* from different geographical regions in South Africa. All isolates were induced to sporulate in culture and separated into eight groups based on conidial morphology. To facilitate their identification, representative isolates from these groups were compared with known *Botryosphaeria* spp. based on ITS rDNA sequence data. Phylogenetic analysis distinguished eight clades for the isolates obtained from *S. cordatum*. The ITS rDNA sequence data did not separate isolates of the cryptic species *B. parva* and *B. ribis*. These species could not be distinguished confidently on morphological data either. A PCR-RFLP fingerprinting technique was, therefore, used to distinguish isolates of these two species. In this study nine *Botryosphaeria* spp. were identified, namely *B. parva, B. ribis, B. lutea, B. australis, B. rhodina, B. dothidea, Fusicoccum mangiferum*, *Lasiodiplodia gonubiensis* and an unknown *Botryosphaeria* sp. The isolates related to *B. ribis, B. parva* and *F. mangiferum* were the most abundant, while only one isolate represented *B. dothidea*.

INTRODUCTION

Botryosphaeria Ces. & De Not. (*Dothideales*) comprises species that have a wide geographic distribution and extensive host range, including gymnosperms and angiosperms (von Arx & Müller 1954, Barr 1972). These fungi are common endophytes and latent, opportunistic pathogens on many forest tree species, including *Eucalyptus* spp. (*Myrtaceae*) (Fisher *et al*. 1993, Smith *et al*. 1996a, b). *Botryosphaeria* spp. cause a wide variety of symptoms on trees of all ages, but are mostly associated with cankers and die-back followed by extensive production of kino, and in severe cases mortality of trees (Davison & Tay 1983, Webb 1983, Sharma *et al*. 1984, Shearer *et al*. 1987, Smith *et al*. 1994, Old & Davison 2000, Roux *et al*. 2000, 2001, Smith *et al*. 2001a).

Exotic *Eucalyptus* plantations are often established in associations with closely related native myrtaceous trees (Burgess & Wingfield 2001). In such cases pathogens from either of these related native and introduced hosts could cross infect the other host group and cause serious diseases (Crous & Swart 1995, Wingfield 1999, Burgess & Wingfield 2001). For example, the rust fungus *Puccinia psidii* G. Winter that occurs on native *Myrtaceae* in South America has become one of the most important pathogens on exotic *Eucalyptus* in this region (Coutinho *et al.*1998).

Myrtaceae is predominantly a Southern Hemisphere family with more than 3000 species, particularly well represented in the tropical and temperate regions of Australasia and Central and South America (Johnson & Briggs 1981). Although less numerous in African countries, myrtaceous species form an integral part of Southern African indigenous flora. The most common and widely distributed myrtaceous tree in South Africa is *Syzygium cordatum* Hochst. (Palgrave 1977).

Eucalyptus spp. make up almost half of 1.35 million hectares of exotic plantations in South Africa (Anonymous 2002). *Eucalyptus* plantations are established mainly in the eastern part of the country in the same area where native *S. cordatum* is widely spread (Palgrave 1977, Anonymous 2002). Thus, a *Botryosphaeria* spp. that occur on this native host could pose a threat to exotic *Eucalyptus*. The only report of *Botryosphaeria* spp. from native mytaceous trees in South Africa is that of Smith *et al.* (2001a). Due to the economic importance of *Eucalyptus* plantations, as well as the need to protect indigenous flora, identification of *Botryosphaeria* spp. that occur on native *S. cordatum* is of importance.

Identification of *Botryosphaeria* spp. based solely on morphological characteristics has led to much confusion. Due to the rarity of *Botryosphaeria* teleomorphs (Shoemaker 1964, Laundon 1973, Jacobs & Rehner 1998, Slippers 2003) and overlap between their morphological features (Pennycook & Samuels 1985, Slippers *et al.* 2004a, b), species are often identified based on morphological characteristics of associated anamorphs (Shoemaker 1964, Pennycook & Samuels 1985, Crous & Palm 1999). Identification of species based on anamorph characters also causes some problems because characteristics overlap between species and changes in spore morphology occurs with age (Pennycook & Samuels 1985, Jacobs & Rehner 1998, Slippers 2003). Furthermore, morphological characteristics of the anamorphs can be strongly influenced by the substrate on which they are grown (Sutton 1980). Thus, identification of botryosphaeriaceous fungi based solely on either morphological characteristics of their anamorph or teleomorph is unreliable.

Recent studies combined morphological characteristics and DNA sequence data to distinguish and identify species within *Botryosphaeria* (Jacobs & Rehner 1998, Denman *et al*. 1999, Smith *et al.* 2001, Zhou & Stanosz 2001a, b, Zhou *et al.* 2001, Phillips *et al.* 2002, Denman *et al.* 2003, De Wet *et al*. 2003, Alves *et al.* 2004, Slippers *et al*. 2004a, b). Molecular data most commonly used to study *Botryosphaeria* spp. are sequence data from internal transcribed spacer ITS gene region of the rDNA operon (Jacobs & Rehner 1998, Denman *et al.* 2000, 2003, Smith *et al.* 2001, Zhou & Stanosz 2001a, Phillips *et al.* 2002). Based on anamorph morphology and ITS rDNA sequence data, Denman *et al.* (2000) proposed that all *Botryosphaeria* anamorphs might either be placed in *Fusicoccum* or *Diplodia*. However, some closely related or cryptic species within these groups could not been distinguished based on single gene genealogies. To distinguish such species multiple gene sequence data have been used (De Wet *et al.* 2003, Slippers *et al.* 2004a, b).

Although closely related species within *Botryosphaeria* can be separated using multiple gene sequence data, this method is impractical to be used in the identification of large number of isolates. Jacobs (2002) and Slippers (2003) developed PCR Restriction Fragment Length Polymorphism (RFLP) techniques that could distinguish species within *Botryosphaeria*. PCR-RFLP profiles of ITS rDNA were useful to distinguish distant and closely related *Botryosphaeria* species (Jacobs 2002, Slippers 2003).

The aim of this study was to identify *Botryosphaeria* spp. that occur on native *S. cordatum* growing in different geographical regions of South Africa. This was achieved using ITS rDNA sequence data, PCR-RFLP analysis and morphological characteristics.

MATERIALS AND METHODS

Isolates

Isolates used in this study were collected during a survey of *Botryosphaeria* spp. on native *Syzygium cordatum* in different geographical regions in South Africa, in 2001 and 2002 (Tables 1, 2, Fig. 1). Isolations were made from dying twigs and asymptomatic, visually healthy twigs and leaves. Leaves and twig portions (5 cm in length) were washed in running tap water and surface disinfected by placing them sequentially for 1 min in 96 % ethanol, undiluted bleach (3.5–5 % available chlorine) and 70 % ethanol, and rinsed in sterile water. The disinfected twig portions were halved and pieces from the pith tissue (2 mm^2) and segments of the leaves (3 mm^2) were placed on 2 % malt extract agar (MEA) (2 % malt extract, 1.5 % agar; Biolab, Midrand, Johannesburg, S.A.). Petri dishes were incubated for two weeks at 20 °C under continuous near fluorescent light and colonies resembling *Botryosphaeria* spp. were selected. These colonies were transferred to 2 % MEA at 25 °C and stored at 5 °C. All isolates are maintained in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa (Table 2).

DNA extraction and ITS rDNA amplification

Single spore cultures were grown on MEA for 7 days at 25 °C in the dark. Template DNA was obtained from the mycelium using modified phenol:chlorophorm DNA extraction method of Raeder & Broda (1985). Mycelium was scraped directly from the medium and transferred to Eppendorf tubes (1.5mL) and 200µL of an extractions buffer (200 mM Tris-HCl pH 8.5, 150 mM NaCl, 25 mM EDTA, 0.5 % SDS) was added. Mycelium was ground with a pestle to obtain a homogeneous solution. Further 500 µL of extraction buffer, 400 µL of phenol and 300 µL of chloroform were added and centrifuged (13000 rpm, 1h). Thereafter, phenol and chlorophorm (1:1) were added, centrifuged and the upper aqueous phase removed repeatedly until the interphase was clean of any cellular debris. Precipitation of nucleic acids was done by the addition of 0.1 volume 3 M of NaAc (pH 5.5) and 2 volumes of absolute ethanol and centrifuged at 13000 rpm for 10 min at 4 °C. DNA was further purified by washing with 70 % ethanol (80 µL) and vacuum dried. The resulting DNA pellets were resuspended in 50 µL sterile SABAX water. RNAse (1 mg/ml)

was added to DNA samples and incubated overnight at 37 °C to degrade residual RNA. DNA was electrophoresed on a 1.5 % agarose gel, stained with ethidium bromide and visualized under ultra-violet light. DNA concentrations were estimated against λ standard size markers.

Using the primer pair ITS1 (5′ TCCGTAGGTGAACCTGCGG) and ITS4 (5′ TCCTCCGCTTATTGATATGC) (White *et al*. 1990) the internal transcribed spacer (ITS) regions ITS1 and ITS2, and also 5.8S gene of the ribosomal RNA (rRNA) were amplified. The PCR reactions were performed using the PCR protocol of Slippers *et al.* (2004a). PCR products were separated in a 1.5 % agarose gel, stained with ethidium bromide and visualized under UV light. Sizes of PCR products were estimated against a 100 bp molecular weight marker XIV (Roche Diagnostics, South Africa). The PCR products were purified using High Pure PCR Product Purification Kit (Roche Diagnostics, Penzberg, Germany).

DNA sequencing and analysis

Based on conidial morphology, the *Botryosphaeria* isolates from *S. cordatum* in South Africa were tentatively separated into eight groups. ITS rDNA sequences were determined for representative samples from all morphological groups (Table 1). To determine the identity and phylogenetic relationship of these isolates, ITS sequences of known *Botryosphaeria* spp. were obtained from GenBank and included in the analyses (Table 1). The purified PCR products were sequenced using the same primers that were used for the PCR reactions. The ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Warrington, U.K.) was used for sequencing reactions, as specified by the manufacturers. Sequence reactions were run on an ABI PRISM 3100™ automated DNA sequencer (Perkin-Elmer, Warrington, U.K.).

The nucleotide sequences were analyzed using Sequence Navigator version 1.0.1. (Perkin-Elmer Applied BioSystems, Inc., Foster City, California) software and manually aligned by inserting gaps. Gaps were treated as fifth character and all characters were unordered and of equal weight. Phylogenetic analyses of aligned sequences were done using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b8 (Swofford 1999). Most parsimonious trees were found using the heuristic search function with 1000 random addition replicates and the tree bisection and reconstruction (TBR) selected as branch

swapping algorithm. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Branch support was determined using 1000 bootstrap replicates (Felsenstein 1985). The trees were rooted using the GenBank sequences of *Guignardia philoprina* (Berk. & M.A. Curtis) Aa and *Mycosphaerella africana* Crous & M.J. Wingf., which are closely related to *Botryosphaeria*.

PCR-RFLP analyses

PCR-RFLP fingerprint techniques were applied to confirm the identity of isolates that were not sequenced and to identify the isolates that could not be separate based on ITS rDNA sequences. Amplicons obtained using primer pairs ITS1 and ITS4, or BOT15 (5′ CTGACTTGTGACGCCGGCTC) and BOT16 (5′ CAACCTGCTCAGCAAGCGAC) were digested with the restriction endonucleases (RE) *Cfo*I. The RFLP reaction mixture consisted of 10 µl PCR products, 0.3 µl RE *Cfo*I and 2.5 µl matching enzyme buffer (Roche Diagnostics, Indianopolis, U.S.A.). The reaction mixture was incubated at 37 ºC overnight. Restriction fragments were separated on 1.5 % agarose gel as described for PCR products. The results were compared to those published by Slippers 2003.

Morphology and cultural characteristics

Fungal isolates were grown on 2 % water agar (WA)(Biolab, S.A.) with sterilized pine needles placed onto the medium, at 25 °C under near-UV light, to induce sporulation. Conidia released from pycnidia formed on the pine needles were mounted in lactophenol on microscope slides and examined microscopically. Measurements and digital photographs were made using the light microscope, an HRc Axiocam digital camera and accompanying software (Carl Zeiss Ltd., München, Germany). Colony morphology and colour were determined from cultures grown on 2 % MEA at 25 ºC under near-UV light. Colony colours (upper surface and reverse) were compared to the colour charts of Rayner (1970).

RESULTS

DNA sequence analyses

DNA fragments of approximately 600 bp were amplified. The ITS dataset consists of 56 ingroup sequences and *Guignardia philoprina* and *Mycosphaerella africana* as outgroup

(Table 1). After alignment the ITS dataset consisted of 536 characters; 389 uninformative characters were excluded, and 147 parsimony informative characters were used in the analysis. The parsimony analysis (using heuristic searches) produced 84 most parsimonious trees of 414 steps (CI = 0.717, RI = 0.921), of which one was chosen for presentation (Fig. 2).

The isolates considered in the phylogenetic analyses formed 14 terminal groupings, designated as groups I to XIV (Fig. 2). These groups were resolved in two major clades that corresponded to *Botryosphaeria* species with *Fusicoccum* or *Diplodia* anamorphs. The *Fusicoccum* clade comprised six groups that represented: *B. parva* Pennycook & Samuels and *B. ribis* Grossenb. & Duggar (group I), *Fusicoccum mangiferum* (Syd. & P. Syd.) Johnson, Slippers & M.J. Wingf. prov nom. (group II), *B. eucalyptorum* Crous, H. Smith & M.J. Wingf. (group III), *B. australis* Slippers, Crous & M.J. Wingf., (group IV), *B. lutea* A.J.L. Phillips (group V) and *B. dothidea* (Moug. : Fr.) Ces. & De Not. (group VI). Groups VII to IX represented species with *Lasiodiplodia* anamorphs: *B. rhodina* (Berk. & M. A. Curtis) Arx (anamorph *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.) (group VII) *Lasiodiplodia gonubiensis* Pavlic, Slippers & M.J. Wingf. (group VIII) and unknown *Botryosphaeria* sp. (group IX). These three groups (VII to IX) formed distinct subclade (supported by 100% bootstrap value) within the *Diplodia* clade. The other major subclade within the *Diplodia* clade contained five groups corresponding to: *B. stevensii* Shoemaker, (group X), *B. corticola* A.J.L. Phillips, Alves & Luque (group XI), *B. tsugae* Funk (group XII), *B. obtusa* (Schwein.) Shoemaker (group XIII) and *Diplodia pinea* (Desm.) J. Kickx (= *Sphaeropsis sapinea* (Fr. : Fr.)) (group XIV) (Fig. 2).

All solates from *Syzygium cordatum* resided in eight groups (Fig. 2). Group I comprises isolates of *B. parva* and *B. ribis*. Isolates of these two species could not be distinguished based solely on ITS rDNA data (Fig. 2). Group II corresponded to *Fusicoccum mangiferum*, while groups IV and V represented *B. australis* and *B. lutea* respectively (Fig. 2). The latter two species were clearly separated from other species within the *Fusiccocum* clade (95 % bootstrap support), but bootstrap values for the individual groups were low (69 % and 62 %). A single isolate grouped with *B. dothidea* (group VI). *B. dothidea* isolates grouped apart from the other *Botryosphaeria* spp. with *Fusicoccum* anamorphs on a long well supported branch (100 % bootstrap support). Three strains from *S. cordatum* corresponded to *Botryosphaeria* spp. with *Lasiodiplodia* anamorphs (group VII to IX). Two of these groups were identified as *B. rhodina*

(anamorph *L. theobromae*) (group VII) and *L. gonubiensis* (group VIII). Isolates of *L. gonubiensis* grouped most closely to *B. rhodina*, but also resided in clearly distinct group with 96 % bootstrap support (Fig. 2). Group IX is represented by a single strain isolated from *S. cordatum* and were distinct from both *B. rhodina* (group VII) and *L. gonubiensis* (group VIII). Due to presence of a single isolate and lack of morphological distinguishing features this isolate could not be further compared and described, and was treated as an unknown *Botryosphaeria* sp.

PCR-RFLP analysis

Isolates not identified using sequencing were subjected to ITS PCR-RFLP analyses. Digests of the PCR products, obtained using primers ITS1 and ITS4, with the RE *Cfo*I produced two distinctive banding patterns (Fig. 3). These profiles matched those of *B. parva* / *B. ribis* (99 isolates) and *B. lutea* / *B. australis* (5 isolates) as shown by Slippers (2003). To further distinguish isolates of *B. parva* from *B. ribis*, amplicons obtained using primers BOT15 and BOT16 (Slippers 2003) were digested using the same restriction endonuclease (RE). The two banding patterns obtained matched those of *B. parva* (42 isolates) and *B. ribis* (57 isolates) as described by Slippers 2003 (Fig. 4). *B. lutea* and *B. australis* could, however, not be separated using this technique.

Morphology and cultural characteristics

All isolates of the *Botryosphaeria* spp. from *S. cordatum* produced anamorph structures on pine needles on WA within two to three weeks. No teleomorph (sexual) structures were observed. Based on conidial morphology, isolates were separated into eight groups. Six of these groups corresponded to *Botryosphaeria* spp. with *Fusicoccum* anamorphs (Figs 21–27) and two of them with *Lasiodiplodia* (*Diplodia*-like) anamorphs (Figs 28–29).

Representative samples from these groups were identified based on ITS rDNA sequence comparison. As described earlier, isolates of *B. parva* and *B. ribis* were separated based on PCR-RFLP analyses. Further morphological examination of isolates identified based on DNA molecular data provided support for their identity. Conidial measurement (length, width and L/W) for all isolates from *S. cordatum* are presented in Table 2. The percentage of isolates of each species identified in this study is presented in

Fig. 30. Distribution of *Botryosphaeria* spp. isolated from *S. cordatum* in different areas in South Africa is presented in Table 3.

Cultures of *B. parva* initially white with fluffy, aerial mycelium, becoming pale olivaceous grey (21’’’’’d) from the middle of colony after 3–4 days, columns of the mycelium formed in the middle of colony reaching the lid, margins regular (Figs 10, 18); reverse side of the colonies were olivaceous grey (21’’’’’). Conidia hyaline, smooth, aseptate and fusiform to ellipsoid (average of 420 conidia 18.2 × 5.5 µm, l/w 3.3) (Fig. 21, Table 2).

Colonies of *B. ribis* initially white, becoming pale olivaceous grey (21’’’’’d) from the middle of colony, with thick aerial mycelium reaching the lid of Petri dishes, margins regular (Figs 9, 17); reverse side of the colonies grey olivaceous (21’’’’). Conidia hyaline, unicellular, aseptate, fusiform, apices tapered (average of 570 conidia 21 × 5.5 µm, l/w 3.8) (Fig. 22, Table 2).

Single *B. dothidea* culture identified in this study produced greenish olivaceous (23’’’) appresed mycelium, margins regular (Figs 6, 14); reverse side of the colony grey olivaceous (21’’’’) to iron grey (23’’’’’k). Conidiomata were readily formed in the middle of colony after 3–4 for days of incubation. Conidia hyaline, smooth with granular contents, aseptate, narrowly fusiform (average of 10 conidia 27.8 × 5.4 µm, l/w 5.1) (Fig. 27, Table 2).

Isolates of *F. mangiferum* produce pale olivaceous grey (21’’’’d) appressed mycelium, slightly fluffy on the edge of colony, margins sinuate (Figs 5, 13); reverse side of the colony olivaceous (21’’k). Conidiomata were readily formed in the middle of colony after 3–4 days and cover all surface of the colony within 7–10 days. Conidia hyaline, fusiform (average of 300 conidia 14.2 × 6.3 µm, l/w 2.25) (Fig. 26, Table 2).

