# Diseases of exotic plantation forestry trees in Ethiopia

by

**Alemu Gezahgne**
M. Phil. (University College of North Wales, Bangor)

Submitted in fulfilment of the requirements for the degree

**Doctor of Philosophy**

In the faculty of Natural and Agricultural Sciences,
Department of Microbiology and Plant Pathology
University of Pretoria
Pretoria, South Africa

March 2003

| | |
|---|---|
| **Promoter** | Dr. J. Roux |
| **Co-promoters** | Prof. M. J. Wingfield |
| | Prof. B. D. Wingfield |

# Diseases of exotic plantation forestry trees in Ethiopia

by

**Alemu Gezahgne**
M. Phil. (University College of North Wales, Bangor)

Submitted in fulfilment of the requirements for the degree

**Doctor of Philosophy**

In the faculty of Natural and Agricultural Sciences,
Department of Microbiology and Plant Pathology
University of Pretoria
Pretoria, South Africa

March 2003

| | |
|---|---|
| **Promoter** | Dr. J. Roux |
| **Co-promoters** | Prof. M. J. Wingfield |
| | Prof. B. D. Wingfield |

**Dedicated to my late younger sister, Getenesh Gebre Tsadik.**

# TABLE OF CONTENTS

# ACKNOWLEDGEMENTS

I am very grateful to God for guiding me and for giving me the strength and patience to accomplish this work and for cherishing me in times of joy and sorrow.

I would like to extend my gratitude and sincere appreciation to the following institutions and people who have made significant contributions to the completion of this study. Without your assistance this study would not have been possible.

My sincere thanks go to Dr. Jolanda Roux for her unqualified moral support and technical guidance from the inception to the finalisation of this project. Your guidance, comments and above all your friendship were instrumental to my accomplishment. I have learnt a lot from your wealth of knowledge on forest pathology.

I am also very much indebted to Professor Michael J. Wingfield, not only for accepting me to be part of FABI but also for his guidance, comments and support, as well as for his moral encouragement whenever I needed it.

I thank Professor Brenda D. Wingfield for her assistance in the area of molecular biology and for her constructive comments and suggestions on the content of this study.

The wonderful people in the Forestry and Agricultural Biotechnology Institute (FABI) deserve special thanks for their extended support during my study at the institution. I have learnt a lot from each one of you. I thank you all. I am really proud of being a member of this highly renowned institution.

I would also like to thank Bernard Slippers, Martin Coetzee, Mauricio Marin, Gavin Hunter and Juanita de Wet for their tireless assistance in specific chapters of this thesis.

My wife Yeshi Ketema and my daughter Rekik Alemu deserve special thanks for their understanding and patience in my absence from home. Yeshi, without your

moral support, encouragement, endurance and love, it would have been difficult to accomplish this work. I very much appreciate your determination to bear alone the responsibility of bringing up our daughter in the three years of my absence. I am proud of you and I love you very much.

I do not have enough words to express my gratitude to my mother Shewaye Demessie. Your determination, dedication and all round support have been the corner stone of all my achievements. You are a wonderful mother. I honour you and love you very much.

I also thank my aunt Lackech Demessie for being with me and supporting me in difficult times. I really appreciate this support.

I thank my brothers Getachew Shale, Fitawek Gebre Tsadick, Afework Gebre Tsadick, Lakew Bekele, my sisters Tezeru Ayele, Haregua Zewde as well as Betelihame Girma, Tizita Girma, Eden Girma, Mulumbet Girma, Firegenet Alemu and Etaferu Getachew for their continuous moral support and encouragement as well as for the support you gave to my wife Yeshi and for taking care of my daughter Rekik.

Many, many thanks go to my mother-in-law Desta Wolde Gebrael, my father-in-law Ketma Meshesha as well as my brother-in-law Binyam Ketma and sister-in-law, Jerusalem Ketema for closely attending my family in my absence. It is a blessing to have had you on my side at such a critical time. Mesfin Woje, Belay, and Kedir also deserve special thanks for taking care of my family while I was away.

Dr. Pia Barklund deserves special honour for her all-rounded assistance from the inception up to the completion of this study. I also thank my colleague Dagne Duguma for his unqualified support during the fieldwork and thereafter.

I thank the Agricultural Research and Training Project (ARTP) of the Ethiopian Agricultural Research Organisation (EARO) for the financing of my study. I also thank the Forestry Research Centre and its staff for facilitating and supporting the fieldwork.

Wondo Genet Forestry College, Munesa Shashemene Forest Development and Marketing Enterprise and the Jima Zonal Agricultural Bureau of Oromia region are acknowledged for allowing me to do field work in their forests and for their assistance during the field work.

Lastly, but not least the University of Pretoria, FABI and the Tree Pathology Co-operative Programme are thanked for providing the infrastructure and equipment for the lab work, as well as for funding.

# PREFACE

In several parts of Africa, exotic tree species are planted mainly in agroforestry development programs, for reduction of soil erosion, run-off control to combat desertification and rehabilitation of degraded land. They also contribute to the production of fuelwood, sawn timber and in some cases for pulp and paper production. In Ethiopia, planting exotic species commenced 110 years ago with the introduction of *Eucalyptus globulus*. Currently, several exotic species including those of *Eucalyptus*, *Pinus*, *Acacia* and *Cupressus* have been planted in Ethiopia. The government, the community and individual small-scale farmers own these plantations. These plantations contribute to the production of round wood for sawn timber, poles and posts and to meet wood requirements for local use, such as for construction material and for wood fuel. *Eucalyptus* spp. are the preferred planting stock, especially for fuel wood owing to their rapid growth and immediate economic return. *Pinus* spp. and *Cupressus* spp. are mostly planted in state owned forest areas.