Cultures of *B. lutea* initially white, becoming pale olivaceous grey (21’’’’d) from the middle of colonies within 3–4 days, mycelium suppressed, moderate fluffy in the middle, margins regular; yellow pigment noticeable between 3–5 days after incubation recognised as amber yellow (21’b) on the reverse side of Petri dishes; after 5-7 days colonies becoming olivaceous buff (21’’’d) to olivaceous gray (21’’’’I) (Figs 7, 15). Conidiomata readily formed from the middle of colony within 3–4 days and cover all surface of the colony within 7–10 days. Conidia hyaline, fusiform to ellipsoid, sometimes irregularly fusiform, smooth with granular contents, unicellular, forming one or two septa before germination (average of 40 conidia 18.9 × 6.3 µm, l/w 3.0) (Figs 24, 25; Table 2).

Cultures of *B. australis* (Figs 8, 16) were very similar in morphology to those of *B. lutea* but yellow pigment produce in young cultures was brighter and recognised as honey yellow (19'') (Fig. 8). Conidiomata readily formed in the middle of colony within 3–4 days and cover the colony surface within 7–10 days. Conidia hyaline, fusiform, apices rounded, aseptate, rarely one septate (average of 70 conidia 20.5 × 5.7 µm, l/w 3.6) (Fig. 23, Table 2). These conidia are slightly longer and narrower on average than *B. lutea,* that also reflected in higher l/w ratio.

Isolates of *L. theobromae* produced initially white to smoke grey (21''''d) fluffy aerial mycelium, becoming pale olivaceous grey (21'''''d) within 5-6 days, margins regular (Figs 12, 20); reverse side olivaceous grey (21''''') to iron (24'''''k), becoming dark slate blue (39''''m) after 7–10 days. Conidia hyaline, aseptate, ellipsoid to ovoid, thick-walled with granular content (average of 60 conidia 27 × 14.7 µm, l/w 1.85) (Fig. 29, Table 2). Dark, septate conidia typical for this species were not observed in this study.

Isolates of *L. gonubiensis* were similar in culture morphology to those of *L. theobromae* (Figs 11, 19). Conidia of *L. gonubiensis* were initially hyaline, unicellular, ellipsoid to obovoid, thick-walled with granular content, rounded at apex, occasionally truncate at base. Aging conidia become cinnamon (15''b) to sepia (15''m) with longitudinal striations, forming one to three septa (average of 20 conidia 33.9 × 18.9 µm, l/w 1.8) (Fig. 28, Table 2).

DISCUSSION

In this study, nine *Botryosphaeria* spp. were identified on native *Syzygium cordatum* in South Africa. The isolates obtained from *S. cordatum* represented *B. ribis, B. parva, B. lutea, B. australis, B. dothidea, B. rhodina* and the *Botryosphaeria* anamorphs *Fusicoccum mangiferum, Lasiodiplodia gonubiensis* and an unknown *Botryosphaeria* sp. The isolates were identified based on ITS rDNA sequence data, PCR-RFLP analysis and anamorph morphology.

Botryosphaeria ribis (anamorph *Fusicoccum ribis* Slippers, Crous & M.J. Wingf.) were the dominant species collected from native *S. cordatum* in South Africa. This species represented 38 % of all isolates obtained in this study (Fig. 30) and were found in most of the areas surveyed (Table 3). This is the first report of this species on native *Myrtaceae* in South Africa. This abundance and wide distribution of *B. ribis* on this native host might indicate that this species is native to this region. *Botryosphaeria ribis* has been reported

from *Eucalyptus* (*Myrtaceae*) in its native range in Australia and in exotic *Eucalyptus* plantations (Davison & Tay 1983, Shearer 1994, Shearer *et al.* 1987, Crous *et al.* 1989, Old & Davison 2000, Rodas 2003). These identifications should, however, be interpreted with caution, as the distinction of *B. parva* and *B. ribis* was not recognised in all these studies (Slippers *et al.* 2004a). Furthermore, *B. ribis* as identified in this study (using RFLPs) was also interpreted as representing a *B. ribis sensu lato* group rather than strictly conspecific populations with the type isolates of this species by Slippers (2003). Further analyses using sequence data of more gene regions and other variable markers are needed to better characterise populations and potential cryptic species in this group.

Botryosphaeria parva (anamorph *Fusicoccum parvum* Pennycook & Samuels) was the second most common species isolated from *S. cordatum* in South Africa, representing 28 % of the total number of isolates. *B. parva* was described as a new species from kiwifruit in New Zealand in 1985 (Pennycoook & Samuels 1985). Identification of *B. parva*, based on morphology alone, can lead to confusion with closely related species, which has lead to its distribution and importance being underestimated (Slippers 2003). Recent studies showed that *B. parva* is an important and widely distributed pathogen of *Eucalyptus* in exotic plantations (Ahamuda 2002, Nakabonge 2002, Gezahgne 2003, Slippers 2003). Based on molecular and morphological data, Slippers (2003) also showed that isolates identified from *Eucalyptus* and native *Heteropyxis natalensis* in South Africa represent *B. parva* rather than *B. dothidea* (Smith *et al.* 2001a). The wide distribution of *B. parva* on exotic and native *Myrtaceae* in South Africa raises the question whether these populations are native or exotic and how they interact with each other. The movement of this pathogen between these important host groups is a potential threat for both groups and should be further investigated.

Thirty isolates obtained in this study were identified as *Fusicoccum mangiferum* (Slippers *et al.* 2004c). This is the first report of *F. mangiferum* on native trees in this country. The presence of such a large number of isolates on the native host raises the question whether it is native to this region or has been introduced with foreign hosts. This species has been associated with various fruit and forestry hosts mostly in tropics and subtropics (Sutton & Dyko 1989). It is best known as a pathogen of mango (*Mangifera indica*) worldwide, particularly in Australia (Johnson *et al.*1992, Slippers *et al.* 2004c). *F. mangiferum* was earlier reported under different names from mango in South Africa (Sutton & Dyko 1989, Darvas 1991). A comprehensive recent study of *Botryosphaeria* spp. from mango plantations in South Africa, using a combination of molecular and

morphological data, however, did not report this species (Jacobs 2002). *Fusicoccum mangiferum* was also recently reported from South Africa from introduced *Tibouchina* trees (Heath 2003).

All isolates of *F. mangiferum,* obtained from *S. cordatum,* originated from the KwaZulu-Natal Province. This is also the area in which the majority of *Eucalyptus* plantations have been established (Anonymous 2002). This fungus could, therefore, pose a threat to commercial *Eucalyptus* plantations in the country. Its pathogenicity on these trees is, however, not known and should be further investigated to assess this threat.

Four isolates of *Botryosphaeria lutea* (anamorph *Fusicoccum luteum* Pennecook & Samuels) were obtained from *S. cordatum* in the Eastern Cape province. This is the first record of *B. lutea* on a native host in South Africa. *B. lutea* occurs on a variety of native and introduced hosts in New Zealand, Australia and Portugal (Pennycoook & Samuels 1985, Phillips *et al.* 2002, Slippers *et al.* 2004b). This fungus has not been reported on *Eucalyptus* in South Africa. An earlier report of *B. lutea* from native *Protea cynaroides* (Denman *et al.* 2003) in South Africa, was recently shown to represent *B. australis* (Slippers *et al.* 2004b).

Botryosphaeria australis (anamorph *Fusicoccum australe* Slippers, Crous & M.J. Wingf.) has been recently described as a new species that is closely related to *B. lutea* (Slippers *et al.* 2004b). In this study, *B. australis* was identified from *S. cordatum* in two different areas, Sodwana bay and East London. This fungus has been identified on native hosts in Australia, including *Eucalyptus* (Slippers 2003, Slippers *et al.* 2004b), but has not been recorded on *Eucalyptus* in South Africa. Distribution of this fungus on native *S. cordatum* and other native hosts (Denman *et al.* 2003, Slippers *et al.* 2004b) and in different geographic regions in South Africa could indicate its origin in this country.

Only one isolate obtained from *S. cordatum* was identified as *B. dothidea.* This species has been one of the most commonly reported botryosphaeriaceous species from a wide variety of hosts, including *Eucalyptus* (von Arx & Muller 1954, Barnard *et al.* 1987, Farr *et al.* 1989, Masuka 1990, Fisher *et al.* 1993, Smith *et al.* 2001a). However, in a recent study on botryosphaeriaceous fungi on *Eucalyptus* in its native range in Australia and exotic plantations in South Africa, Hawaii and Uruguay only one isolate, from Australia, has been identified as *B. dothidea* (Slippers 2003). The latter study showed that *B. dothidea* is not the most common *Botryosphaeria* spp. on *Eucalyptus* in South Africa, as has been previously reported (Smith *et al.* 2001a). *Botrypsphaeria dothidea* thus appears to be of little importance on both native and exotic *Myrtacea* in South Africa.

Botryosphaeria rhodina (anamorph *Lasiodiplodia theobromae*) was isolated from *S. cordatum* from subtropical area in South Africa. *Eucalyptus* is intensively planted in these areas too (Anonymous 2002). *Botryosphaeria rhodina* is an opportunistic pathogen with an extremely wide host range, including more than 500 host plants, mostly in tropical and subtropical regions (Punithalingam 1976). This fungus has previously been isolated from exotic *Acacia, Eucalyptus* and *Pinus* spp. in South Africa (Cilliers 1993, Crous *et al.* 2000, Burgess *et al.* 2003). This is the first record of this species on native *Myrtaceae* in the country.

Another *Lasiodiplodia* species isolated from *S. cordatum* has recently been described as *Lasiodiplodia gonubiensis* (Pavlic *et al.* 2004). This species is only known from *S. cordatum* in South Africa. Although closely related to *B. rhodina*, this species was isolated from a geographical region with a moderate climate. *Botryosphaeria rhodina* were absent from this region. Extending sampling from *S. cordatum* and closely related species that occur in this region could provide additional information of this fungus.

A combination of ITS rDNA sequence data and RFLP analyses of another variable locus was sufficient to identify all known species encountered in this study. ITS rDNA sequence could not distinguish *B. ribis* and *B. parva*. In this case, RFLP analysis of an unknown, variable locus previously identified during microsatellite development (Slippers 2003) was useful to distinguish the species. Other studies have also shown that ITS rDNA sequence data is insufficient to distinguish cryptic species within *Botryosphaeria*, and relied on multiple gene genealogies to distinguish species (De Wet *et al.* 2003, Slippers *et al.* 2004a, b). This is, however, not always necessary as some morphologically cryptic species, such as *B. lutea* and *B. australis*, can be distinguished based on ITS sequence data.

This study confirms that conidial and cultural characteristics can be useful in identification of *Botryosphaeria* spp., but are best used in combination with other methods. Given the large number of isolates treated in this study the morphological characters were especially useful to make initial groupings. Subsequent to identification of selected isolates based on molecular data, cultural morphology combined with conidial characteristics, such as average conidial size (length x width), length / width ratio and septation were then used to further confirm the identity of isolates not sequenced. Conidial characteristics of some species, such as *B. parva, B. ribis, B. lutea* and *B. australis,* are variable and overlap between species. This was also shown in the other studies on *Botryosphaeria* spp. with *Fusicoccum*-like anamorphs (Pennycook & Samuels 1985,

Phillips *et al.* 2002, Slippers 2003, Slippers *et al.* 2004a, b). Thus, to avoid misidentification, particularly when working with small number of isolates and closely related species, morphological characterization should be combined with DNA molecular data.

Isolates of all *Botryosphaeria* spp. identified in this study were isolated as endophytes from asymptomatic twigs and leaves of *S. cordatum*. All species, except *B. dothidea* and *L. gonubiensis,* were also isolated from symptomatic, dying twigs. *Botryosphaeria* spp. are known as endophytes and latent, opportunistic pathogens on *Eucalyptus* (Fisher *et al.* 1993, Smith *et al*. 1996a, b). Most of the species obtained in this study have, however, not been recorded on *Eucalyptus* in South Africa. These species from a native *Myrtaceae* host such as *S. cordatum* pose a potential threat to commercial *Eucalyptus* spp. in South Africa. Future studies will, therefore, be undertaken to test pathogenicity of the *Botryosphaeria* spp. obtained in this study to both *Syzygium* and *Eucalyptus* spp.

REFERENCES

Ahumada R (2002). Diseases in commercial *Eucalyptus* plantations in Chile, with special reference to *Mycosphaerella* and *Botryosphaeria* species. M.Sc. thesis. Department of Microbiology and Plant Pathology, University of Pretoria, South Africa.

Anonymous (2002). *Commercial timber resources and roundwood processing in South Africa 2000 / 2001*. Forestry Economics Services, South Africa.

Alves A, Correia A, Luque J, Phillips A (2004). *Botryosphaeria corticola,* sp. nov. on *Quercus* species, with notes and description of *Botryosphaeria stevensii* and its anamorph, *Diplodia mutila*. *Mycologia* **96**: 598–613.

Arx JA von, Müller E (1954). Die Gattungen der amerosporen Pyrenomyceten. *Beiträge zur Kryptogamenflora der Schweiz* **11(1)**: 1−434.

Barnard EL, Geary T, English JT, Gilly SP (1987). Basal cankers and coppice failure of *Eucalyptus grandis* in Florida. *Plant Disease* **71**: 358−361.

Barr ME (1972). Preliminary studies on the *Dothideales* in temperate North America. *Contributions from the University of Michigan Herbarium* **9**: 523−638.

Burgess T, Wingfield MJ (2001). Impact of fungal pathogens in natural forests ecosystems: A focus on *Eucalyptus*. In *Microorganisms in plant conservation and*

biodiversity (Sivasithamparam K, Dixon KW, eds). Kluwer Academic press, The Netherlands: 285−306.

Burgess T, Wingfield MJ, Wingfield BD (2003). Development and characterization of microsatellite loci for tropical tree pathogen *Botryosphaeria rhodina*. *Molecular Ecology Notes* **3**: 91−94.

Cilliers A (1993). A review of *Lasiodiplodia theobromae* with particular reference to its occurrence on coniferous seeds. *South African Forestry Journal* **166**: 47−52.

Coutinho TA, Wingfield MJ, Alfenas AC, Crous PW (1998). *Eucalyptus* rust: A disease with the potential for serious international implications. *Plant Disease* **82**: 819−825.

Crous PW, Knox-Devies PS, Wingfield MJ (1989). Newly recorded foliage fungi of *Eucalyptus* spp. in South Africa. *Phytophylactica* **21**: 85−88.

Crous PW, Palm ME (1999). Reassessment of the anamorphs genera *Botryodiplodia*, *Dothiorella* and *Fusicoccum*. *Sydowia* **51**: 167−175.

Crous PW, Phillips AJL, Baxter AP (2000). *Phytopathogenic Fungi from South Africa*. University of Stellenbosch, Department of Plant Pathology Press, University of Stellenbosch Printers, Stellenbosch, South Africa.

Crous PW, Swart WJ (1995). Foliicolous fungi of *Eucalyptus* spp. from Eastern Madagascar: implications for South Africa. *South African Forestry Journal* **172**: 1−6.

Darvas JM (1991). *Dothiorella dominicana*, a new mango pathogen in South Africa. *Phytophylactica* **23**: 295−298.

Davison EM, Tay FCS (1983). Twig branch and upper trunk cankers of *Eucalyptus marginata*. *Plant Disease* **67**: 1285−1287.

Denman S, Crous PW, Groenewald JZ, Slippers B, Wingfield BD, Wingfield MJ (2003). Circumscription of *Botryosphaeria* species associated with *Proteaceae* based on morphology and DNA sequence data. *Mycologia* **95**: 294−307.

Denman S, Crous PW, Taylor JE, Kang JC, Pascoe I, Wingfield MJ (2000). An overview of the taxonomic history of *Botryosphaeria* and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. *Studies in Mycology* **45**: 129−140.

Denman S, Crous PW, Wingfield MJ (1999). A taxonomic reassessment of *Phyllachora proteae*, a leaf pathogen of *Proteaceae*. *Mycologia* **91**: 510−516.

De Wet J, Burgess T, Slippers B, Preisig O, Wingfield BD, Wingfield MJ (2003). Multiple gene genealogies and microsatellite markers reflect relationships between morphotypes of *Sphaeropsis sapinea* and distinguish a new species of *Diplodia*. *Mycological Research* **107**: 557−566.

Farr DF, Bills GF, Chamuris GP, Rossman AY (1989). *Fungi on Plant and Plant Products in the United States*. The American Phytopathological Spciety Press, Minnesota, USA.

Felsenstein J (1985). Confidence intervals on phylogenetics: an approach using bootstrap. *Evolution* **39**: 783−791.

Fisher PJ, Petrini O, Sutton BC (1993). A comparative study of fungal endophytes in leaves, xylem and bark of *Eucalyptus nitens* in Australia and England. *Sydowia* **45**: 1−14.

Gezahgne A (2003). Diseases of exotic plantation forestry trees in Ethioipia. Ph. D. dissertation. Department of Microbiology and Plant Pathology, University of Pretoria, South Africa.

Heath RN (2003) Studies to consider the possible origin of three canker pathogens of *Eucalyptus* in South Africa. M.Sc. thesis. Department of Microbiology and Plant Pathology, University of Pretoria, South Africa.

Jacobs R (2002). Characterisation of *Botryosphaeria* species from mango in South Africa. M.Sc. thesis. Department of Microbiology and Plant Pathology, University of Pretoria, South Africa.

Jacobs KA, Rehner SA (1998). Comparison of cultural and morphological characters and ITS sequences in anamorphs of *Botryosphaeria* and related taxa. *Mycologia* **90**: 601−610.

Johnson LAS, Briggs BG (1981). Three old southern families - *Myrtaceae*, *Proteaceae* and *Restionaceae*. In *Ecological Biogeography of Australia*. (Keast A. eds). W Junk, The Hague: 427−464.

Johnson GI, Mead AJ, Cooke AW, Dean JR (1992). Mango stem end rot pathogens-Fruit infections by endophytic colonisation of the inflorescence and pedicel. *Annals of Applied Biology* **120**: 225−234.

Laundon GF (1973). *Botryosphaeria obtusa*, *B. stevensii*, and *Otthia spiraeae* in New Zealand. *Transactions of the British Mycological Society* **61**: 369−374.

Masuka A (1990). A new canker of *Eucalyptus grandis* Hill ex Maiden in Zimbabwe. *Commonwealth Forestry Review* **69**: 195−200.

Nakabonge G (2002). Diseases associated with plantation forestry in Uganda. M.Sc. thesis. Department of Microbiology and Plant Pathology, University of Pretoria, South Africa.

Old KM, Davison EM (2000). Canker diseases of Eucalypts. In *Diseases and Pathogens of* Eucalypts (Keane PJ, Kile GA, Podger FD, Brown BN, eds). CSIRO Publishing, Australia: 241−257.

Pavlic D, Slippers B, Coutinho TA, Gryzenhout M, Wingfield MJ (2004). *Lasiodiplodia gonubiensis* sp. nov., a new *Botryosphaeria* anamorph from native *Syzygium cordatum* in South Africa. *Studies in Mycology* **50**: 313−322.

Palgrave KC (1977). *Trees of Southern Africa*. 1st edn. C. Struik publishers, South Africa.

Pennycook SR, Samuels GJ (1985). *Botryosphaeria* and *Fusicoccum* species associated with ripe fruit rot of *Actinidia deliciosa* (Kiwifruit) in New Zealand. *Mycotaxon* **24**: 445−458.

Phillips AJL, Fonseca F, Povoa V, Castilho R, Nolasco G (2002). A reassessment of the anamorphic fungus *Fusicoccum luteum* and description of its teleomorph *Botryosphaeria lutea* sp. nov. *Sydowia* **54**: 59−77.

Punithalingam E (1976). *Botryodiplodia theobromae.* CMI descriptions of pathogenic fungi and bacteria. No. 519. Commonwealth Mycological Institute, Kew, Surrey, England.

Raeder U, Broda P (1985). Rapid preparation of DNA from filamentous fungi. *Letters in Applied Microbiology* **1**: 17−20.

Rayner RW (1970). *A mycological colour chart*. CMI and British Mycological Society, Kew, Surrey, UK.

Rodas PCA (2003). Diseases of *Eucalyptus* in Colombia. M.Sc. thesis. Department of Microbiology and Plant Pathology, University of Pretoria, South Africa.

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

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

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

Shearer BL (1994). The major plant pathogens occurring in native ecosystems of south-western Australia. *Journal of the Royal Society of Western Australia* **77**: 113−122.

Shearer BL, Tippett JT, Bartle JR (1987). *Botryosphaeria ribis* infection associated with death of *Eucalyptus radiata* in species selection trials. *Plant Disease* **71**: 140−145.

Shoemaker RA (1964). Conidial states of some *Botryosphaeria* species on *Vitis* and *Quercus*. *Canadian Journal of Botany* **42**: 1297−1301.

Slippers B (2003). Taxonomy, phylogeny- and ecology of botryosphaeriaceous fungi occurring on various woody hosts. Ph.D. dissertation. Department of Microbiology and Plant Pathology, University of Pretoria, South Africa.

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

Slippers B, Fourie G, Crous PW, Coutinho TA, Wingfield BD, Wingfield MJ (2004b). Multiple gene sequences delimit *Botryosphaeria australis* sp. nov. from *B. lutea*. *Mycologia* **96**: 1028−1039.

Slippers B, Johnson GI, Crous PW, Coutinho TA, Wingfield BD, Wingfield MJ (2004c). Phylogenetic and morphological re-evalution of the *Botryosphaeria* anamorphs causing diseases of *Mangifera indica* in Australia. *Mycologia* (in press).

Smith DR, Stanosz GR (2001). Molecular and morphological differentiation of *Botryosphaeria dothidea* (anamorph *Fusicoccum aesculi*) from some other fungi with *Fusicoccum* anamorphs. *Mycologia* **93**: 505−515.

Smith H, Crous PW, Wingfield MJ, Coutinho TA, Wingfield BD (2001a). *Botryosphaeria eucalyptorum sp. nov.*, a new species in the *B. dothidea*-complex on *Eucalyptus* in South Africa. *Mycologia* **93**: 277−285.

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

Smith H, Wingfield MJ, Crous PW, Coutinho TA (1996a). *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. *South African Journal of Botany* **62**: 86−88.

Smith H, Wingfield MJ, Petrini O (1996b). *Botryosphaeria dothidea* endophytic in *Eucalyptus grandis* and *Eucalyptus nitens* in South Africa. *Forest Ecology and Management* **89**: 189−195.

Sutton BC (1980). The Coelomycetes, Fungi imperfecti with pycnidia, acervuli and stromata. Commonwealth Mycological Institute, Kew, Surrey, England.

Sutton BC, Dyco BJ (1989). Revision of *Hendersonula*. *Mycologycal Research* **93(4)**: 466−488.

Swofford DL (1999). PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland, Massachusetts: Sinauer Associates.

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

White TJ, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols: a guide to methods and applications.* (Innis MA, Gelfand DH, Snisky JJ, White TJ, eds) Academic Press, San Diego: 315−322.

Wingfield MJ (1999). Pathogens in exotic plantations forestry. *International Forestry Review* **1**: 163−168.

Zhou S, Smith DR, Stanosz GR (2001). Differentiation of *Botryosphaeria* species and related anamorphic fungi using inter simple or short sequence repeat (ISSR) fingerprinting. *Mycological Research* **105**: 919−926.

Zhou S, Stanosz GR (2001a). Relationships among *Botryosphaeria* species and associated anamorphic fungi inferred from the analyses of ITS and 5.8S rDNA sequences. *Mycologia* **93**: 516−527.

Zhou S, Stanosz GR (2001b). Primers for amplification of mt SSU rDNA, and a phylogenetic study of *Botryosphaeria* and associated anamorphic fungi. *Mycological Research* **105**: 1033−1044.

Table 1. Isolates of *Botryosphaeria*, *Guignardia* and *Mycosphaerella* species considered in the phylogenetic study.

Culture no.[1]	Other no.[1]	Identity[2]	Host	Location	Isolator	GenBank ITS
CMW 7772	107	*Botryosphaeria ribis*	*Ribis* sp.	New York	B. Slippers & G.Hudler	AY236935
CMW 7054	CBS 121	*B. ribis* (chromagena)	*R. rubrum*	New York	N.E. Stevens	AF241177
CMW 14011	**BOT 2626**	***B. ribis***	***Syzygium cordatum***	**Sodwana bay, S. Africa**	**D. Pavlic**	
CMW 14012	**BOT 2627**	***B. ribis***	***S. cordatum***	**Sodwana bay, S. Africa**	**D. Pavlic**	
CMW 13990	**BOT 2605**	***B. ribis***	***S. cordatum***	**Sodwana bay, S. Africa**	**D. Pavlic**	
CMW 13991	**BOT 2606**	***B. ribis***	***S. cordatum***	**Sodwana bay, S. Africa**	**D. Pavlic**	
CMW 14016	**BOT 2667**	***B. ribis***	***S. cordatum***	**Kwambonambi, S. Africa**	**D. Pavlic**	
CMW 14031	**BOT 2682**	***B. ribis***	***S. cordatum***	**Kwambonambi, S. Africa**	**D. Pavlic**	
CMW 14025	**BOT 2676**	***B. ribis***	***S. cordatum***	**Kwambonambi, S. Africa**	**D. Pavlic**	
CMW 13992	**BOT 2607**	***B. ribis***	***S. cordatum***	**Sodwana bay, S. Africa**	**D. Pavlic**	
CMW 9081	ICMP8003	*Botryosphaeria parva*	*Populus nigra*	New Zealand	G.J. Samuels	AY236943
CMW 9078	ICMP7925	*B. parva*	*Actinidia deliciosa*	New Zealand	S.R. Pennycook	AY236940
CMW 994	ATCC58189	*B. parva*	*Malus Sylvestris*	New Zealand	G.J. Samuels	AF243395
CMW 9071	160	*B. parva*	*Ribes* sp.	Australia	M.J. Wingfield	AY236938
CMW 10122	BOT21	*B. parva*	*Eucalyptus grandis*	Mpumalanga, S. Africa	H. Smith	AF283681
CMW 14030	**BOT 2681**	***B. parva***	***S. cordatum***	**Kwambonambi, S. Africa**	**D. Pavlic**	
CMW 14029	**BOT 2680**	***B. parva***	***S. cordatum***	**Kwambonambi, S. Africa**	**D. Pavlic**	
CMW 7801	BRIP23396	*Fusicoccum mangiferum*	*Mangifera indica*	Australia	G.I. Johnson	AY615187
CMW 7024	BRIP24101	*F. mangiferum*	*M. indica*	Australia	G.I. Johnson	AY615185
CMW 13998	**BOT 2613**	***F. mangiferum***	***S. cordatum***	**Sodwana bay, S. Africa**	**D. Pavlic**	
CMW 14005	**BOT 2620**	***F. mangiferum***	***S. cordatum***	**Sodwana bay, S. Africa**	**D. Pavlic**	
CMW 14102	**BOT 2804**	***F. mangiferum***	***S. cordatum***	**Sodwana bay, S. Africa**	**D. Pavlic**	
CMW 9072		*Botryosphaeria australis*	*Acacia*sp.	Melbourne, Australia	J. Roux & D. Guest	AY339260
CMW 6837		*B. australis*	*Acacia*sp.	Batemans Bay, Australia	M.J. Wingfield	AY339262

Table 1. Continued.

CMW 1110		*B. australis*	*Widdringtonia nodiflora*	Cape province, S. Africa	W. J. Swart	AY615166
CMW 1112		*B. australis*	*W. nodiflora*	Cape province, S. Africa	W. J. Swart	AY615167
CMW 3386		*B. australis*	*Wollemia nobilis*	Queensland, Australia	M. Ivory	AY615165
CMW 14074	**BOT 2776**	***B. australis***	***S. cordatum***	**East London, S. Africa**	**D. Pavlic**	
CMW 13986	**BOT 2601**	***B. australis***	***S. cordatum***	**Sodwana bay, S. Africa**	**D. Pavlic**	
CMW 13987	**BOT 2602**	***B. australis***	***S. cordatum***	**Sodwana bay, S. Africa**	**D. Pavlic**	
CMW 14013	**BOT 2628**	***B. australis***	***S. cordatum***	**Sodwana bay, S. Africa**	**D. Pavlic**	
CMW 9076	ICMP 7818	*Botryosphaeria lutea*	*Malus domestica*	New Zealand	S.R. Pennycook	AY236946
CMW 992	KJ 93.52	*B. lutea*	*Actinidia deliciosa*	New Zealand	G.J. Samuels	AF027745
CMW 10309	CAP 002	*B. lutea*	*Vitis vinifera*	Portugal	A.J.L. Phillips	AY339258
CMW 14071	**BOT 2773**	***B. lutea***	***S. cordatum***	**East London, S. Africa**	**D. Pavlic**	
CMW 14073	**BOT 2775**	***B. lutea***	***S. cordatum***	**East London, S. Africa**	**D. Pavlic**	
CMW 10125	BOT 24	*Botryosphaeria eucalyptorum*	*E. grandis*	Mpumalanga, S. Africa	H. Smith	AF283686
CMW 11705		*B. eucalyptorum*	*E. nitens*	South Africa	B. Slippers	AY339248
CMW 9075	ICMP 8019	*Botryosphaeria dothidea*	*P. nigra*	New Zealand	G.J. Samuels	AY236950
CMW 8000		*B. dothidea*	*Prunus* sp.	Crocifisso, Switzerland	B. Slippers	AY236949
CMW 14009	**BOT 2624**	***B. dothidea***	***S. cordatum***	**Sodwana bay, S. Africa**	**D. Pavlic**	
CMW 10130	BOT 977	*Botryosphaeria rhodina*	*Vitex donniana*	Uganda	J. Roux	AY236951
CMW 9074		*B. rhodina*	*Pinus* sp.	Mexico	T. Burgess	AY236952
CMW 14114	**BOT 2917**	***B. rhodina***	***S. cordatum***	**Kwambonambi, S. Africa**	**D. Pavlic**	
CMW 14116	**BOT 2819**	***B. rhodina***	***S. cordatum***	**Kwambonambi, S. Africa**	**D. Pavlic**	
CMW 14004	**BOT 2619**	***Botryosphaeria* sp.**	***S. cordatum***	**Sodwana bay, S. Africa**	**D. Pavlic**	
CMW 14077	CBS 115812	*Lasiodiplodia gonubiensis*	*S. cordatum*	Eastern Cape, S. Africa	D. Pavlic	AY639595
CMW 14078	CBS 116355	*L. gonubiensis*	*S. cordatum*	Eastern Cape, S. Africa	D. Pavlic	AY639594
CMW 7774		*Botryosphaeria obtusa*	*Ribes* sp.	New York, U.S.A.	B. Slippers & G. Hudler	AY236953
	KJ 93.56	*B. obtusa*	Hardwood shrub	New York, U.S.A.	G.J. Samuels	AF027759

Table 1. Continued.

CMW 7060	CBS 431	*Botryosphaeria stevensii*	*Fraxinus excelsior*	Netherlands	H.A. van der Aa	AY236955
	ZS 94-6	*B. stevensii*	*Malus pumila*	New Zealand	N. Tisserat	AF243407
	CBS 112545	*Botryosphaeria corticola*	*Quercus ilex*	Spain	M.A. Sanchez & A. Trapero	AY259089
	CBS 112551	*B. corticola*	*Quercus suber*	Portugal	A. Alves	AY259101
	CBS 418.64	*Botryosphaeria tsugae*	*Tsuga heterophylla*	Canada	A. Funk	AF243405
	KJ 94.07	*Diplodia pinea*	*Pinus resinosa*	Wisconsin, U.S.A.	D.R. Smith	AF027758
CMW 3025		*Mycosphaerella africana*	*Eucalyptus viminalis*	Stellenbosch, S. Africa	P.W. Crous	AF 283690
CMW 7063	CBS 447	*Guignardia philoprina*	*Taxus baccata*	Netherlands	H.A. van der Aa	AY236956

[1]Culture collections: BOT and CMW = Tree Pathology Co-operative Programme, Forestry and Agricultural Biotechnology Institute, University of Pretoria; KJ = Jacobs & Rehner (1998); ATCC = American Type Culture Collection, Fairfax, VA, U.S.A.; BRIP = Plant Pathology Herbarium, Department of Primary Industries, Queensland, Australia; CAP = Culture collection of AJL Phillips, Lisbon, Portugal; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; ICMP = International Collection of Microorganisms from Plants, Auckland, New Zealand; ZS = Zhou & Stanosz (2001).

[2]Isolates sequenced in this study are given in bold.

Table 2. Conidial measurement of *Botryosphaeria* isolates from *Syzygium cordatum* in South Africa

Identity	CMW no.[1]	BOT no.[1]	Location	Conidial measurement[2] (µm)		
				Length	Width	L/W
Botryosphaeria ribis	13990	2605	Sodwana bay	(17.2) 17.9 (18.9)	(5.1) 5.3 (5.9)	3.4
B. ribis	13991	2606	Sodwana bay	(18.0) 19.7 (21.8)	(4.7) 5.1 (5.6)	3.9
B. ribis	13992	2607	Sodwana bay	(22.1) 23.6 (27.3)	(5.2) 5.5 (6.1)	4.2
B. ribis	13993	2608	Sodwana bay	(19.0) 20.0 (21.4)	(4.8) 5.2 (5.6)	3.8
B. ribis	13994	2609	Sodwana bay	(17.4) 18.8 (20.4)	(4.9) 5.6 (6.0)	3.3
B. ribis	13995	2610	Sodwana bay	(16.8) 19.8 (22.7)	(4.6) 5.2 (5.6)	3.8
B. ribis	13996	2611	Sodwana bay	(19.3) 20.7 (22.6)	(4.7) 5.5 (6.4)	3.8
B. ribis	13997	2612	Sodwana bay	(16.1) 19.0 (21.1)	(5.2) 5.5 (5.9)	3.5
B. ribis	14006	2621	Sodwana bay	(17.5) 20.0 (22.1)	(5.4) 5.8 (5.9)	3.4
B. ribis	14007	2622	Sodwana bay	(18.3) 19.4 (20.9)	(4.5) 5.4 (6.0)	3.6
B. ribis	14106	2808	Sodwana bay	(17.6) 20.5 (21.9)	(4.7) 5.2 (5.6)	3,9
B. ribis	14008	2623	Sodwana bay	(18.5) 20.6 (22.1)	(5.1) 5.4 (5.9)	3.8
B. ribis	14010	2625	Sodwana bay	(18.8) 19.9 (21.2)	(5.2) 5.6 (6.4)	3.5
B. ribis	14011	2626	Sodwana bay	(16.9) 18.5 (20.0)	(5.1) 5.5 (6.2)	3.4
B. ribis	14012	2627	Sodwana bay	(17.3) 19.0 (20.2)	(4.6) 5.1 (5.6)	3.7
B. ribis	14016	2667	Kwambonambi	(15.0) 18.1 (22.2)	(5.0) 5.7 (6.5)	3.2
B. ribis	14023	2674	Kwambonambi	(21.3) 23.0 (25.6)	(5.1) 6.1 (6.7)	3.8
B. ribis	14025	2676	Kwambonambi	(23.6) 24.8 (26.4)	(5.7) 6.2 (6.6)	4
B. ribis	14028	2679	Kwambonambi	(18.1) 20.6 (23.5)	(4.9) 5.2 (5.7)	3.9
B. ribis	14031	2682	Kwambonambi	(22.7) 24.6 (27.9)	(5.4) 6.2 (7.0)	4
B. ribis	14035	2686	Kwambonambi	(19.4) 21.0 (22.8)	(5.0) 5.6 (5.9)	3.7
B. ribis	14041	2692	Kwambonambi	(21.0) 22.9 (24.7)	(5.2) 5.6 (6.3)	4
B. ribis	14042	2693	Kwambonambi	(22.3) 23.9 (24.8)	(5.1) 5.5 (6.1)	4.3
B. ribis	14046	2697	Kwambonambi	(19.4) 21.0 (23.4)	(5.6) 6.1 (7.0)	3.4
B. ribis	14055	2706	Kosi bay	(18.5) 20.4 (21.8)	(4.9) 5.3 (5.9)	3.8
B. ribis	14056	2707	Kosi bay	(22.0) 23.1 (24.1)	(4.7) 5.1 (5.7)	4.5
B. ribis	14057	2708	Kosi bay	(16.4) 18.7 (20.4)	(5.2) 5.5 (6.1)	3.4
B. ribis	14058	2709	Kosi bay	(17.2) 20.3 (23.1)	(4.9) 5.4 (5.7)	3.7
B. ribis	14098	2800	Kosi bay	(19.5) 21.5 (23.1)	(4.9) 5.8 (6.3)	4.2
B. ribis	14099	2801	Kosi bay	(19.6) 21.4 (24.0)	(4.6) 5.4 (5.9)	4
B. ribis	14059	2710	Kosi bay	(18.8) 17.8 (21.7)	(5.0) 5.8 (6.8)	3.1
B. ribis	14060	2711	Kosi bay	(16.8) 18.9 (22.7)	(4.6) 5.5 (5.9)	3.4
B. ribis	14100	2802	Kosi bay	(19.2) 22.0 (24.2)	(5.1) 5.8 (6.7)	3.8
B. ribis	14061	2712	Kosi bay	(18.5) 20.3 (21.3)	(5.3) 5.8 (6.5)	3.5
B. ribis	14062	2713	Kosi bay	(17.7) 19.1 (20.3)	(4.6) 5.1 (5.9)	3.7
B. ribis	14101	2803	Kosi bay	(16.8) 19.2 (21.4)	(5.2) 5.8 (6.4)	3.3
B. ribis	14068	2719	Kosi bay	(17.9) 19.5 (21.4)	(5.2) 5.8 (6.1)	3.4
B. ribis	14047	2698	Mkuze-KZN	(17.6) 19.0 (21.0)	(5.1) 5.6 (6.2)	3.4