Plantations of exotic species are successful in most areas where they have been planted. The success of these plantations is ascribed, at least in part, to the separation of the trees from their natural enemies. Despite this, pathogenic fungi, including native and introduced organisms pose serious threats to the development of exotic plantations. In Ethiopia, even though plantations of exotic species commenced over a century ago, little attention has been afforded to diseases of plantation trees. Currently, very little knowledge is available on the status of diseases in these plantations. The aim of studies making up this thesis has, therefore, been to increase the knowledge base pertaining to diseases in Ethiopian plantations.

The studies in this thesis focus mainly on identifying and recording the major diseases found in plantations of *Eucalyptus* and *Pinus* species. The thesis is comprised of seven separate chapters and each should be seen as an independent unit. Except for chapter one, the remaining six chapters were structured based on results of disease surveys conducted in 2000 and 2001. They have been presented as separate manuscripts and, therefore, some redundancy in introduction and methodologies used, could not be avoided.

Chapter one of the thesis presents a review of the impact and importance of diseases recorded on the most commonly planted exotic forestry species in Africa. The review discusses briefly, diseases recorded on *Eucalyptus*, *Pinus*, *Acacia* and *Cupressus* species. Root diseases, stem diseases, foliage diseases and wilt diseases recorded on plantations of exotic species in Africa have been included in the review. Knowledge pertaining to the disease situation on trees in Ethiopia was also evaluated and the lack of information on this aspect of forestry in the country is highlighted.

In order to manage forests and plantations effectively and to obtain maximum returns from them, it is essential to obtain information on the prevalence of diseases and their importance. In 2000 and 2001, disease surveys were conducted in major plantation areas situated in South, South Western and Western Ethiopia. Chapter two of the thesis deals with the results of these disease surveys, focusing on plantations of *Eucalyptus* and *Pinus* spp. This chapter presents the first record of major diseases in Ethiopian plantation forests.

Armillaria root rot was one of the most common diseases found associated with *Pinus* spp. It was, however, also found on some indigenous and exotic species. Although Armillaria root rot had previously been recorded from Ethiopia, there was uncertainty regarding the identity of the fungus found in the country. Chapter three discusses the results of the survey on the distribution and host range of this pathogen in plantations of Ethiopia. Also included is a taxonomic study, identifying the *Armillaria* sp. responsible for the disease in areas that we have considered.

*Eucalyptus camaldulensis* is one of the most widely planted *Eucalyptus* species in Ethiopia. A serious stem canker was frequently observed on *E. camaldulensis* planted in South and South Western Ethiopia. Preliminary investigations suggested that the disease was Coniothyrium stem canker. Chapter four presents the results of a study aimed at identifying this stem canker pathogen.

Chapter five of this thesis deals with Botryosphaeria stem canker of *Eucalyptus* spp. *Botryosphaeria* spp. are well known as wound and stress related opportunistic pathogens. Symptoms of Botryosphaeria stem canker, including production of brown

## SUMMARY

In Ethiopia, the planting of exotic species commenced with the introduction of *Eucalyptus globulus* approximately 110 years ago. Today several different *Eucalyptus*, *Pinus*, *Cupressus* and Australian *Acacia* species are planted to provide wood for fuel/energy and raw material for furniture and construction. In many areas, people are dependent solely on wood to provide for their basic fuel and construction needs. Despite this, little attention has been given to improve the silvicultural and management practices of plantations in Ethiopia. In particular, disease surveillance and management has never received due attention. The aim of the studies that make up this thesis have been to address the issue of diseases of plantation trees in Ethiopia. Studies have thus focused on the prevalence, identity and importance of major diseases of especially *Eucalyptus* and *Pinus* spp.

As a background to this thesis, available information on diseases of exotic tree species in Africa has been reviewed and this is presented in the first chapter. In the review, diseases of the major exotic plantation species including *Eucalyptus*, *Pinus*, *Cupressus* and *Acacia* species have been considered. A section was also devoted to highlight tree diseases reported from Ethiopia. The review shows clearly that there is a great lack of information on diseases of exotic plantation species in most African countries, with the exception of South Africa. This suggests the need for more pathology studies in African plantations. The review also highlights the importance of diseases in plantation forests.

In Ethiopia, little information is available on tree diseases in plantation forests. To partially address this problem, disease surveys were conducted in 2000 and 2001 in *Eucalyptus* and *Pinus* plantations in South and South Western Ethiopia. The results of this survey showed that a number of pathogens, known from other countries, including Armillaria root rot, stem canker and foliage diseases are found in plantations of Ethiopia. The major diseases discovered during the survey are discussed in Chapter two of this thesis and an indication is given of their impact and distribution.