B. ribis	14051	2702	Mkuze-KZN	(17.8) 20.2 (22.4)	(4.7) 5.3 (5.9)	3.8
B. ribis	14054	2705	Mkuze-KZN	(20.9) 23.7 (26.2)	(5.0) 5.8 (7.1)	4
B. ribis	14096	2798	St. Johan's Port	(17.5) 18.2 (18.9)	(5.1) 5.7 (6.5)	2.9
B. ribis	14079	2781	Gonubie	(15.6) 17.7 (19.6)	(4.8) 5.7 (6.5)	3.1
B.ribis	14136	2868	Tzaneen	(22.5) 25.2 (26.9)	(5.6) 6.4 (7.4)	3.9
B.ribis	14140	2872	Tzaneen	(19.3) 22.4 (24.9)	(5.6) 6.2 (6.6)	3.6
B.ribis	14144	2876	Sabie	(19.3) 23.6 (25.5)	(4.8) 5.3 (5.7)	4.4
B.ribis	14145	2877	Sabie	(23.2) 25.1 (27.0)	(4.0) 4.8 (5.1)	5.2
B.ribis	14146	2878	Sabie	(23.0) 24.2 (25.0)	(4.7) 5.2 (6.0)	4.6
B.ribis	14147	2879	Sabie	(22.8) 24.2 (24.8)	(5.1) 5.7 (6.30	4.2
B.ribis	14148	2880	Sabie	(17.9) 21.2 (22.7)	(4.5) 5.1 (5.7)	4.2
B.ribis	14149	2881	Sabie	(19.8) 22.1 (24.0)	(4,6) 5.1 (5.8)	4.3
B.ribis	14150	2882	Sabie	(20.0) 22.0 (24.3)	(5.4) 5.5 (5.8)	4
B.ribis	14151	2883	Sabie	(23.2) 24.5 (26.1)	(4.5) 5.1 (5.8)	4.8
B.ribis	14152	2884	Sabie	(23.5) 24.9 (27.0)	(4.7) 5.3 (6.2)	4.7
B.ribis	14153	2885	Sabie	(16.0) 18.2 (20.7)	(5.4) 5.7 (6.7)	3.2
B.ribis	14154	2886	Sabie	(20.3) 21.6 (23.3)	(6.0) 6.5 (7.0)	3.3
B.ribis	14155	2887	Sabie	(19.1) 20.4 (22.3)	(5.1) 5.9 (6.2)	3.5
B.ribis	14156	2888	Sabie	(17.9) 20.0 (22.5)	(4.9) 5.5 (5.9)	3.6
Botryosphaeria parva	14018	2669	Kwambonambi	(16.5) 15.4 (17.6)	(4.2) 4.4 (5.5)	3.5
B.parva	14019	2670	Kwambonambi	(17.4) 18.2 (19.0)	(4.9) 5.2 (5.7)	3.5
B.parva	14020	2671	Kwambonambi	(14.9) 16.9 (19.4)	(4.9) 5.4 (5.9)	3.1
B.parva	14021	2672	Kwambonambi	(16.5) 17.5 (18.4)	(4.6) 5.1 (5.8)	3.4
B.parva	14022	2673	Kwambonambi	(17.2) 19.0 (20.1)	(4.8) 5.3 (5.8)	3.6
B.parva	14024	2675	Kwambonambi	(16.7) 17.7 (18.6)	(4.3) 4.8 (5.0)	3.7
B.parva	14027	2678	Kwambonambi	(16.7) 17.7 (19.9)	(4.8) 5.0 (5.3)	3.5
B.parva	14029	2680	Kwambonambi	(16.4) 17.8 (19.2)	(5.2) 5.7 (6.6)	3.1
B.parva	14030	2681	Kwambonambi	(13.7) 15.6 (17.1)	(3.4) 3.9 (5.0)	4
B.parva	14032	2683	Kwambonambi	(16.1) 17.3 (18.2)	(5.1) 5.5 (5.8)	3.1
B.parva	14036	2687	Kwambonambi	(14.8) 18.1 (19.5)	(4.4) 4.9 (5.3)	3.7
B.parva	14037	2688	Kwambonambi	(16.7) 18.7 (20.6)	(5.0) 5.6 (6.4)	3.3
B.parva	14038	2689	Kwambonambi	(17.6) 18.1 (19.1)	(5.2) 5.5 (5.8)	3.3
B.parva	14039	2690	Kwambonambi	(17.1) 18.3 (20.0)	(5.5) 5.8 (6.1)	3.1
B.parva	14040	2691	Kwambonambi	(17.0) 19.5 (21.0)	(4.5) 5.2 (5.7)	3.7
B.parva	14045	2696	Kwambonambi	(16.5) 17.7 (21.2)	(5.0) 5.6 (6.3)	3.2
B.parva	14081	2783	Pietermaritzburg	(16.1) 21.5 (24.6)	(4.7) 5.7 (6.5)	3.8
B.parva	14082	2784	Pietermaritzburg	(14.3) 16.8 (22.1)	(5.0) 5.7 (6.4)	2.9
B.parva	14084	2786	Pietermaritzburg	(15.2) 17.7 (19.8)	(4.9) 5.7 (6.1)	3.1
B.parva	14085	2787	Pietermaritzburg	(17.6) 19.6 (22.5)	(5.8) 6.4 (7.2)	3.1
B.parva	14086	2788	Pietermaritzburg	(17.2) 18.7 (20.2)	(5.4) 5.9 (6.6)	3.2

B.parva	14087	2789	Pietermaritzburg	(20.5) 23.0 (24.6)	(5.1) 5.7 (6.2)	4
B.parva	14088	2790	Pietermaritzburg	(18.5) 20.0 (22.4)	(5.5) 5.9 (6.4)	3.4
B.parva	14089	2791	Pietermaritzburg	(17.2) 18.9 (20.3)	(5.5) 6.0 (6.4)	3.1
B.parva	14090	2792	Pietermaritzburg	(17.9) 20.4 (23.2)	(4.9) 5.6 (6.3)	3.6
B.parva	14093	2795	Pietermaritzburg	(16.8) 18.5 (19.7)	(6.2) 7.1 (7.9)	2.6
B.parva	14094	2796	Pietermaritzburg	(17.4) 19.4 (21.8)	(5.4) 6.0 (6.7)	2.9
B.parva	14095	2797	Pietermaritzburg	(17.1) 18.7 (20.0)	(4.9) 5.6 (6.0)	3.3
B. parva	14097	2799	St. Johan's Port	(16.8) 18.4 (21.0)	(6.0) 6.6 (7.3)	2.8
B. parva	14080	2782	Gonubie	(14.7) 16.4 (18.8)	(4.7) 5.3 (5.8)	3.1
B.parva	14128	2860	Tzaneen	(12.4) 15.0 (20.4)	(4.5) 5.5 (6.1)	2.7
B.parva	14129	2861	Tzaneen	(16.8) 18.3 (19.8)	(5.6) 5.8 (6.1)	3.1
B.parva	14130	2862	Tzaneen	(17.2) 18.7 (20.1)	(4.8) 5.4 (6.2)	3.4
B.parva	14133	2865	Tzaneen	(19.8) 20.5 (21.6)	(4.9) 5.3 (5.6)	3.9
B.parva	14134	2866	Tzaneen	(16.7) 17.8 (18.9)	(4.7) 5.4 (6.4)	3.3
B.parva	14135	2867	Tzaneen	(17.7) 19.0 (20.5)	(5.3) 5.7 (6.3)	3.3
B.parva	14137	2869	Tzaneen	(18.5) 20.0 (21.2)	(4.7) 5.3 (5.9)	3.8
B.parva	14138	2870	Tzaneen	(18.5) 19.5 (20.6)	(4.9) 5.6 (6.1)	3.5
B.parva	14139	2871	Tzaneen	(14.8) 15.6 (16.3)	(5.0) 5.4 (6.0)	2.9
B.parva	14141	2873	Tzaneen	(16.5) 17.7 (19.0)	(4.9) 5.5 (5.9)	3.2
B.parva	14142	2874	Palaborwa	(15.4) 17.7 (19.3)	(4.5) 5.0 (5.7)	3.5
B.parva	14143	2875	Palaborwa	(12.9) 15.0 (16.1)	(5.3) 5.7 (6.2)	2.6
Fusicoccum mangiferum	14102	2804	Sodwana bay	(13.5) 14.0 (14.9)	(5.3) 5.9 (6.9)	2.4
F. mangiferum	13998	2613	Sodwana bay	(12.1) 13.3 (14.7)	(5.5) 6.2 (7.2)	2.1
F. mangiferum	13999	2614	Sodwana bay	(14.1) 14.9 (15.5)	(6.1) 6.5 (6.8)	2.3
F. mangiferum	14000	2615	Sodwana bay	(12.3) 13.9 (14.8)	(5.7) 6.4 (6.9)	2.2
F. mangiferum	14001	2616	Sodwana bay	(14.2) 15.0 (16.7)	(7.3) 7.8 (8.3)	1.9
F. mangiferum	14002	2617	Sodwana bay	(13.8) 14.9 (15.9)	(5.7) 6.1 (6.8)	2.4
F. mangiferum	14003	2618	Sodwana bay	(14.2) 15.6 (18.5)	(5.3) 5.4 (7.3)	2.9
F. mangiferum	14107	2809	Sodwana bay	(12.6) 13.9 (14.8)	(6.2) 6.8 (7.6)	2
F. mangiferum	14005	2620	Sodwana bay	(12.7) 13.6 (14.5)	(5.7) 6.2 (7.0)	2.2
F. mangiferum	14103	2805	Sodwana bay	(13.5) 14.2 (14.7)	(5.7) 6.3 (7.0)	2.2
F. mangiferum	14104	2806	Sodwana bay	(14.6) 15.2 (15.9)	(5.6) 6.1 (6.5)	2.5
F. mangiferum	14105	2807	Sodwana bay	(12.3) 13.4 (14.4)	(6.1) 6.3 (7.0)	2.1
F. mangiferum	14014	2665	Kwambonambi	(12.0) 12.7 (15.2)	(6.2) 6.0 (7.0)	2.1
F. mangiferum	14015	2666	Kwambonambi	(12.5) 14.1 (15.3)	(5.5) 6.3 (6.7)	2.3
F. mangiferum	14017	2668	Kwambonambi	(13.9) 14.6 (15.2)	(5.9) 6.5 (7.3)	2.2
F. mangiferum	14026	2677	Kwambonambi	(13.8) 14.6 (16.4)	(5.6) 6.4 (6.9)	2.3
F. mangiferum	14033	2684	Kwambonambi	(12.6) 14.0 (15.0)	(6.0) 6.4 (6.8)	2.2
F. mangiferum	14034	2685	Kwambonambi	(13.3) 14.5 (15.0)	(6.6) 7.0 (7.4)	2.1
F. mangiferum	14043	2694	Kwambonambi	(14.1) 14.6 (15.5)	(5.5) 6.5 (7.2)	2.2

F. mangiferum	14063	2714	Kosi bay	(14.4) 15.2 (16.3)	(5.2) 5.7 (6.2)	2.7
F. mangiferum	14064	2715	Kosi bay	(14.2) 15.0 (15.7)	(6.0) 6.3 (6.7)	2.4
F. mangiferum	14065	2716	Kosi bay	(11.9) 14.1 (17.3)	(5.6) 6.3 (7.1)	2.2
F. mangiferum	14066	2717	Kosi bay	(11.9) 12.9 (13.9)	(5.8) 6.4 (6.9)	2
F. mangiferum	14067	2718	Kosi bay	(13.2) 13.7 (14.7)	(5.9) 6.2 (7.0)	2.2
F. mangiferum	14069	2720	Kosi bay	(12.8) 14.0 (15.1)	(5.9) 6.3 (6.7)	2.2
F. mangiferum	14048	2699	Mkuze-KZN	(13.8) 14.7 (15.7)	(5.5) 5.8 (6.6)	2.5
F. mangiferum	14049	2700	Mkuze-KZN	(13.8) 14.5 (16.0)	(6.2) 6.7 (8.3)	2.2
F. mangiferum	14050	2701	Mkuze-KZN	(12.0) 13.5 (15.0)	(6.1) 6.4 (6.7)	2.1
F. mangiferum	14052	2703	Mkuze-KZN	(14.0) 14.7 (15.6)	(5.8) 6.2 (6.8)	2.4
F. mangiferum	14053	2704	Mkuze-KZN	(12.6) 12.8 (14.4)	(6.3) 6.9 (7.6)	2
Botryosphaeria australis	13986	2601	Sodwana bay	(19.0) 20.2 (22.5)	(4.0) 5.1 (6.0)	3.9
B.australis	13987	2602	Sodwana bay	(16.0) 19.2 (20.4)	(4.8) 5.2 (5.7)	3.7
B.australis	13988	2603	Sodwana bay	(16.2) 18.5 (20.7)	(4.8) 5.2 (5.5)	3.6
B. australis	14013	2628	Sodwana bay	(14.0) 15.8 (20.3)	(5.0) 5.6 (7.2)	2.8
B.australis	14074	2776	East London	(21.6) 24.0 (25.1)	(5.6) 6.5 (7.5)	3.7
B.australis	14075	2777	East London	(22.3) 24.3 (26.3)	(6.0) 6.5 (7.0)	3.7
B.australis	14076	2778	East London	(19.0) 21.2 (23.0)	(5.4) 6.1 (6.5)	3.5
Botryosphaeria rhodina	-	2816	Kwambonambi	(22.7) 24.2 (26.6)	(12.8) 14.4 (15.3)	1.7
B.rhodina	14114	2817	Kwambonambi	(22.5) 27.8 (30.1)	(12.4) 13.4 (14.1)	2.1
B.rhodina	14115	2818	Kwambonambi	(26.3) 28.7 (31.6)	(12.8) 13.4 (14.0)	2.1
B.rhodina	14116	2819	Kwambonambi	(26.8) 28.0 (29.6)	(14.3) 15.4 (16.9)	1.8
B.rhodina	14118	2821	Kwambonambi	(22.5) 24.4 (25.5)	(13.3) 14.7 (15.6)	1.7
Botryosphaeria lutea	14070	2772	East London	(18.1) 20.3 (21.7)	(6.0) 7.0 (7.8)	2.9
B. lutea	14072	2774	East London	(18.5) 19.3 (20.5)	(5.3) 6.0 (6.6)	3.2
B.lutea	14071	2773	East London	(16.6) 18.3 (20.7)	(5.5) 6.1 (6.8)	3
B.lutea	14073	2775	East London	(17.0) 17.6 (18.8)	(5.4) 6.2 (7.0)	2.8
Lasiodiplodia gonubiensis	14077	2779	Gonubie	(29.3) 34.0 (36.4)	(16.6) 18.1 (20.7)	1.9
L. gonubiensis	14078	2780	Gonubie	(31.6) 33.8 (36.0)	(18.8) 19.7 (20.8)	1.7
Botryosphaeria dothidea	14009	2624	Sodwana bay	(26.4) 27.8 (29.4)	(4.9) 5.4 (6.1)	5.1
***Botryosphaeria* sp.**	14004	2619	Sodwana bay	(26.5) 28.9 (29.3)	(15.5) 17.2 (18.9)	1.7

[1] Culture collections: BOT and CMW = Tree Pathology Co-operative Programme, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa.

[2] Measurements in brackets are actual ranges. Values outside brackets are averages of 10 conidia.

Table 3. Distribution of eight *Botryosphaeria* spp. isolated from *S. cordatum* in this study.

	Sodwana bay	Kwambonambi	Kosi bay	Mkuze-KZN	Pietermaritzburg	St. Johan's Port	East London	Gonubie	Tzaneen	Palaborwa	Sabie
Botryosphaeria ribis	15	9	13	3	-	1	-	1	2	-	13
Botryosphaeria parva	-	16	-	-	12	1	-	1	10	2	-
Fusicoccum mangiferum	12	7	6	5	-	-	-	-	-	-	-
Botryosphaeria australis	4	-	-	-	-	-	3	-	-	-	-
Botryosphaeria rhodina	-	5	-	-	-	-	-	-	-	-	-
Botryosphaeria lutea	-	-	-	-	-	-	4	-	-	-	-
Lasiodiplodia gonubiensis	-	-	-	-	-	-	-	2	-	-	-
Botryosphaeria dothidea	1	-	-	-	-	-	-	-	-	-	-
***Botryosphaeria* sp.**	1	-	-	-	-	-	-	-	-	-	-

Fig. 1. A map of South Africa indicating the area of natural distribution of *Syzygium cordatum* (left) and sites from where isolates of *Botryosphaeria* spp. identified in this study were obtained (stars, right).

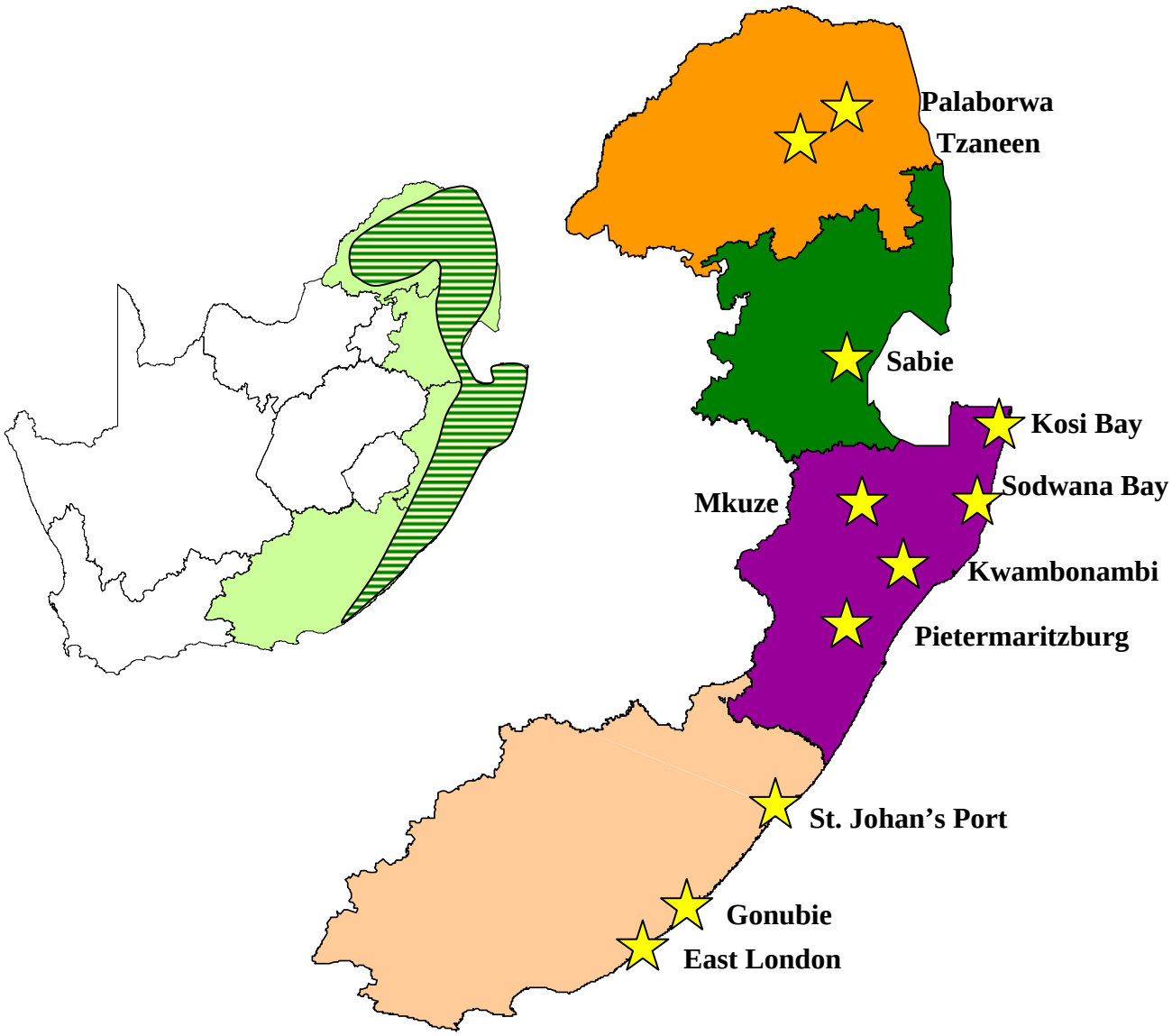

Palaborwa
Tzaneen
Sabie
Kosi Bay
Sodwana Bay
Mkuze
Kwambonambi
Pietermaritzburg
St. Johan's Port
Gonubie
East London

Fig. 2. One of 84 most parsimonious trees obtained from heuristic searches of the ITS1, 5.8S and ITS2 rDNA sequence data (tree length = 414 steps, CI = 0.717, RI = 0.921). Branch lengths, proportional to the number of steps, are indicated above the internodes, and bootstrap values (1000 replicates) below the internodes. The tree is rooted to the outgroups *Guignardia philoprina* and *Mycosphaerella africana*. Isolates sequenced in this study are given in bold.