During the disease survey, Armillaria root rot was found to be associated with both exotic and native tree species. Morphological and molecular identification techniques revealed that the *Armillaria* sp. collected in this study is *A. fuscipes*. This is discussed in chapter three, where I also provide preliminary data regarding the host range and distribution of Armillaria root rot in Ethiopia. Prior to this study it was suggested that *A. mellea* is responsible for Armillaria root rot of

hard woods in Ethiopia. The current study, however, showed that at least two *Armillaria* spp., *A. mellea* and *A. fusicpes* are causing Armillaria root rot in the country. Of significance is the fact that *A. fuscipes* was isolated from two indigenous tree species, *A. abyssinica* and *J. excelsa.*

Chapter four of this thesis deals with the identity of the fungus causing stem canker on *Eucalyptus camaldulensis.* Disease symptoms identical to those caused by *Coniothyrium zuluense* were commonly found on *E. camaldulensis* in restricted areas in Western Ethiopia. The causative agent was determined based on DNA sequence analysis of the ITS 1, ITS 2 and 5.8S gene region and β-tubulin genes. According to the phylogenetic tree generated for these sequence data, the Ethiopian *Coniothyrium* isolates seem to be closely related to *C. zuluense,* however, the Ethiopian isolates formed a separate group. This may suggest that *C. zuluense* represents a species complex, but this needs further investigation. Coniothyrium canker is considered to be one of the most serious diseases of *Eucalyptus* spp. especially to the sawn timber and construction industry as it weakens and flaws the timber. It occurrence in Ethiopia is, therefore, of great importance.

Disease symptoms similar to those of Botryosphaeria canker on *Eucalyptus* were commonly observed in all the areas where surveys were conducted. *Botryosphaeria* spp. are known as opportunistic stress related and endophytic pathogens on a wide range of woody plants, worldwide. In Ethiopia, symptoms similar to those associated with *Botryosphaeria* infection elsewhere, were found in almost all plantations surveyed. The disease was found on several *Eucalyptus* spp. including *E. globulus*, *E. saligna*, *E. grandis* and *E. citriodora.* Both morphological and molecular identification techniques were used to determine the identity of the fungus and the results are presented in chapter five. It was shown that *B. parva* is responsible for Botryosphaeria stem canker of *Eucalyptus* spp. in Ethiopia and the pathogenicity of Ethiopian isolates was also tested. This pathogen can have a serious effect on *Eucalyptus* in Ethiopia, as growing conditions in the country are often harsh and many people rely on coppicing to reproduce their stands. All these factors are conducive to stress and thus to *Botryosphaeria* infection.

*Diplodia pinea* is a fungus that commonly resides in the cones of *Pinus* spp. and it tends to move from these sites to infect stems, when trees are under stress. Therefore, isolations were made from *Pinus patula* cones to determine whether *D. pinea* was present in these structures in Ethiopia. Chapter 6 of the thesis provides results of this study. It was expected that *D. pinea* would be the most common inhabitant of the cones. Contrary to this, a *Fusicoccum* sp. was found more

frequently than *D. pinea*. The results presented in this chapter show clearly that the A morphotype of *D. pinea* is found in cones of *P. patula* in Ethiopia. The *Fusicoccum* sp. found associated with *P. patula* cones is most closely related to *B. parva*. Results of greenhouse inoculation studies showed that both these fungi are pathogenic to *Pinus tadea*, with *D. pinea* being the more pathogenic.

Serious leaf spot and shoot die-back symptoms were observed on leaves of *E. globulus* at several localities. The leaf blotch symptoms closely resemble those caused by *Mycosphaerella* spp. Even though 30 different *Mycosphaerella* spp. are known to be associated with *Eucalyptus* species world-wide, the cause of Mycosphaerella leaf blotch on *E. globulus* in Ethiopia is not known. Morphological and DNA based comparisons were used to determine the identity of the species found in Ethiopia and the results are provided in chapter seven. I was thus able to show that three *Mycosphaerella* spp. namely, *M. marksii, M. grandis* and *M. nubilosa* are involved in causing Mycosphaerella leaf disease of *E. globulus* in Ethiopia. This is the first report of these species from Ethiopia and the first report of *M. grandis* from a country other than Australia.

The results presented in the various chapters making up this thesis provide the first detailed studies on diseases of plantation trees in Ethiopia. Most tree diseases discussed in the thesis are first reports for the country. The thesis provides information on the identity of the pathogens and their significance in plantation development in Ethiopia. It also highlights the need for adequate management and silvicultural practices, as well as the need for selecting disease tolerant provenances and/or individuals. The information presented in the thesis also expands the host range and geographic distribution of all the pathogens included in the study, giving the study international significance.

exudate as well as stem cracking, were commonly found on several *Eucalyptus* spp. in all areas surveyed. This chapter discusses the results of the morphological and DNA based comparisons conducted to determine the identity of the *Botryosphaeria* spp. in Ethiopia.

Chapter six investigates the occurrence of species of *Botryosphaeria sensu lato* in *Pinus patula* cones in Ethiopia. *Diplodia pinea* is a common endophyte and stress related pathogen in *Pinus* spp. Interestingly, the most common inhabitant of *P. patula* cones in Ethiopia proved to not be *D. pinea*. In this chapter I discuss these findings, the identification of the two *Botryosphaeria* type fungi present, as well as their relative pathogenicity to *Pinus patula*.

Serious leaf spotting and shoot die-back typical of Mycosphaerella leaf blotch disease was observed on *E. globulus*, widely planted in cooler areas of Ethiopia. The last chapter of the thesis deals with the identification of the causal agents of MLD in Ethiopia. Ascospore germination patterns, cultural morphology and DNA sequence data were used to determine the identity of the fungus.

The research presented in this thesis represents the first comprehensive series of studies on diseases of plantation trees in Ethiopia. From an Ethiopian point of view, the information contained in this thesis will hopefully create due awareness among forest managers regarding the importance of diseases in plantation development. I also hope that they will form a foundation and pave the way for further studies on these and other tree diseases in Ethiopia in the future.

# Chapter One
# Impact and Importance of Diseases in Exotic Plantations in Ethiopia and Other African Countries-Literature Review

# INTRODUCTION

The demand for forest products has increased considerably over the past 100 years. The result has been the near depletion of indigenous wood sources. This increasing demand for timber and fuel energy has necessitated the establishment of large areas of plantation forests. Plantation forests can be developed using either exotic or native tree species. However, there is a growing worldwide trend towards the establishment of plantations of exotic tree species, especially in the tropics and subtropics (Evans 1984, Turnbull 1991, Persson 1995). These provide a source of energy, paper and pulp and wood extracts such as tannins. Plantations also play a substantial role in agroforestry development, the reduction of soil erosion, run-off control, combating desertification, rehabilitation of degraded land, fodder and they provide shade and shelter (Evans 1984).

The establishment of exotic plantation forestry has grown substantially worldwide. It is estimated that approximately 78 million hectares of exotic plantations exist today (Vercoe 1995). The most commonly planted species are *Pinus radiata* D. Don, *P. patula* Schiede & Deppe, *Eucalyptus grandis* Hill ex Maid and other *Eucalyptus* hybrids and species, Australian *Acacia* spp. and *Tectona* spp. Plantations of *Eucalyptus* spp. alone covers about 10 million ha worldwide (Eldridge *et al.* 1997). The largest exotic plantation forestry countries are Chile, Brazil, Indonesia, South Africa, Australia and New Zealand (Vercoe 1995). In these countries, plantations are utilized mainly for the paper and pulp industries and for sawn timber, forming multi-billion dollar industries.

Many countries in Africa grow large areas of exotic plantations of *Eucalyptus*, *Pinus, Cupressus* and *Acacia* spp., to provide fuel and timber as well as for the production of paper and pulp for the local and especially for the export markets (Evans 1984, Vercoe 1995). Exotic plantations in South Africa for example cover approximately 1.5 million ha, of which *E. grandis* and *P. patula* are the dominant species (Denison & Kietzka 1993, Anonymous 1998). In Kenya, almost all wood required for fuel-wood and industrial purposes are obtained from plantations of exotic species, which include *P. patula, Cupressus lusitanica* Mill. and different *Eucalyptus* spp. (Ciesla, Mbugua & Ward 1995).

Exotic trees are commonly used as plantation species in Ethiopia. The establishment of exotic plantation forestry in this country commenced with the introduction of *Eucalyptus* species, approximately 110 years ago, around 1890 (Persson 1995). Since then, a number of other exotic

tree species including *Pinus* spp., *Acacia* spp., and *Cupressus* spp. have been introduced and planted in different parts of the country. Plantations of exotic tree species cover more than 200 000 ha of land (Anonymous 1994, Vercoe 1995).

To meet the increasing demands for wood and wood products, both natural forests and plantation forests have to be protected. Threats against these wood sources include fire, indiscriminate cutting, encroachment, pests and diseases. The occurrence of pathogens on woody plants is a common phenomenon on trees growing in natural forests, plantations and ornamentals and has resulted in serious losses to forestry programmes worldwide (Manion 1981, Wingfield 1990).

In many countries exotic plantation forestry has been highly successful, largely because these trees have been removed from their natural enemies, found in their areas of origin. This might account specifically to the success of *Eucalyptus* spp. in Africa (Wingfield 1990, Persson 1995, Bright 1998). Exotic plantation species, though separated form their natural enemies, have on the other hand, been planted in a new environment. They can thus be exposed to potentially new pests and diseases, to which they do not have a natural resistance (Wingfield 1990). This situation may increase the disease risk associated with exotics (Wingfield 1987a). Despite being isolated from their natural enemies, a number of serious disease problems have emerged on exotic species in most countries where they have been planted (Wingfield 1990). Both native pathogens originating from endemic hosts as well as accidentally introduced pathogens cause damage in plantations (Wingfield, Swart & Kemp 1991). A large number of new and emerging tree disease have been recorded in the last decade. This may be attributed to the increased movement of people and plant material between countries (Palm 1999, Wingfield & Roux 2000a, Wingfield *et al.* 2001, Allen & Humble 2002).

Pathogenic fungi pose an enormous threat to trees planted in monoculture, due to the uniform age of the trees and the reduced diversity of plantations (Hodges 1979, Wingfield 1990). It is generally believed that monocultures are more vulnerable to disease and pest damage. The damage to monocultures could be serious because risk is not spread widely. Monocultures are also believed to facilitate the emergence of new and more virulent forms of pathogens (Hodges 1979, Heybroek 1980, Libby 1982, Leakey 1987, Roberds, Namkoong & Skrøppa 1990, Persson 1995). On the other hand, it has been shown that the susceptibility of monoculture to disease has been very much generalised and unbalanced. Choice of species and provenances and site selection has more influence than effect of stand composition on incidence of disease in plantations (Chou 1981).

Similarly, pathogens with wide host ranges for example *Armillaria* spp. (Raabe 1962) and *Phytophthora cinnamomi* Rands (Newhook & Podger 1972) could have a devastating effect even in mixed stands.