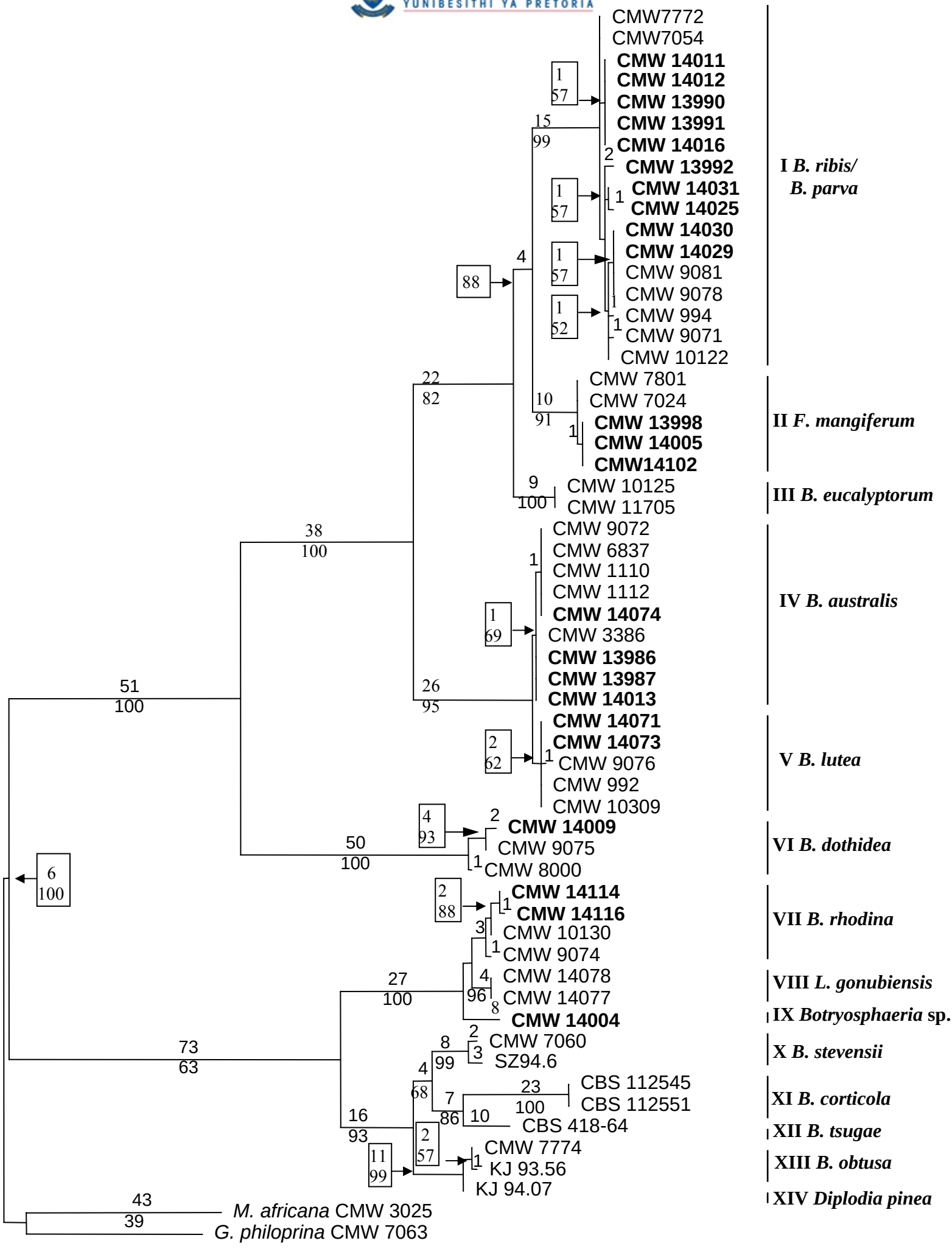

CMW7772
CMW7054
CMW 14011
CMW 14012
CMW 13990
CMW 13991
CMW 14016
CMW 13992
CMW 14031
CMW 14025
CMW 14030
CMW 14029
CMW 9081
CMW 9078
CMW 994
CMW 9071
CMW 10122
CMW 7801
CMW 7024
CMW 13998
CMW 14005
CMW14102
CMW 10125
CMW 11705
CMW 9072
CMW 6837
CMW 1110
CMW 1112
CMW 14074
CMW 3386
CMW 13986
CMW 13987
CMW 14013
CMW 14071
CMW 14073
CMW 9076
CMW 992
CMW 10309
CMW 14009
CMW 9075
CMW 8000
CMW 14114
CMW 14116
CMW 10130
CMW 9074
CMW 14078
CMW 14077
CMW 14004
CMW 7060
SZ94.6
CBS 112545
CBS 112551
CBS 418-64
CMW 7774
KJ 93.56
KJ 94.07
M. africana CMW 3025
G. philoprina CMW 7063
I B. ribis/ B. parva
II F. mangiferum
III B. eucalyptorum
IV B. australis
V B. lutea
VI B. dothidea
VII B. rhodina
VIII L. gonubiensis
IX Botryosphaeria sp.
X B. stevensii
XI B. corticola
XII B. tsugae
XIII B. obtusa
XIV Diplodia pinea
5 changes

Fig. 3. An agarose gel showing two distinctive banding patterns after restriction digestion of the PCR amplicons, obtained using primers ITS1 and ITS4, with the restriction enzyme *Cfo* I. M = 100 bp marker; Ba/l = *B. australis* / *B. lutea*; Bp/r = *B. parva* / *B. ribis*.

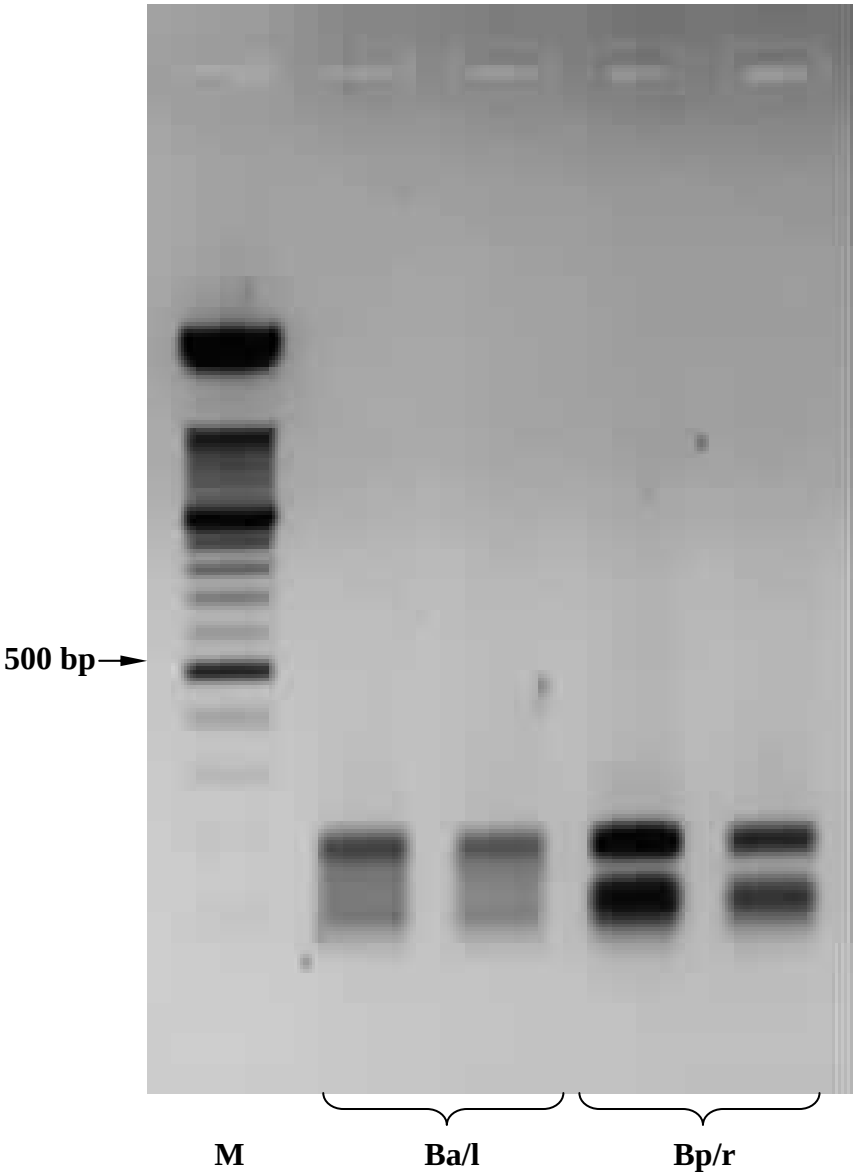
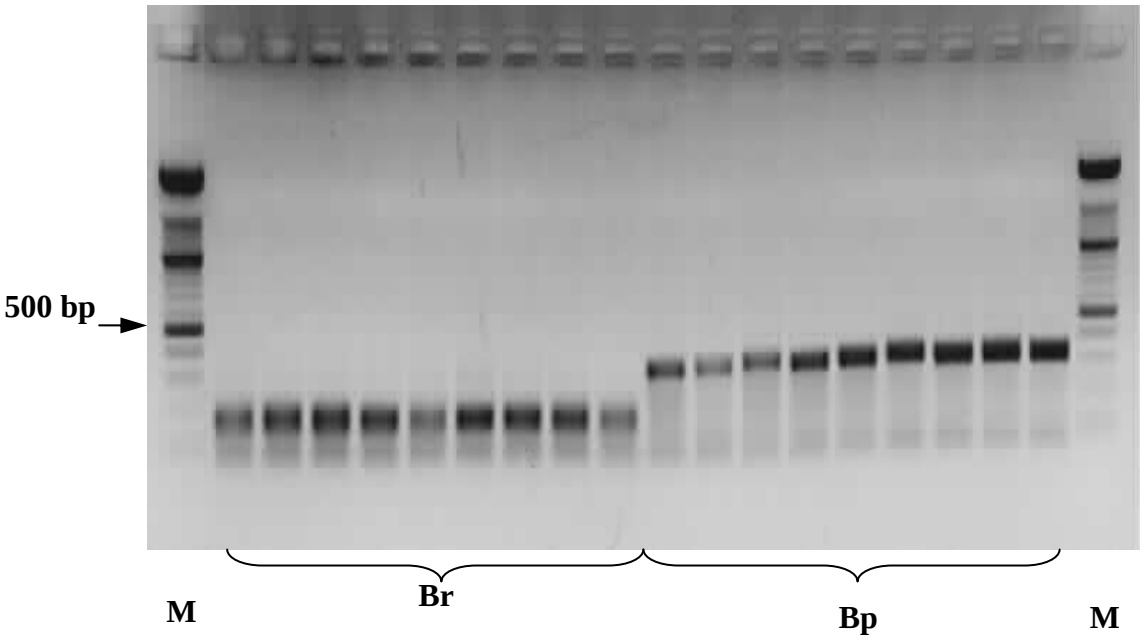
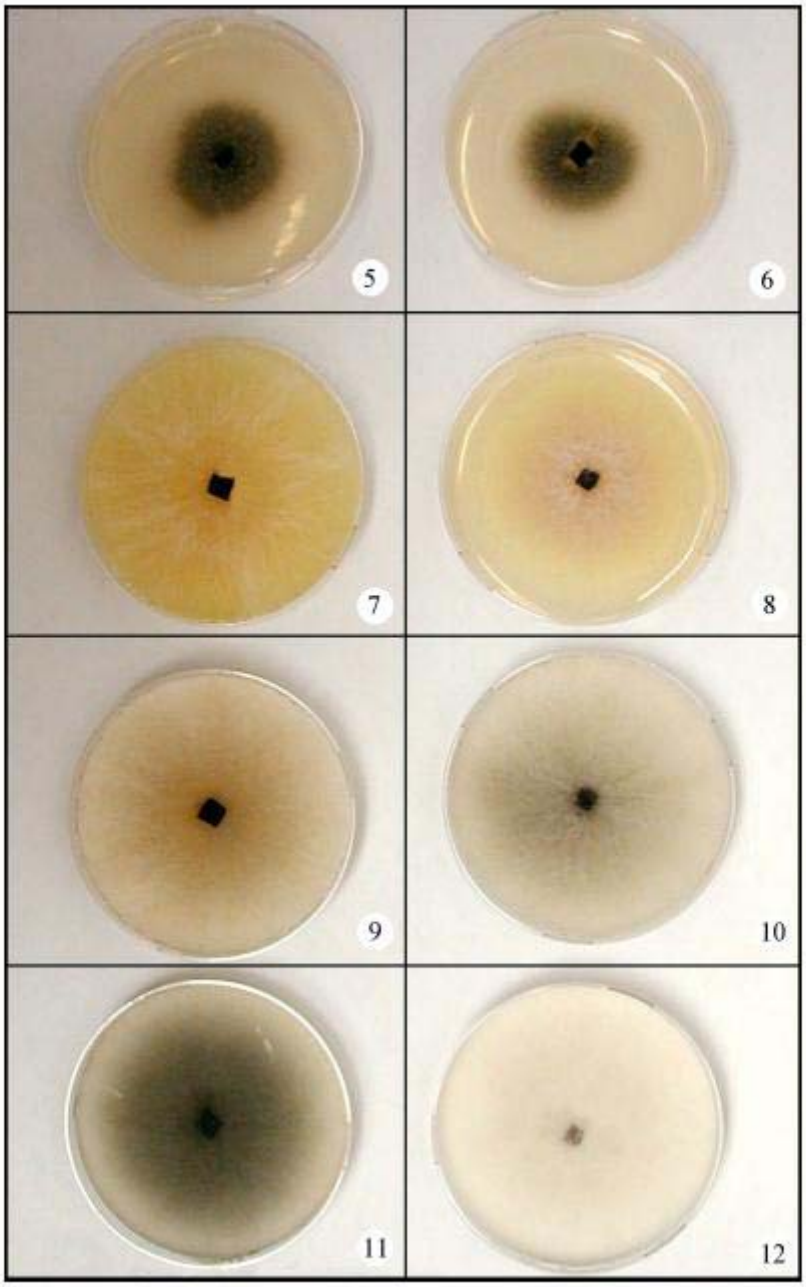

500 bp
M
Ba/l
Bp/r

Fig. 4. An agarose gel showing two distinctive banding patterns after restriction digestion of the PCR aplicons, obtained using primers BOT15 and BOT16, with the restriction enzyme *Cfo* I. M = 100 bp marker; Br = *B. ribis*; Bp = *B. parva*.


500 bp
M
Br
Bp
M

Figs 5–12. Cultural characteristics of eight *Botryosphaeria* spp. isolated from *S. cordatum* in South Africa. All isolates were grown on 2 % malt extract agar (MEA) at 25 ºC under near-UV light for four days. 5. *Fusicoccum mangiferum*. 6. *B. dothidea.* 7. *B. lutea*. 8. *B. australis*. 9. *B. ribis*. 10. *B. parva* 11. *Lasiodiplodia gonubiensis*. 12. *B. rhodina*.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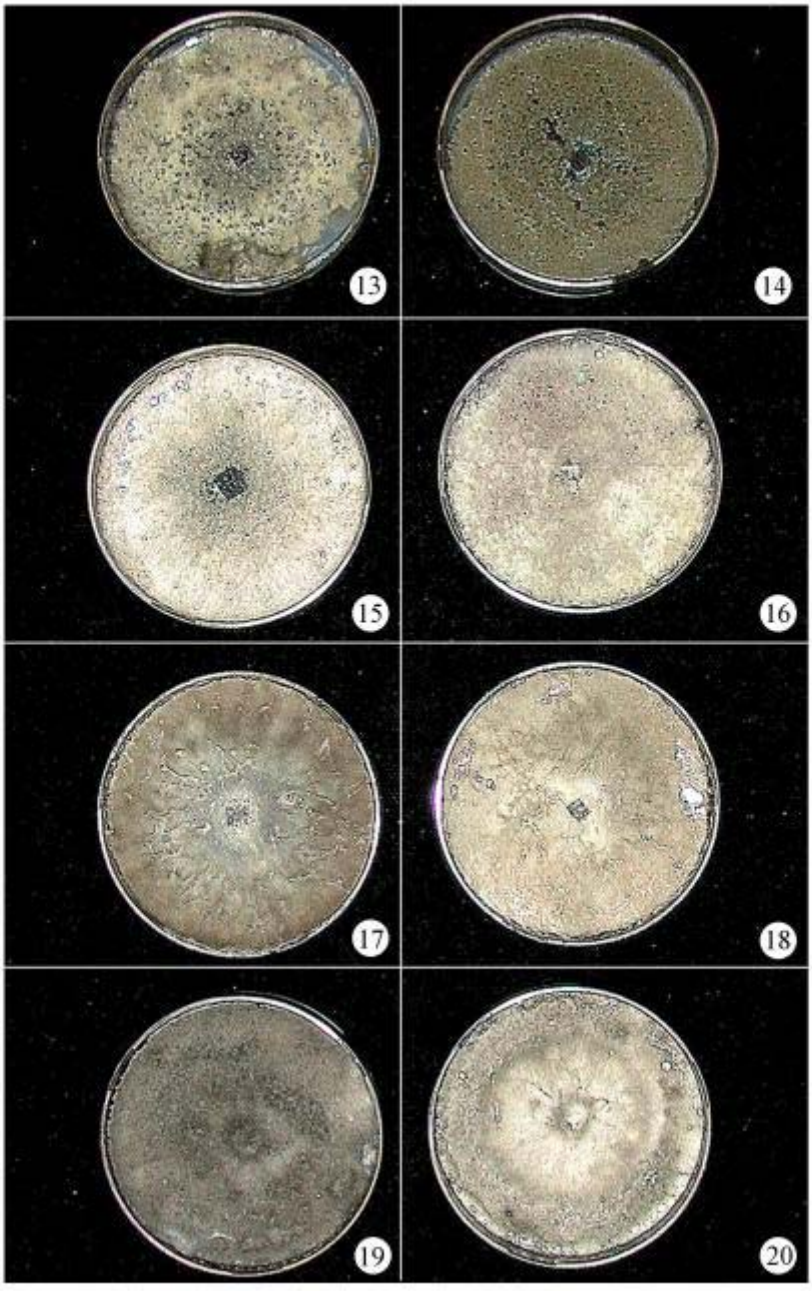
Figs 13–20. Cultural characteristics of eight *Botryosphaeria* spp. isolated from *S. cordatum* in South Africa. All isolates were grown on 2 % malt extract agar (MEA) at 25 ºC under near-UV light for 14 days. 13. *Fusicoccum mangiferum*. 14. *B. dothidea.* 15. *B. lutea*. 16. *B. australis*. 17. *B. ribis*. 18. *B. parva* 19. *Lasiodiplodia gonubiensis*. 20. *B. rhodina*.