Fungal tree diseases, especially, have had a major impact on woody hosts world-wide. This includes those caused by both introduced and native pathogens. Chestnut, which was one of the major forest species in North America, has been nearly eliminated by the Chestnut blight fungus, *Cryphonectria parasitica* (Murr.) Barr (Hepting 1974, Anagnostakis 1987, 1988). Similarly, Dutch elm disease caused by *Ophiostoma ulmi* (Busim.) Nannf. and *Ophiostoma novo-ulmi* Brasier has devastated native elm trees in Europe and North America (Gibbs 1978, Brasier 1990). *Cronartium ribicola* Fischer, the cause of White pine blister rust has had a dramatic impact on White pines in America (Ziller 1974). Similarly, Dothistroma needle blight caused by *Dothistroma pini* Hulbury (syn. = *D. septospora*), which was initially identified in the United States of America, occurs in eastern and southern Africa, Chile, Australia and New Zealand and causes devastating damage on exotic *P. radiata* plantations in these countries (Ivory 1968, Gibson 1972, Lundquist & Roux 1984). Pathogens of native plants have also developed the capacity to infect exotic plantation species. For example, rust caused by *Puccinia psidii* G. Winter, a pathogen on native Mytraceae in South and Central America, suddenly appeared on various exotic *Eucalyptus* spp. planted in this region (Coutinho *et al.* 1998).

This review focuses on the importance and impact of pathogens on exotic plantations in some African countries. Special consideration is given to the disease situation in plantations of Ethiopia. Hence, in the following sections, some of the most important diseases of the major exotic plantation species in Africa are discussed. Special emphasis is given to species of *Eucalyptus, Pinus, Cupresses* and Australian *Acacia,* because they are dominant in the plantations of Ethiopia and other African countries.

## NURSERY DISEASES

As a result of the use of non-sterilised growth medium and water in many African nurseries, diseases cause significant losses in production. Nursery diseases reduce germination, cause seedling death and enhance malformation and stunting. These result in seedling rejection and lower field survival rates. The most commonly observed disease symptoms include damping-off, root rot,

blight, stem cankers and leaf spots (Darvas, Scott & Kotze 1978, Sharma, Mohanan & Florence 1984).

Nursery plants are predisposed to nursery diseases by environmental and management factors. Control of nursery diseases relies heavily on sound sanitation and management. Nursery diseases are commonly spread by infected seed, water and planting media. Mechanical and insect wounds are other factors increasing disease risks. Watering and weeding regimes, spacement and nutrition all impact on nursery health (Bloomberg 1981, Anderson, Belcher & Miller 1984, Dwinell, Barrows-Broaddus & Kuhlman 1985). Available reports of diseases in African nurseries have been summarised in Table 1 and will not be discussed individually.

## DISEASES OF PLANTATION *PINUS* SPP. IN AFRICA

Plantations of *Pinus* spp. have been established especially in South, East and Central Africa (Gibson 1979). The most commonly planted species are *P. patula, P. radiata, P. caribea* L., *P. elliottii* Englam and *P. taedea* L. (Gibson 1975, Evans 1984). These trees are grown mostly for industrial purposes, sawtimber, pulpwood and plywood veneers (Evans 1984, Ivory 1987).

### *Root diseases*

***Armillaria* spp.** include some of the most prominent causes of death and decay of coniferous trees and shrubs in natural forests, native and exotic plantations, and gardens world-wide (Wargo & Shaw 1985, Ivory 1987, Shaw & Kile 1991). *Armillaria* spp. have a wide host range and occur in both tropical and temperate areas of the world (Ivory 1987, Shaw & Kile 1991). Reports indicate that *Armillaria* spp. are dominant in deciduous forests as secondary pathogens attacking trees weakened by biotic or abiotic stress. Such stress includes defoliation by insects, frost, drought, foliage diseases, stem cankers, water logging, soil compaction and air pollution (Wargo & Shaw 1985). *Armillaria* spp. can, however, also be aggressive primary pathogens, frequently killing healthy trees of all ages (Wargo & Shaw 1985).

A typical sign of *Armillaria* root rot in forests and plantations are the concentration of dead and dying trees in circular patches. Infections of trees take place through the roots, resulting in root rot and eventual tree death. Root death results in yellowing and wilting of crowns, resin exudation

around the root collar and basal areas of stems and well developed white mycelial mats under the bark of infected trees (Bottomley 1937, Lückhoff 1964, Ivory 1987). The wood beneath the fungal mycelium has a soft, wet, stringy, white rot. Under favourable conditions rhizomorphs will form (Ivory 1987, Shaw & Kile 1991). It has been reported that *Armillaria* spp. colonise the stumps of indigenous trees and serve as a source of inoculum for the infection of exotic tree species especially, *Pinus* spp. (Kotze 1935, Bottomely 1937, Lückhoff 1964, Gibson 1970, Ivory 1987).

*Armillaria mellea* (Vahl:Fr.) Kummer *sensu lato* and *A. heimii* Pegler are names commonly used for the causal agents of Armillaria root rot in Africa (Ivory 1987, Coetzee 1997). Mohammed (1994) assumed that *A. mellea* is the cause of *Armillaria* root rot in temperate regions of Africa. However, recent reports have indicated that *A. heimii*, *A. mellea sensu stricto*, *A. mellea* (Vahl.:Fr.) P. Kumm. sub sp. *africana* Mohammed, *A. mellea* (Vahl.:Fr.) P. Kumm. var. *cameruensis* Henn. and *A. fuscipes* Petch occur in African plantations (Gibson 1975, Pegler 1977, Ivory 1987, Masuka 1989, Mwangi, Lin & Hubbes 1989, Abomo-Ndongo & Guillaumin 1997, Coetzee *et al.* 2000).