13
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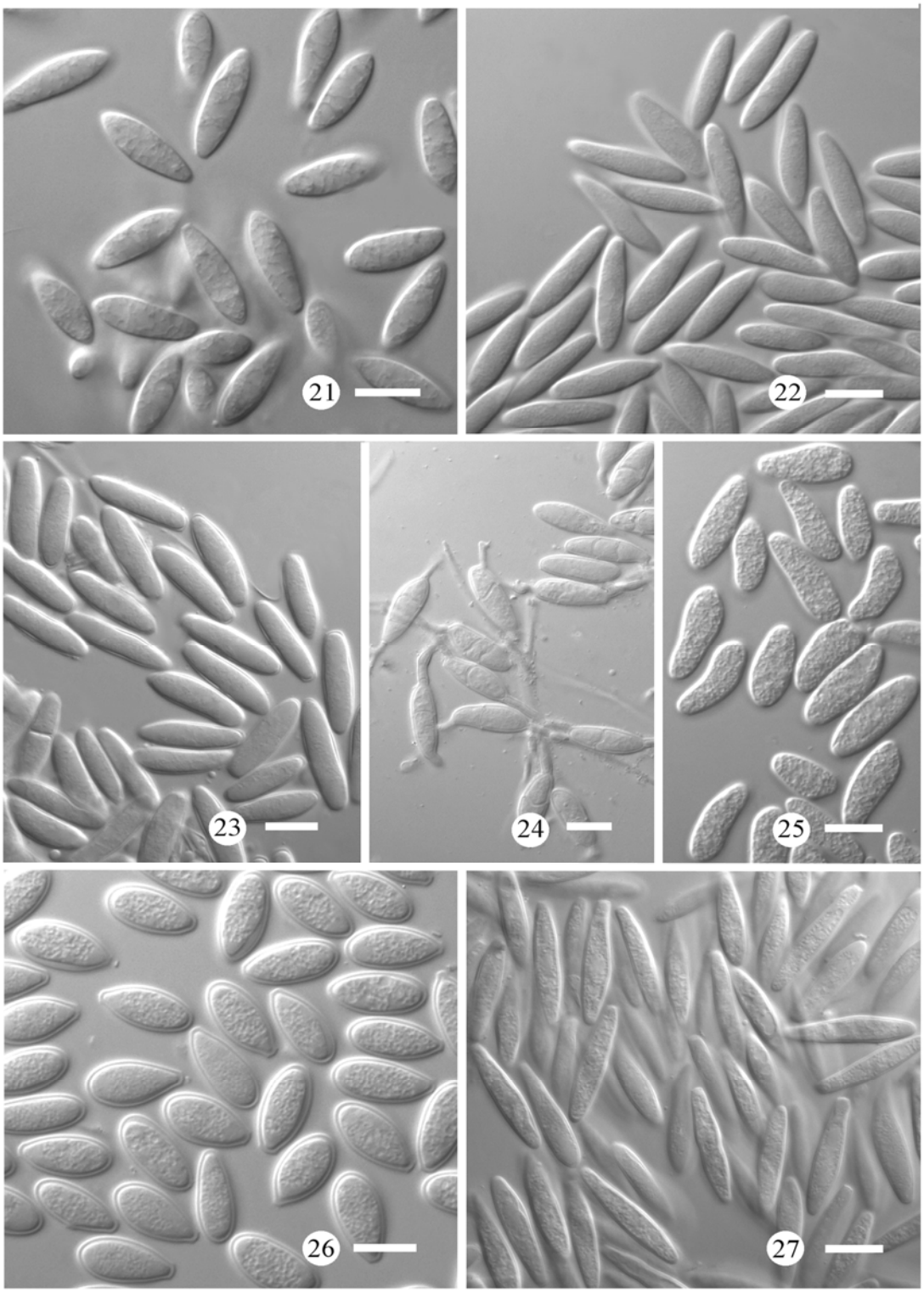
Figs 21–27. Light micrographs of conidia of six *Botryosphaeria* spp. with *Fusococcum* anamorphs. 21. *B. parva*. 22. *B. ribis*. 23. Aseptate and one septate conidia of *B. australis*. 24, 25. Aseptate and germinating one and two septate conidia of *B. lutea*. 26. *Fusicoccum mangiferum*. 27. *B. dothidea.* Bars = 10 µm.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27

Figs 28–29. Light micrographs of conidia of two *Botryosphaeria* spp. with *Lasiodiplodia* anamorphs. 28. *Lasiodiplodia gonubiensis*. 29. *B. rhodina* (anamorphs *Lasiodiplodia theobromae*). Bars = 10 μm.


28

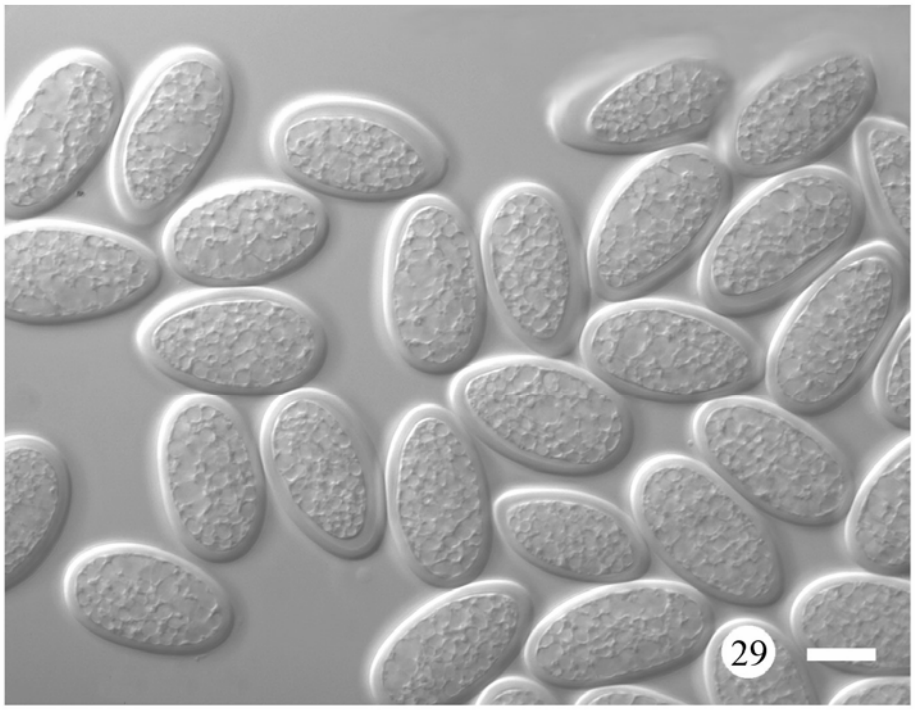
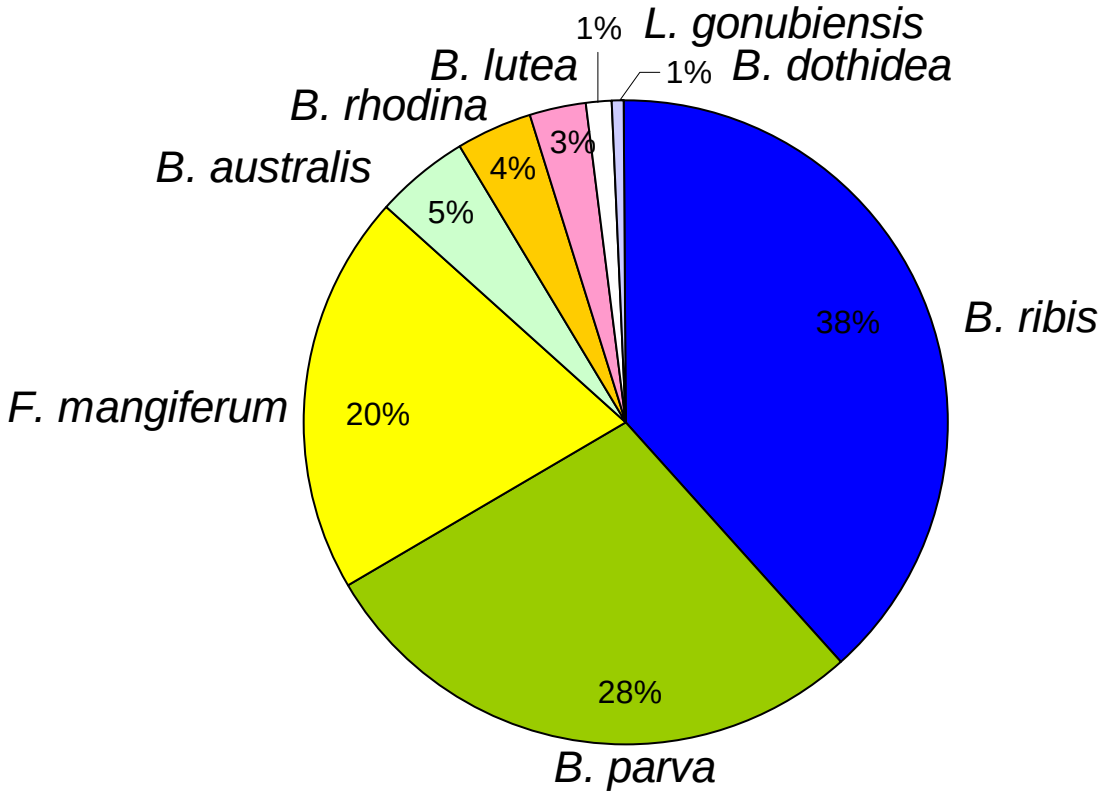

29

Fig. 30. Pie chart presenting the percentage of the each *Botryosphaeria* spp. in the total number of isolates obtained from *S. cordatum* in South Africa.


1%
L. gonubiensis
B. lutea
1%
B. dothidea
B. rhodina
3%
B. australis
4%
5%
38%
B. ribis
F. mangiferum
20%
28%
B. parva

Chapter 4

Pathogenicity of *Botryosphaeria* spp. from native *Syzygium cordatum* on *Eucalyptus* and *S. cordatum*

ABSTRACT

Botryosphaeria spp. are canker and die-back pathogens on many economically important plants including *Eucalyptus*. A recent study has revealed eight *Botryosphaeria* spp. on the native *Eucalyptus* relative, *Syzygium cordatum,* in South Africa. These included *B. parva, B. ribis, B. dothidea, B. lutea, B. australis, B. rhodina, Fusicoccum mangiferum* and *Lasiodiplodia gonubiensis.* Some of these species, such as *B. dothidea, B. parva* and *B. rhodina,* are well known pathogens on *Eucalyptus* worldwide and have also been reported from *Eucalyptus* in South Africa. To investigate the potential threat of these species to *Eucalyptus,* one strain of *B. dothidea* and two strains of each of the other seven species were inoculated into *Eucalyptus* clone GC-540 in greenhouse trials. The same strains were also used for inoculations on *S. cordatum* saplings, under similar conditions, to determine their pathogenicity to this native host. All the isolates were more pathogenic on *Eucalyptus* than on *S. cordatum*. The most pathogenic species to *Eucalyptus* were *B. rhodina, B. ribis* and *B. lutea*, while *F. mangiferum* and *B. ribis* were the most pathogenic to *S. cordatum*. *Botryosphaeria dothidea* and *Lasiodiplodia gonubiensis* were the least pathogenic on both hosts. These results confirm that *Botryosphaeria* spp. from native hosts are potential pathogens of introduced *Eucalyptus.*

INTRODUCTION

Species of *Botryosphaeria* Ces. & De Not. (*Dothideales*) are common and widely distributed on many woody plants in tropical, subtropical and temperate regions of the world (von Arx & Müller 1954, Barr 1972). *Botryosphaeria* spp. are best known as canker and die-back pathogens of woody plants, including *Eucalyptus* (*Myrtaceae*) (Davison & Tay 1983, Webb 1983, Sharma *et al.* 1984, Shearer *et al*. 1987, Smith *et al*. 1994, Old & Davison 2000, Roux *et al*. 2000, 2001, Smith *et al*. 2001). These fungi also exist in healthy asymptomatic tissue as latent pathogens and cause disease rapidly after the onset of unfavourable environmental conditions (Smith *et al*. 1996a,b). Disease symptoms can develop on all parts of trees and on trees of all ages (Schoeneweiss 1981, Webb 1983, Old *et al*. 1990, Smith *et al*. 1994, Roux *et al.* 2000, 2001).

Forestry companies in South Africa maintain about 1.35 million hectares of plantations of exotic trees, approximately half of which are comprised of *Eucalyptus* spp. (Anonymous 2002). The plantations are established mainly in the eastern part of the country in the same area where the closely related, *Syzygium cordatum* Hochst. (*Myrtaceae*) has a widespread natural distribution (Palgrave 1977, Anonymous 2002). *Syzigium cordatum* and *Eucalyptus* are sufficiently closely related that they have already been shown to share pathogens such as *Cryphonectria cubensis* (Wingfield 2003). This justifies a more complete understanding of the pathogens of *S. cordatum* in South Africa.

In an effort to evaluate potential pathogens of *Eucalyptus* in South Africa, eight *Botryosphaeria* spp. have recently been recorded on native *S. cordatum*. These include *B. parva, B. ribis, B. dothidea, B. lutea, B. australis, B. rhodina, Fusicoccum mangiferum* and *Lasiodiplodia gonubiensis.* Of these species, *B. dothidea* and *B. parva*, are well known pathogens on *Eucalyptus* in South Africa. However, the pathogenicity of the other *Botryosphaeria* spp. identified on *S. cordatum* is not known either on *Eucalyptus* or on *Syzygium*. The economic importance of *Eucalyptus* and the need to protect native *Syzygium* from invasion by exotic pathogens has prompted us to evaluate the pathogenicity of *Botryosphaeria* spp. to these trees.

Isolates of *Botryosphaeria* spp. from *S. cordatum* were mostly obtained from asymptomatic twigs and leaves, but some also originated from dying twigs. Although these symptoms could have resulted from other causes, they provided anecdotal evidence that *Botryosphaeria* spp. are pathogens on *S. cordatum*. The aim of this study was to test pathogenicity of the *Botryosphaeria* spp. isolated from *S. cordatum* on both *Eucalyptus*

and *S. cordatum* and further, to compare the pathogenicity of the *Botryosphaeria* spp. on *Eucalyptus* and *S. cordatum*.

MATERIALS AND METHODS

Isolates

Fifteen isolates, representing eight *Botryosphaeria* spp. isolated from native *S. cordatum* in different geographical regions in South Africa (Chapter 3), were used in this study (Table 1). One isolate of *B. dothidea* and two isolates for each of the other seven *Botryosphaeria* spp. were randomly selected for inoculations. The isolates were grown on 2 % malt extract agar (MEA) (2 % malt extract; Biolab, Midrand, Johannesburg, R.S.A) at 25 ºC under continuous near fluorescent light for seven days prior to inoculation. All isolates are maintained in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa (Table 1).

Inoculations

Two-year-old trees of a *E. camaldulensis* clone (GC-540) and one-year-old saplings of *S. cordatum* raised from seeds taken from a single tree, were selected for the pathogenicity trials under greenhouse conditions. Trees were maintained in the greenhouse for acclimatization for three weeks prior to inoculation. The greenhouse was subjected to natural day/night conditions and a constant temperate of approximately 25 ºC. Each of the isolates was inoculated into the stems of ten trees. Ten trees were also inoculated with sterile MEA plugs to serve as controls. The 160 inoculated trees were arranged in a randomised block design. The entire trial was repeated once under the same conditions.

For inoculations, wounds were made on the stems of trees using a 6mm diam. (*Eucalyptus*) or a 4 mm diam. (*Syzygium*) cork borer to remove the bark and expose the cambium. Wounds were made between two nodes on the stems of trees approximately 250 mm (*Eucalyptus*) or 150 mm (*Syzygium*) above the soil level. Plugs of mycelium were taken from 7-day-old cultures grown on MEA using the same size cork borer. These were placed into the wounds with mycelium surface facing the cambium. Inoculated wounds were sealed with a laboratory film (Parafilm “M”, Pechiney plastic packaging, Chicago, U.S.A.) to prevent desiccation and contamination. Lesion lengths (mm) were measured six

weeks after inoculation. The fungi were re-isolated by cutting small pieces of wood from the edges of lesions and plating them on 2 % MEA at 25 ºC.

Statistical analyses

Pathogenicity for all isolates inoculated on *Eucalyptus* and *S. cordatum* was determined based on the length of lesions (mm) that developed after six weeks. Statistical analyses of the data were performed using SAS statistical software (Version 8, SAS Institute, Cary, NC). The analyses were conducted for all isolates separately on each of the hosts and also by grouping the isolates by species. The 95 % confidence limits were determined for all means based on full model analysis of variance (ANOVA). Differences between means were, therefore, considered significant at the $P \leq 0.05$ level.

RESULTS

All *Botryosphaeria* isolates tested for pathogenicity on the *E. camaldulensis* clone (GC-540) produced lesions within six weeks. No lesions developed on trees inoculated with sterile MEA plugs as controls (Fig. 1). Statistical analyses showed that the mean lesion length for the majority of isolates used in the trial differed significantly from the controls (Fig. 2). The fungi re-isolated from the lesions of inoculated trees were the same as those used for inoculations.

The length of lesions that developed on inoculated trees were significantly different for the different *Botryosphaeria* spp. (Fig. 1). The longest lesions were produced by isolates of *B. rhodina*, while the size of lesions produced by *B. dothidea* and *Lasiodiplodia gonubiensis* were only slightly longer than those of the controls (Fig. 1). The highest values for mean lesion length, calculated as an average of two isolates of each *Botryosphaeria* sp., were obtained for *B. rhodina*, *B. ribis* and *B. lutea* respectively. The lowest mean lesion length was associated with inoculations using *B. dothidea* (Fig. 4).

The mean lesion lengths for different strains of the same *Botryosphaeria* species were not significantly different, except for the isolates of *B. rhodina* (Fig. 2). Thus *B. rhodina* isolate CMW 14116 was significantly more pathogenic than isolate CMW 14114 (Fig. 2).

All *Botryosphaeria* isolates inoculated on *Syzygium cordatum* saplings produced lesions within six weeks. The mean lesion lengths produced by some of the isolates were,

however, not significantly different from the controls (Fig. 3). Some trees inoculated as controls also developed small lesions. No *Botryosphaeria* spp. could, however, be re-isolated from the lesions that appeared to represent wound reactions. The fungi re-isolated from the lesions on inoculated trees were the same as those used for inoculations.

The longest lesions were produced by the isolates of *F. mangiferum,* while the isolates of *B. dothidea, L. gonubiensis* and *B. australis* produced lesions only slightly different to the controls. The highest values of the mean lesion length, calculated as average of two isolates for each *Botryosphaeria* sp., were obtained for *F. mangiferum, B. ribis* and *B. rhodina,* respectively, while the lowest value was obtained for *B. dothidea* (Fig. 4).

The mean lesion length obtained for one isolate of *F. mangiferum* (CMW 14034) was significantly different from that of the other isolate (CMW 14102) of the same species (Fig. 3). There were no statistically significant differences between the lesion lengths for the different isolates of the other *Botryosphaeria* spp. (Fig. 3).

Isolates of all the *Botryosphaeria* spp. used in this study, except those of *Fusicoccum mangiferum,* were more pathogenic on *Eucalyptus* than on *Syzygium* (Fig. 4). Analyses of variance showed that the interactions between mean lesion length produced by *Botryosphaeria* spp. on *Eucalyptus* and those on *Syzygium* were statistically significant ($P \leq 0.001$).