Armillaria root rot has been reported in *P. patula*, *P. elliottii*, *P. oocarpa* Shchiete and *P. radiata* plantations in Zimbabwe, Uganda, Kenya, Tanzania, Malawi and South Africa (Lee 1970, Gibson 1975, Ivory 1987, Masuka 1989, Mwangi *et al.* 1989, Abomo-Ndongo & Guillaumin 1997, Coetzee *et al.* 2000). In South Africa, *Armillaria* root rot is known in plantations of several pine species including *P. patula, P. caribea* and *P. elliottii.* (Kotze 1935, Bottomley 1937, Lückhoff 1964). Despite this, the identity of *Armillaria* found in South Africa was not determined until recently. Initially it was arbitrarily called *A. mellea* (Lundquist 1987) and later the pathogen was assumed to be *A. heimii* (Wingfield & Knox-Davies 1980a). Coetzee *et al.* (2000), however, showed that the isolates obtained from plantations in South Africa are clearly different from both *A. mellea* and *A. heimii* and rather represents *A. fuscipes*.

In Zimbabwe, *Armillaria* root rot was considered to be one of the most serious threats to *Pinus* plantations in that country. Affected species included *P. elliotii* and *P. oocarpa* (Masuka 1989). Mwenje & Ride (1996) characterized the *Armillaria* isolates from Zimbabwe into three morphological groups, namely groups I, II, and III. Coetzee *et al.* (2000) showed that most *Armillaria* isolates from Zimbabwe (Harare area) resemble neither *A. fuscipes* that is found in South Africa, nor *A. mellea*, but represents *A. heimii*.

In Kenya Armillaria root rot is widely spread in plantations, indigenous forests and cash crops (Mwangi *et al.* 1994). It was isolated from *P. patula, P. elliottii* and *P. radiata* (Abomo-Ndongo & Guillaumin 1997). Mewnje and Ride (1996) showed that both *A. mellea* and *A. heimii* are found in Kenya.

***Sphaeropsis sapinea*** (Fr.:Fr.) Dyko and Sutton (syn. *Diplodia pinea* (Desm.) Kickx ) has a world-wide distribution and is associated with a wide range of disease symptoms (Punithalingam & Waterston 1970, Gibson 1979, Swart, Knox-Davies & Wingfield 1985, Swart, Wingfield & Knox-Davies 1987). It was reported to cause serious root disease on *P. taeda* and *P. elliotii* in South Africa and Swaziland (Wingfield & Knox-Davies 1980a, Swart *et al.* 1985, Swart *et al.* 1987). The above ground symptoms of *S. sapinea* infection include discoloration of the root collar, foliage chlorosis, needle fall, exudation of resin and death of tree tops. It also causes dark blue radial lesions in young roots, which extend into the lateral root and even up to the trunks of trees (Wingfield & Knox-Davies 1980a, Swart *et al.* 1985). This root disease is associated with stress such as overstocking, drought and poor site conditions (Wingfield & Knox-Davies 1980a, Swart *et al.* 1987).

***Rhizina undulata*** Fr. (= *R. inflata* (Schaff.) Krast) is a fire associated pathogen that causes root rot of *Pinus* and other conifers (Morgan & Driver 1972, Lundquist 1984a, Wingfield, Swart & Von Broembsen 1988). It is found in Europe, North America, Africa and Asia (Gibson 1979). According to Germishuizen (1984), *Rhizina* root rot caused serious losses to *Pinus* plantations in South Africa. It was also reported to occur in Swaziland (Germishuizen 1979). Studies indicated that slash burning, which was conducted to remove logging debris, induced infection by *R. undulata* (Morgan & Driver 1972, Germishuizen 1984, Ivory 1987).

***Phytophthora* spp.** are known to cause serious damage to woody plants in many parts of the world and have a wide host range (Zentmyer 1980, Tidball & Linderman 1990, Strouts & Winter 1994, Erwin & Ribeiro 1996). *Phytophthora* spp. are important pathogens in forestry nurseries (Von Broembsen & Donald 1981) and in sites that are wet or with poor soil nutrients (Erwin & Ribeiro 1996). In South Africa, *P. cinnamomi* is associated with root rot and death of several *Pinus* spp., especially *P. patula,* both in nurseries and plantations (Von Broembsen 1984, Wingfield & Knox-Davies 1980b, Linde, Kemp & Wingfield 1994a). Other *Pinus* spp. affected includes *P. pinea* L., *P. radiata, P. elliottii* and *P. patula* (Wingfield & Knox-Davies 1980b, Von Broembsen 1984, Ivory 1987).

***Pythium* spp.** are not considered as important pathogens of mature pine trees (Markes & Kassaby 1974). Yet, some *Pythium* spp. cause death of young *Pinus* seedlings planted out in the field (Linde, Kemp & Wingfield 1994b). Some reports from South Africa indicate that a number of *Pythium* spp. are associated with death of *P. patula* established on previously cultivated lands. *P. irregulare* Buisman was consistently isolated from dying plants as well as from the soil in *Pinus* plantations and is considered as a major cause for the failure of *P. patula* planted on previously cultivated land (Linde, Kemp & Wingfield 1994c).