DISCUSSION

Results of this study have shown that all eight *Botryosphaeria* spp. isolated from native *Syzygium cordatum* in South Africa have the ability to infect and cause lesions on the stems of *Eucalyptus camaldulensis* and *S. cordatum* in greenhouse trials. Lesions resulting from inoculations on *S. cordatum* were, however, very small and generally not significantly different to the controls. The isolates used for inoculations were readily re-isolated from the lesions and thus can be considered as potential pathogens on both *Eucalyptus* and *Syzygium*. With the exception of *Fusicoccum mangiferum*, all the other *Botryosphaeria* spp. were more pathogenic to *Eucalyptus* than to *S. cordatum*.

Botryosphaeria rhodina (anamorph *Lasiodiplodia theobromae*) was the most pathogenic species to *Eucalyptus* in this trial. This species is a well-known pathogen of *Eucalyptus* in exotic plantations worldwide (Sharma *et al.* 1984, 1986, Roux *et al.* 2000, 2001). It has also been isolated from *Eucalyptus* in South Africa (Burgess *et al.* 2003), but

there are no reports of its pathogenicity to this tree. Although the two isolates of *B. rhodina* displayed different levels of pathogenicity in this study, both were highly pathogenic. *B. rhodina* might be considered a potentially important pathogen of *Eucalyptus* in South Africa and studies to consider its pathogenicity to different species and hybrid clones would be warranted.

Botryosphaeria ribis (anamorph *Fusicoccum ribis* Slippers, Crous & M.J. Wingf.) was the second most pathogenic *Botryosphaeria* sp. on *Eucalyptus* in this study. This fungus has been reported as a widely distributed pathogen of *Eucalyptus*, that causes a variety of symptoms including seed capsule abortion, dieback of shouts and twigs, stem and branch cankers (Davison & Tay 1983, Webb 1983, Old *et al.* 1990, Old & Davison 2000, Rodas 2003). Some of these earlier reports might, however, refer to the closely related and morphologically similar *B. parva*, which makes its distribution and status on *Eucalyptus* uncertain (Slippers *et al*. 2004a). *Botryosphaeria ribis* has not been reported as a canker or dieback pathogen on *Eucalyptus* in South Africa. This species is, however, the most widely distributed *Botryosphaeria* sp. on native *S. cordatum* in this country (Chapter 3). It appears to be only mildly pathogenic to *S. cordatum* but results of a recent study (Rodas 2003) has shown that different *Eucalyptus* clones differ substantially in their susceptibility to infection by *B. ribis*. This fungus should thus be considered as a potentially important pathogen of *Eucalyptus* in South Africa in the future.

Botryosphaeria lutea (anamorph *Fusicoccum luteum* Pennycook & Samuels) was the third most pathogenic species to *Eucalyptus* in this study. This species has not been found on *Eucalyptus* in South Africa. This fungus is, however, highly pathogenic to *Eucalyptus* and its occurrence on the related *S. cordatum* in South Africa is of concern. *B. lutea* was not the most commonly encountered *Botryosphaeria* sp. on *S. cordatum* (Chapter 3) but its presence alone provides sufficient evidence that it is well established in the country.

The presence of *Fusicoccum mangiferum* (Slippers *et al.* 2004c) on *S. cordatum* (Chapter 3) is intriguing, as it is a well-known pathogen of fruit crops and especially mango (*Mangifera indica*) (Johnson *et al.* 1992, Slippers *et al.* 2004c). It has never been found on *Eucalyptus* and also does not appear to be present on mango in South Africa (Jacobs 2002). Although it was less pathogenic than *B. rhodina, B. ribis* and *B. lutea* on *Eucalyptus,* the results have shown that it can be a pathogen of this tree. *Fusicoccum mangiferum* is the most widely distributed *Botrysphaeria* sp. on *Syzygium* trees growing in the KwaZulu-Natal Province (Chapter 3), where the majority of *Eucalyptus* plantations

have been established (Anonymous 2002). Its appearance on *Eucalyptus* spp. in plantations in the future would thus not be surprising.

Fusicoccum mangiferum was the most pathogenic *Botryosphaeria* sp. on *S. cordatum*. This was also the only species more pathogenic on *Syzygium* than on *Eucalyptus. Fusicococcum mangiferum* has not only been found on native *S. cordatum* in South Africa but it has also been found on *Tibouchina* spp. (Heath 2003) that are known to share pathogens with *Eucalyptus* (Wingfield 2003). The fact that this fungus is highly pathogenic on *S. cordatum* might imply that it has been introduced into South Africa on other woody plants. Studies focused on the origin of *F. mangiferum* are likely to yield intriguing results, relevant to commercial forestry and to the protection of natural biodiversity in South Africa.

Botryosphaeria parva (anamorph *Fusicoccum parvum* Pennycook & Samuels) was less pathogenic than *F. mangiferum*, but produced significant lesions on *Eucalyptus.* This species has recently been recognized as a pathogen on *Eucalyptus* worldwide, including South Africa (Ahumada 2002, Nakabonge 2002, Gezahgne *et al.* 2004, Slippers *et al*. 2004a). *Botryosphaeria parva* is a common and widely distributed *Botryosphaeria* sp. on *S. cordatum* in all areas of South Africa where *Eucalyptus* spp. have been planted (Chapter 3). Results of this study have confirmed the importance of this pathogen to *Eucalyptus* and also suggest that the fungus may be native to South Africa.

Botryosphaeria dothidea, L. gonubiensis and *B. australis* were only mildly pathogenic to *Eucalyptus.* While *B. dothidea* was considered to be an important canker pathogen of *Eucalyptus* in South Africa (Smith *et al.* 1994), recent studies have suggested that previous reports probably referred to *B. parva* (Slippers *et al.* 2004a). *Botryosphaeria dothidea* was seldom encountered on *Eucalyptus* in other studies on this host (Ahumada 2002, Nakabonge 2002, Rodas 2003, Gezahgne *et al.* 2004, Slippers *et al*. 2004d) and results of this study suggest that it is probably not an important pathogen of this tree.

Lasiodiplodia gonubiensis is a recently described fungus from *S. cordatum* in South Africa (Pavlic *et al.* 2004). This fungus appears to be very mildly pathogenic even though it is most closely related to the highly pathogenic *L. theobromae* (Punithalingam 1979). *Syzygium cordatum* is the only known host of *L. gonubiensis* and in contrast to *L. theobromae*, which has a very wide host range, it could be a specialized inhabitant of this native South African tree.

Botryosphaeria australis gave rise to only small lesions on *Eucalyptus*. *Botryosphaeria australis* is a recently described species (Slippers *et al.* 2004b) and the

present study is the first to consider the pathogenicity of the fungus. *Botryosphaeria australis* is phylogenetically closely related to *B. lutea* but was much less pathogenic than that latter species.

The results of this study have provided an interesting insight into the biology of *Botryosphaeria* spp. occuring on native *S. cordatum* in South Africa. Some of these fungi appear to be potentially important pathogens of *Eucalyptus* and future surveys should recognize this fact. Evidence is also emerging that *Botryosphaeria* spp. have most likely been moved between countries on woody plants or their seeds without this fact being recognized. The global distribution of *Diplodia pinea* (Desm.) J. Kickx (= *Sphaeropsis sapinea* (Fr. : Fr.) Dyko & Sutton) provides an intriguing and somewhat worrying example of this fact (Burgess & Wingfield 2002). The fact that *Botryosphaeria* spp. exist in asymptomatic plant tissues and on seed or seed parts (Fisher *et al*. 1993, Smith *et al*. 1996a, b, Burgess & Wingfield 2001) means that they can easily move between countries without detection. They are also difficult to identify and prior to the recent DNA sequence-based identifications (Denman *et al.* 2000, Zhou *et al.* 2001, Zhou & Stanosz 2001a, b, Denman *et al.* 2003, De Wet *et al.* 2003, Slippers *et al.* 2004a, b), names that have been applied to species of these fungi are most likely not reliable. An understanding of the global distribution and areas of origin of these fungi is only beginning to emerge, and additional studies such as this one on the pathogenicty of these fungi will be needed to better understand their importance.

REFERENCES

Ahumada R (2002). Diseases in commercial *Eucalyptus* plantations in Chile, with special reference to *Mycosphaerella* and *Botryosphaeria* species. M.Sc. thesis. Department of Microbiology and Plant Pathology, University of Pretoria, South Africa.

Anonymous (2002). *Commercial timber resources and roundwood processing in South Africa 2000 / 2001*. Forestry Economics Services, South Africa.

Arx JA von, Müller E (1954). Die Gattungen der amerosporen Pyrenomyceten. *Beiträge zur Kryptogamenflora der Schweiz* **11**: 1−434.

Barr ME (1972). Preliminary studies on the *Dothideales* in temperate North America. *Contributions from the University of Michigan Herbarium* **9**: 523−638.

Burgess T, Wingfield MJ (2001). Impact of fungal pathogens in natural forests ecosystems: A focus on *Eucalyptus*. In *Microorganisms in plant conservation and*

biodiversity (Sivasithamparam K, Dixon KW, eds). Kluwer Academic press, The Netherlands: 285−306.

Burgess T, Wingfield MJ (2002). Quarantine is important in restricting the spread of exotic seed-borne tree pathogens in the southern hemisphere. *International Forestry Review* **4**: 56−65.

Burgess T, Wingfield MJ, Wingfield BD (2003). Development and characterization of microsatellite loci for tropical tree pathogen *Botryosphaeria rhodina*. *Molecular Ecology Notes* **3**: 91−94.

Davison EM, Tay FCS (1983). Twig branch and upper trunk cankers of *Eucalyptus marginata*. *Plant Disease* **67**: 1285−1287.

Denman S, Crous PW, Taylor JE, Kang JC, Pascoe I, Wingfield MJ (2000). An overview of the taxonomic history of *Botryosphaeria* and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. *Studies in Mycologia* **45**: 129−140.

Denman S, Crous PW, Groenewald JZ, Slippers B, Wingfield BD, Wingfield MJ (2003). Circumscription of *Botryosphaeria* species associated with *Proteaceae* based on morphology and DNA sequence data. *Mycologia* **95**: 294−307.

De Wet J, Burgess T, Slippers B, Preisig O, Wingfield BD, Wingfield MJ (2003). Multiple gene genealogies and microsatellite markers reflect relationships between morphotypes of *Sphaeropsis sapinea* and distinguish a new species of *Diplodia*. *Mycological Research* **107**: 557−566.

Fisher PJ, Petrini O, Sutton BC (1993). A comparative study of fungal endophytes in leaves, xylem and bark of *Eucalyptus nitens* in Australia and England. *Sydowia* **45**: 1−14.

Gezahgne A, Roux J, Slippers B, Wingfield MJ (2004). Identification of the causal agent of *Botryosphaeria* stem canker in Ethiopian *Eucalyptus* plantations. *South African Journal of Botany* **70**: 241−248.

Heath RN (2003). Studies to consider the possible origin of three canker pathogens of *Eucalyptus* in South Africa. M.Sc. thesis. Department of Microbiology and Plant Pathology, University of Pretoria, South Africa.

Jacobs R (2002). Characterisation of *Botryosphaeria* species from mango in South Africa. M.Sc. thesis. Department of Microbiology and Plant Pathology, University of Pretoria, South Africa.

Johnson GI, Mead AJ, Cooke AW, Dean JR (1992). Mango stem end rot pathogens-Fruit infections by endophytic colonisation of the inflorescence and pedicel. *Annals of Applied Biology* **120**: 225−234.

Nakabonge G (2002). Diseases associated with plantation forestry in Uganda. M.Sc. thesis. Department of Microbiology and Plant Pathology, University of Pretoria, South Africa.

Old KM, Gibbs R, Craig I, Myers BJ, Yuan ZQ (1990). Effect of drought and defoliation on the susceptibility of *Eucalypts* to canker caused by *Endothia gyrosa* and *Botryosphaeria ribis*. *Australian Journal of Botany* **38**: 571−581.

Old KM, Davison EM (2000). Canker diseases of Eucalypts. In *Diseases and Pathogens of* Eucalypts (Keane PJ, Kile GA, Podger FD, Brown BN, eds). CSIRO Publishing, Australia: 241−257.

Pavlic D, Slippers B, Coutinho TA, Gryzenhout M, Wingfield MJ (2004). *Lasiodiplodia gonubiensis* sp. nov., a new *Botryosphaeria* anamorph from native *Syzygium cordatum* in South Africa. *Studies in Mycology* **50**: 313−322.

Palgrave KC (1977). *Trees of Southern Africa*. 1st edn. C. Struik publishers, South Africa.

Punithalingam E (1979). Plant diseases attributed to *Botryodiplodia theobromae* Pat. J. Cramer, Germany.

Rodas PCA (2003). Diseases of *Eucalyptus* in Colombia. M.Sc. thesis. Department of Microbiology and Plant Pathology, University of Pretoria, South Africa.

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

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

SAS Statistical Software ISBN 1-58025-494-2(1999). SAS/STAT Users Guide, Version 8. SAS Institute Inc., Cary, NC, USA.

Schoeneweiss DF (1981). The role of environmental stress in diseases of woody plants. *Plant Disease* **65**: 308−314.

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

Sharma JK (1986). Potential threat of native pathogens on exotic *Eucalypts* in Kerala. *Eucalypts in India Past, Present and Future* **72**: 367−376.

Shearer BL, Tippett JT, Bartle JR (1987). *Botryosphaeria ribis* infection associated with death of *Eucalyptus radiata* in species selection trials. *Plant Disease* **71**: 140−145.

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

Slippers B, Fourie G, Crous PW, Coutinho TA, Wingfield BD, Wingfield MJ (2004b). Multiple gene sequences delimit *Botryosphaeria australis* sp. nov. from *B. lutea*. *Mycologia* **96**: 1028−1039.

Slippers B, Johnson GI, Crous PW, Coutinho TA, Wingfield BD, Wingfield MJ (2004c). Phylogenetic and morphological re-evalution of the *Botryosphaeria* anamorphs causing diseases of *Mangifera indica* in Australia. *Mycologia* (in press).

Slippers B, Fourie G, Crous PW, Coutinho TA, Wingfield BD, Carnegie A, Wingfield MJ (2004d). Speciation and distribution of *Botryosphaeria* spp. on native and introduced *Eucalyptus* trees. *Studies in Mycology* **50**: 343−358.

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

Smith H, Wingfield MJ, Crous PW, Coutinho TA (1996a). *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. *South African Journal of Botany* **62**: 86−88.

Smith H, Wingfield MJ, Petrini O (1996b). *Botryosphaeria dothidea* endophytic in *Eucalyptus grandis* and *Eucalyptus nitens* in South Africa. *Forest Ecology and Management* **89**: 189−195.

Smith H, Crous PW, Wingfield MJ, Coutinho TA, Wingfield BD (2001). *Botryosphaeria eucalyptorum* sp. nov., a new species in the *B. dothidea*-complex on *Eucalyptus* in South Africa. *Mycologia* **93**: 277−285.

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

Wingfield MJ (2003). Daniel McAlpine Memorial Lecture. Increasing threat of disease to exotic plantation forests in the Southern Hemisphere: lessons from Cryphonectria canker. *Australian Plant Pathology* **32**: 1−7.

Zhou S, Smith DR, Stanosz GR (2001). Differentiation of *Botryosphaeria* species and related anamorphic fungi using inter simple or short sequence repeat (ISSR) fingerprinting. *Mycological Research* **105**: 919−926.

Zhou S, Stanosz GR (2001a). Relationships among *Botryosphaeria* species and associated anamorphic fungi inferred from the analyses of ITS and 5.8S rDNA sequences. *Mycologia* **93**: 516−527.

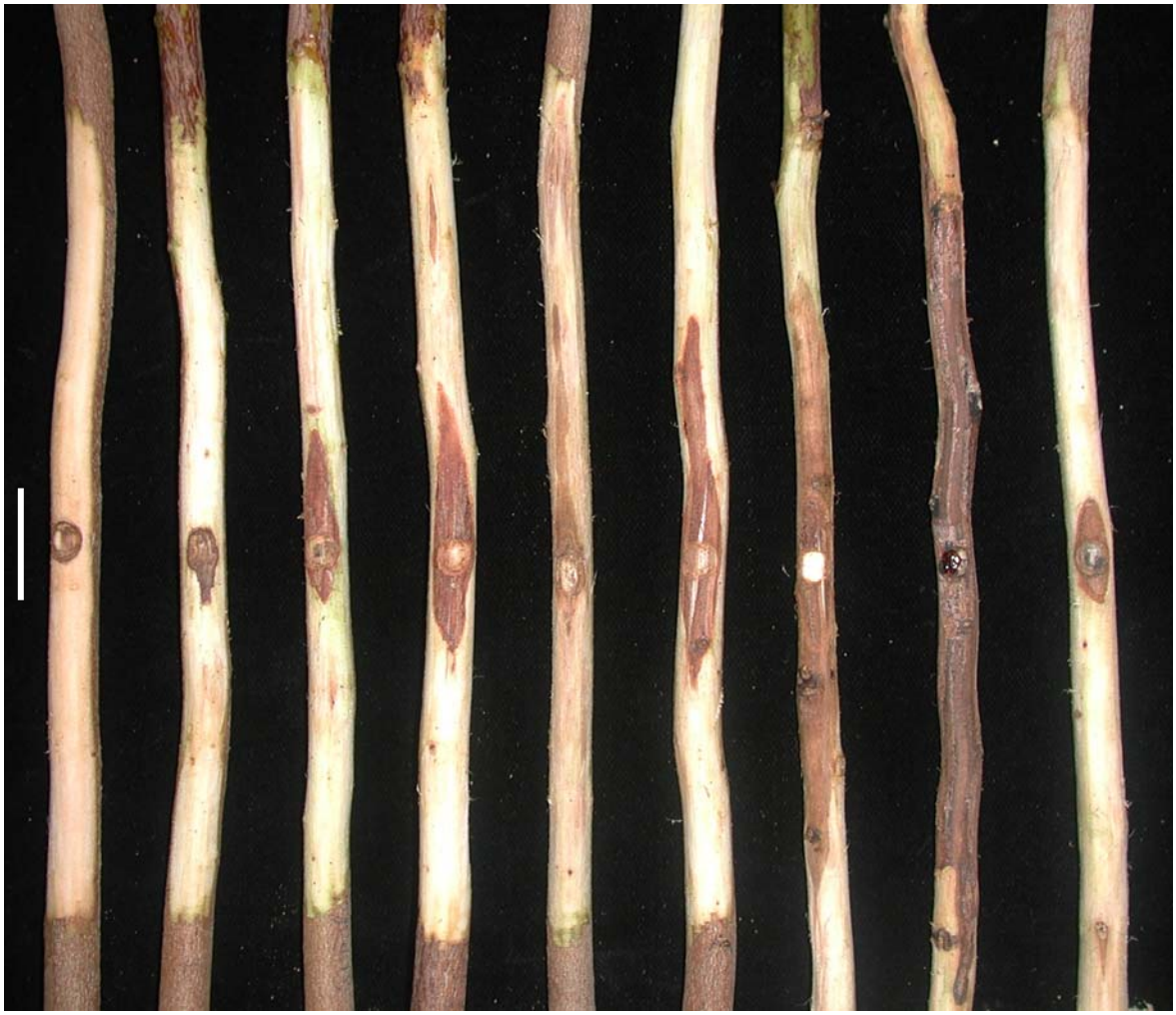
Zhou S, Stanosz GR (2001b). Primers for amplification of mt SSU rDNA, and a phylogenetic study of *Botryosphaeria* and associated anamorphic fungi. Mycologycal Research **105**: 1033−1044.