***Helicobasidium compactum*** Boedijn causes purple root rot and is found in Asia, Europe, Australia and Africa. It was reported from several Central and Southern Africa countries (Gibson 1979). It infects the roots of a wide range of plants including both hardwoods and conifers (Browne 1968). *H. compactum* is associated with root and collar rot of *Pinus* spp. (Bottomley 1937, Gibson 1979) in Zimbabwe, Nigeria, Kenya, Malawi, Tanzania (Browne 1968, Gibson 1975) and South Africa (Bottomley 1937). It causes stunting of terminal shoots, yellowing of needles, wilting and death of trees (Bottomley 1937). Infection by *Helicobasidium* is also associated with collar constriction. It is possible to find purplish brown fungal growth at the base of the tree and at the lowest parts of the branches (Bottomley 1937).

***Pseudophaeolus*** root and collar disease, caused by *Pseudophaeolus baudonii* (Pat.) Rev. (syn. *Phaeolus manihotis* Heim, *Polyporus baudoni* Pat.) is distributed throughout central and Southern Africa (Gibson 1979, Rattan & Pawsey 1981). This pathogen attacked *Pinus* spp. in Congo Brazzaville (Rattan & Pawsey 1981), Ghana (Ofosu-Asiedu 1975) and South Africa (Lückhoff 1955, 1964, Van Der Westhuizen 1973, Lundquist 1987, Wingfield 1987b). Infection spreads by means of root contact. The white to yellow mycelial fans found beneath the bark at the base of trees is used to distinguish the disease. It also produces large yellow fruiting structures on the roots near the base of infected trees (Van Der Westhuizen 1973, Ivory 1987, Wingfield 1987b).

***Stem diseases/canker***

***Sphaeropsis sapinea*** is arguably the most common stem pathogen on *Pinus* spp. in Africa. Apart from causing root rot, *S. sapinea* is best known for the stem disease it causes on *Pinus* spp. It is known to be an opportunistic pathogen (Marks & Minko 1969, Swart *et al.* 1985) that subsists in cones and stems of healthy pine trees as endophytes (Smith *et al.* 1996a). Disease symptoms are

only expressed when trees are under stress (Zwolinski, Swart & Wingfield 1990a, Smith *et al.* 1996a).

*Sphaeropsis sapinea* is known in pines wherever they are native and has been recorded from most countries where these trees are grown as exotics (Currie & Toes 1978, Gibson 1979, Palmer & Nicholls 1985). It has been associated with a wide range of disease symptoms, both in nurseries and on mature trees in the plantation. Plantation related symptoms include collar rot, shoot blight (Gibson 1979, Wingfield & Knox-Davies 1980b), blue stain, stem and branch cankers (Marks & Minko 1969, Wright & Marks 1970) and root disease (Punithalingam & Waterston 1970, Gibson 1979, Palmer, McRoberts & Nicholls 1988).

In Africa, *S. sapinea* has been recorded as a pathogen of *Pinus* spp. in countries such as Malawi, South Africa, Swaziland, Zimbabwe and Tanzania (Gibson 1964, Lee 1970). *Pinus radiata* and *P. patula* are especially susceptible to infection (Swart *et al.* 1987, Wingfield 1990). In South Africa, a close association with hail damage has been shown (Swart *et al.* 1987). In summer rainfall areas of the country, where hail damage is frequent, the planting of *P. radiata* was abandoned (Wingfield 1990). Similarly, *P. elliottii* replaced *P. patula* in hail prone areas due to the susceptibility of *P. patula* to infection (Lundquist 1987).

It was estimated in 1986 that infection by *S. sapinea* in South Africa has resulted in more than nine million Rand of loss per annum (Zwolinski *et al.* 1990a). Infection by *S. sapinea* not only results in death of trees, but reduces profit through the reduction of increment (Wright & Marks 1970), the loss of marketable volume (Currie & Toes 1978, Zwolinski *et al.* 1990a) and blue stain of timber (Laughton 1937, Da Costa 1955, Eldridge 1961). South African exports of saw logs often loose as much as half their marketable volume due to blue stain of timber (Zwolinski, Swart & Wingfield 1990b).

***Needle diseases***

**Dothistroma** needle blight, caused by *Dothistroma pini* Hulbury (=*Dothistroma septospora* Dorog. Morelet) is one of the most important needle diseases of exotic *Pinus* spp. *Dothistroma* needle blight was first recognized as a serious disease of ornamental, windbreak and Christmas trees in the United States of America. The disease was also reported from several other countries, including East and Southern Africa, Chile, Australia and New Zealand in exotic *Pinus* plantations (Ivory

1968, Gibson 1972, Peterson 1977, Lundquist & Roux 1984). According to Ivory (1968), three varieties of *Dothistroma* are found on needles of *Pinus* spp. in different parts of the world. These varieties are *D. pini* Hulbery var. *keniensis* Ivory, *D. pini* var. *pini* and *D. pini* Hulbery var. *linearis* Thyr & Shaw. Of these three varieties only *D. pini* var. *keniensis* has been reported from Africa, whereas *D. pini* var. *pini* is distributed in different parts of the world and *D. pini* var. *linearis* is mainly found in the United States of America and Canada.