Table 1. Isolates of different *Botryosphaeria* spp.used for inoculations in this study.

Culture no.[1]	Identity	Host	Location	Isolator
CMW 14009	*Botryosphaeria dothidea*	*Syzygium cordatum*	Sodwana bay, S. Africa	D. Pavlic
CMW 14097	*B. parva*	*S. cordatum*	St. John's Port, S. Africa	D. Pavlic
CMW 14030	*B. parva*	*S. cordatum*	Kwambonambi, S. Africa	D. Pavlic
CMW 13992	*B. ribis*	*S. cordatum*	Sodwana bay, S. Africa	D. Pavlic
CMW 14031	*B. ribis*	*S. cordatum*	Kwambonambi, S. Africa	D. Pavlic
CMW 13987	*B. australis*	*S. cordatum*	Sodwana bay, S. Africa	D. Pavlic
CMW 14013	*B. australis*	*S. cordatum*	Sodwana bay, S. Africa	D. Pavlic
CMW 14116	*B. rhodina*	*S. cordatum*	Kwambonambi, S. Africa	D. Pavlic
CMW 14114	*B. rhodina*	*S. cordatum*	Kwambonambi, S. Africa	D. Pavlic
CMW 14077	*Lasiodiplodia gonubiensis*	*S. cordatum*	Eastern Cape, S. Africa	D. Pavlic
CMW 14078	*L. gonubiensis*	*S. cordatum*	Eastern Cape, S. Africa	D. Pavlic
CMW 14102	*Fusicoccum mangiferum*	*S. cordatum*	Sodwana bay, S. Africa	D. Pavlic
CMW 14034	*F. mangiferum*	*S. cordatum*	Kwambonambi, S. Africa	D. Pavlic
CMW 14071	*B. lutea*	*S. cordatum*	East London, S. Africa	D. Pavlic
CMW 14073	*B. lutea*	*S. cordatum*	East London, S. Africa	D. Pavlic

[1] Designation of culture collections: BOT and CMW = Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa.

Fig. 1. Lesions formed on stems of *E. camaldulensis* clone (GC-540) six weeks after inoculations with different *Botryosphaeria* spp. (the longest lesions for each species are shown). C = Control; Bd = *B. dothidea*; Bp = *B. parva*; Br = *B. ribis*; Bl = *B. lutea*; Ba = *B. australis*; Fm = *Fusicoccum mangiferum*; Bh = *B. rhodina*; Lg = *Lasiodiplodia gonubiensis.* Bar = 20mm.



C **Bd** **Bp** **Br** **Bl** **Ba** **Fm** **Bh** **Lg**

Fig. 2. Mean lesion lengths (mm) obtained for each isolate of different *Botryosphaeria* spp. after inoculations on *E. camaldulensis* clone (GC-540). Bars represent 95% confidence limits for each isolate. C = Control; Bd = *B. dothidea*; Bp = *B. parva*; Br = *B. ribis*; Ba = *B. australis*; Bh = *B. rhodina*; Lg = *Lasiodiplodia gonubiensis*; Fm = *Fusicoccum mangiferum*; Bl = *B. lutea*.

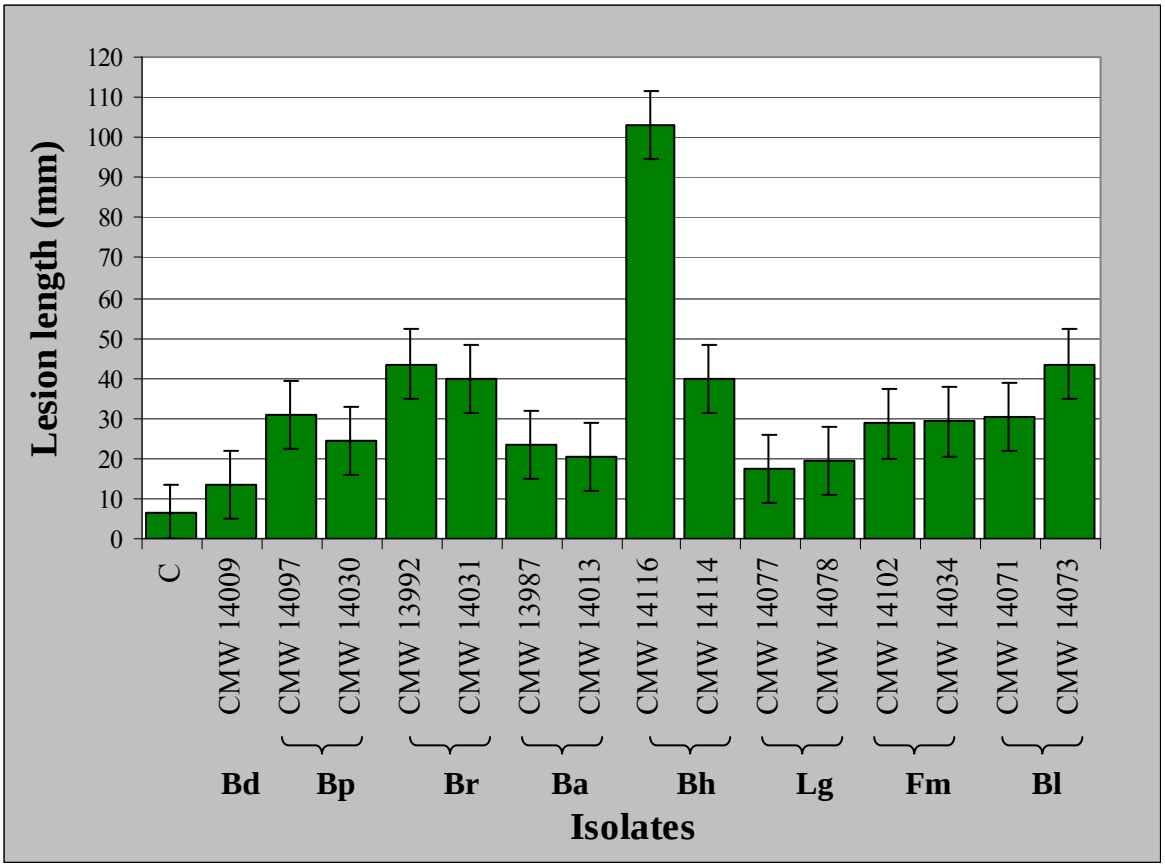

Lesion length (mm)
120
110
100
90
80
70
60
50
40
30
20
10
0
C
CMW 14009
CMW 14097
CMW 14030
CMW 13992
CMW 14031
CMW 13987
CMW 14013
CMW 14116
CMW 14114
CMW 14077
CMW 14078
CMW 14102
CMW 14034
CMW 14071
CMW 14073
Bd
Bp
Br
Ba
Bh
Lg
Fm
Bl
Isolates

Fig. 3. Mean lesion lengths (mm) obtained for each isolate of different *Botryosphaeria* spp. after inoculations on *S. cordatum*. Bars represent 95% confidence limits for each isolate. C = Control; Bd = *B. dothidea*; Bp = *B. parva*; Br = *B. ribis*; Ba = *B. australis*; Bh = *B. rhodina*; Lg = *Lasiodiplodia gonubiensis*; Fm = *Fusicoccum mangiferum*; Bl = *B. lutea*.

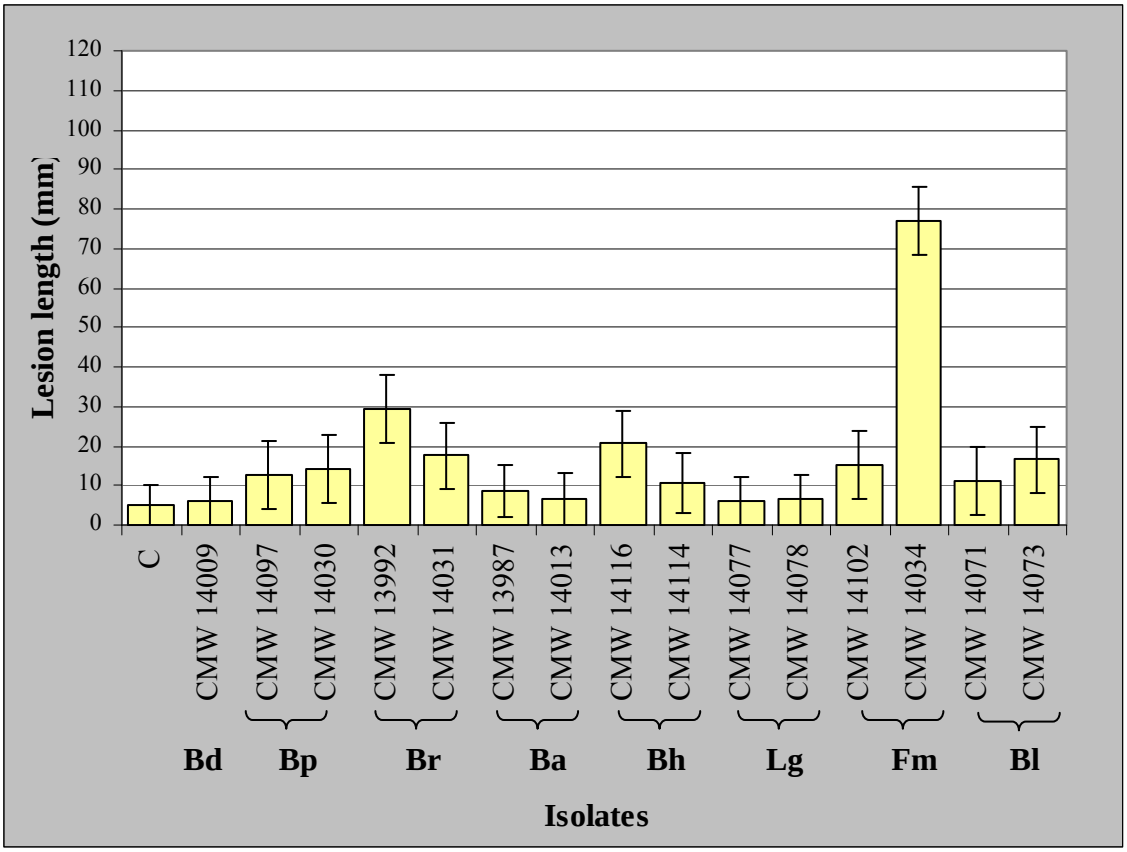
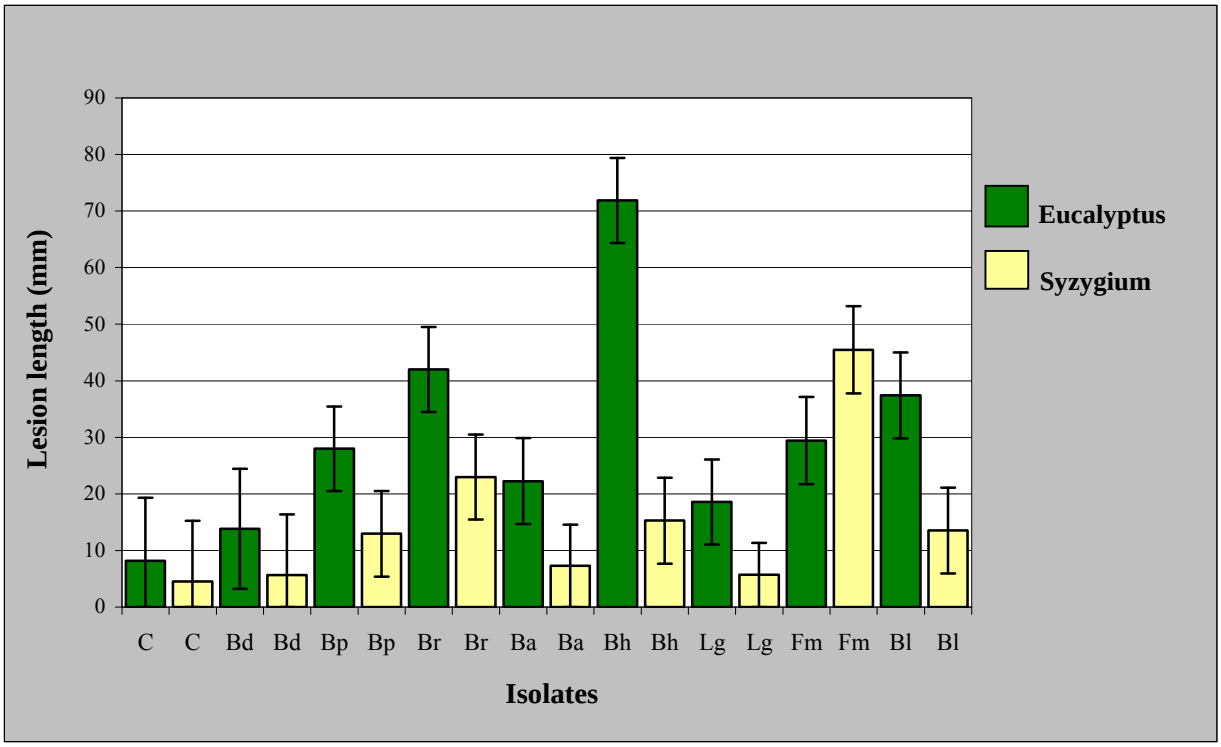

Lesion length (mm)
120
110
100
90
80
70
60
50
40
30
20
10
0
C
CMW 14009
CMW 14097
CMW 14030
CMW 13992
CMW 14031
CMW 13987
CMW 14013
CMW 14116
CMW 14114
CMW 14077
CMW 14078
CMW 14102
CMW 14034
CMW 14071
CMW 14073
Bd
Bp
Br
Ba
Bh
Lg
Fm
Bl
Isolates

Fig. 4. Mean lesion lengths (mm) obtained for one strain of *B. dothidea* and as averages of two straines for other *Botryosphaeria* spp. on *Eucalyptus* and *Syzygium*. C = Control; Bd = *B. dothidea*; Bp = *B. parva*; Br = *B. ribis*; Ba = *B. australis*; Bh = *B. rhodina*; Lg = *Lasiodiplodia gonubiensis*; Fm = *Fusicoccum mangiferum*; Bl = *B. lutea*.


Lesion length (mm)
90
80
70
60
50
40
30
20
10
0
C C Bd Bd Bp Bp Br Br Ba Ba Bh Bh Lg Lg Fm Fm Bl Bl
Isolates
Eucalyptus
Syzygium

SUMMARY

The South African commercial forest industry is almost exclusively reliant on plantations of exotic trees, of which *Eucalyptus* spp. make up almost 50 %. *Botryosphaeria* spp. are important canker pathogens in these *Eucalyptus* plantations in South Africa. However, exotic plantations and their pathogens cannot be viewed separately from the related native flora. This study showed the importance of extending our knowledge on pathogens that occur on related native and exotic hosts, and which can pose a threat by cross infection between these host groups.

In Chapter 1, a review of the literature concerning *Botryosphaeria* spp. that occur on *Eucalyptus* in its native range and exotic plantations is presented. It is clearly shown that *Botryosphaeria* spp. are important pathogens on *Eucalyptus* in exotic plantations worldwide, causing various symptoms on this host. *Botryosphaeria* spp. are also important canker pathogens in *Eucalyptus* plantations in South Africa. Traditional identification of this group of fungi, based on morphological characteristics, led to much confusion about their identity. However, in recent studies morphological characteristics were combined with DNA sequence data to distinguish and identify these fungi. Based on these data a few revisions have been done and new *Botryosphaeria* spp. were described on *Eucalyptus*. *Botryosphaeria* spp. recognised as pathogens on *Eucalyptus* in South Africa include *B. dothidea*, *B. parva* and *B. eucalyptorum*. Future studies should be focused on correct identification of *Botryosphaeria* spp. that occur on *Eucalyptus*, which is the first step towards preventing the spread of this group of pathogens and developing management strategies to control disease outbreaks.

During the study on *Botryosphaeria* spp. on *Syzygium cordatum*, isolates of two *Botryosphaeria* spp. appeared to be undescribed. One of the undescribed species was represented by only one isolate and it was not named. The other species was described as the new *Botryosphaeria* anamorph within *Lasiodiplodia*, namely *L. gonubiensis*. This species grows endophytically on native *S. cordatum* in South Africa and is the first species in *Lasiodiplodia* to be found on native trees in the country. Identification of the new species was based on conidial and cultural morphology and DNA sequence data of the rDNA internal transcribed spacers, ITS1 and ITS2. Identification and description of *L. gonubiensis* is presented and discussed in Chapter 2.

In total nine *Botryosphaeria* spp. were isolated from native *Syzygium cordatum* in South Africa. These include *B. parva*, *B. ribis*, *B. lutea*, *B. australis*, *B. rhodina*, *B. dothidea*, *Fusicoccum mangiferum*, *Lasiodiplodia gonubiensis* and an unknown *Botryosphaeria* sp. The

isolates related to *B. ribis*, *B. parva* and *F. mangiferum* were the most abundant, while only one isolate represented *B. dothidea*. Species were identified based on morphological characteristics of their anamorphs combined with ITS rDNA sequence data. Some species, such as *B. parva* and *B. ribis*, could not be distinguished based on morphology or ITS rDNA data. A PCR-RFLP fingerprinting technique was, therefore, used to distinguish isolates of these two species. Once again this technique has proven useful and reliable in identification of *Botryosphaeria* isolates, including cryptic species. However, isolates of closely related *B. lutea* and *B. australis* could not be distinguished using this technique. It could be of interest to develop PCR-RFLP identification system that could be used in identification of the latter species. Identification and characterization of *Botryosphaeria* spp. are presented in Chapter 3.

Isolates of all *Botryosphaeria* spp., obtained from native *Syzygium cordatum* in this study, caused lesions on the stems of *Eucalyptus camaldulensis* and *S. cordatum* in trials conducted under greenhouse conditions. Except for *Fusicoccum mangiferum*, all the other *Botryosphaeria* spp. were more pathogenic on *Eucalyptus* than on *S. cordatum*. The most pathogenic species on *Eucalyptus* were *B. rhodina*, *B. ribis* and *B. lutea*, while *F. mangiferum* and *B. ribis* were the most pathogenic on *S. cordatum*. *Botryosphaeria dothidea* and *L. gonubiensis* were the least pathogenic on both hosts. The results obtained from this trial clearly show that *Botryosphaeria* spp. on *S. cordatum* pose a potential threat to exotic *Eucalyptus* plantations. Future study should be conducted under field conditions to evaluate data obtained in greenhouse trials. These results were presented and discussed in Chapter 4.

The results presented in this study provide the first detailed information on *Botryosphaeria* spp. on the native *Myrtaceae* in South Africa. Most of the species isolated from *Syzygium cordatum* are not known on *Eucalyptus* in the country. All of the *Botryosphaeria* spp. obtained in this study are pathogenic to *Eucalyptus*, and thus pose a threat to this host. Large number of *B. ribis*, *B. parva* and *F. mangiferum* isolates obtained from the native *S. cordatum* could imply their origin in this region. Further sampling is needed on myrtaceous trees native to the Southern African region, as well as on *Eucalyptus*. Population studies on the most abundant and most pathogenic *Botryosphaeria* spp., should provide more information on the movement and origin of these pathogens. The results from this study also highlights the need for quarantine measures to avoid the introduction of new *Botryosphaeria* spp. or new strains that can be more pathogenic to either native or cultivated plants.