In Africa, Dothistroma needle blight was first observed in Tanzania (Gibson, Christensen & Munga 1964, Ivory 1968) and later in Kenya, Zimbabwe, Malawi and Uganda (Gibson 1964, Ivory 1968). The disease spread very fast in *P. radiata* plantations, causing defoliation and stunting of trees (Gibson *et al.* 1964). Because of Dothistroma needle blight, the planting of *P. radiata,* has been mostly abandoned or severely restricted in African countries such as Kenya, Malawi and Zimbabwe (Ivory 1968, Lee 1970, Gibson 1972, Ciesla, Mbugua & Ward 1995). In many other countries, *P. radiata* has been substituted by *P. patula.* Lundquist & Roux (1984) reported the occurrence of *D. pini* in South Africa. It is, however, only found in a very small area of the country and is not considered to be economically important (Lundquist & Roux 1984, Wingfield & Roux 2000b).

Dothistroma needle blight first appears on the lower branches of young trees (Gibson *et al.* 1964, Wingfield & Roux 2000a). Early infection of needles produce yellow bands that later develop a reddish tint as the disease develops. Following this chlorosis, necrosis of the needles appears, first at the base of the tree, later spreading higher up the tree. In favourable conditions, the disease results in severe defoliation, to the extent that only the needles at the extremes of the branches remain (Gibson *et al.* 1964). Black fruiting bodies commonly appear on the dead epidermis in the red bands (Gibson 1964, Wingfield & Roux 2000a). In severe cases infection may result in malformation and tree death. Dothistroma needle blight is more severe in younger trees less than ten years in age (Gibson 1972).

In Tanzania, death of pine trees associated with Dothistroma needle blight was not experienced in areas where the rainfall was below 1500 mm/annum. In these areas only the diameter and height growth was reduced considerably (Christensen & Gibson 1964). A study conducted in Kenya on the effect of defoliation by *D. pini* on the increment rates of *P. radiata,* showed that diameter growth is considerably reduced. Nearly all growth of trees is inhibited when 75% of the foliage is affected (Christensen & Gibson 1964, Gibson *et al.* 1964).

***Cercospora*** needle blight is the other needle disease recorded on various *Pinus* spp. in several African countries (Gibson 1972, 1979, Ivory & Wingfield 1986, Ivory 1987) including Madagascar, East Africa, South Africa, Swaziland, Zambia and Central Africa (Gibson 1964, 1979, Ivory & Wingfield 1986, Ivory 1994). *Cercospora* needle blight is caused by *Cercoseptoria pini-densiflorae* (Hori. & Nambu) Deightn (Telemorph= *Mycosphaerella gibsonii* H. Evans). In South Africa it infects *P. patula* and *P. radiata* (Ivory & Wingfield 1986, Wingfield & Roux 2000a). The disease causes severe defoliation on young pine seedlings in nurseries and plantations. The fungus initially infects old needles and in severe cases, it also attacks young needles (Wingfield & Roux 2000a). Infection causes light green bands on the needles, which later change to yellow, brown and finally to a grey colour. Fruiting bodies are seen on dead needles and are "brush like" and grey in appearance (Wingfield & Roux 2000a).

## DISEASES OF PLANTATION *EUCALYPTUS* SPP. IN AFRICA

In Africa, the production of *Eucalyptus* trees takes place either by the raising of seedlings from seed, the production of cuttings or by tissue culture (Leakey 1987, Denison & Kietzka 1993). In South Africa alone, approximately 500 000 ha are planted to a variety of *Eucalyptus* spp. and clones (Anonymous 1998). Other African countries also depend on *Eucalyptus* spp. for export income and importantly, as a substitute for indigenous trees. *E. grandis*, *E. saligna* Sm., *E. globulus* Labil., *E. camaldulensis* Dhen., *E. citriodora* Hook and *E. urophylla* Blake are the most commonly planted species (Gibson 1975, Evans 1984). Several pathogens pose a threat to *Eucalyptus* planting on the continent. The most important of these are presented in the following section.

### *Root diseases*

***Phytophthora*** spp. are among the most common pathogens of *Eucalyptus* spp. (Marks & Kassaby 1974, Heather, Pratt & Chin 1977, Zentmyer 1980). In South Africa, *P. cinnamomi* results in death and stunting of *E. fastigata* Deane and Maid., *E. smithii* R. T. Baker and *E. fraxinoides* Deane and Maid. (Wingfield & Knox-Davies 1980b). Hence, as Linde *et al.* (1994a) indicated, the susceptibility of these species has necessitated planting of other *Eucalyptus* spp. not prone to *P. cinnamomi* root disease. Recently *P. nicotianae* Breda de Haan has been isolated from diseased and dying *E. nitens* trees in the Kwazulu Natal Midlands of South Africa (Maseko *et al.* 2001).

Another Oomycetous root pathogen, ***Pythium splendens*** H. Braun, has been reported to cause mortality of young *E. grandis* in South Africa (Linde, Wingfiled & Kemp 1994d). This fungus has caused a root and root collar disease on established *E. grandis* in the warmer sub-tropical areas of the country (Linde *et al.* 1994d). The disease is characterised by reddening of the leaves, rapid wilting as well as girdling of the roots and root collars and consequent death of trees.

***Lasiodiplodia theobromae*** (Pat.) Griff. and Maubl. (telemorph *Botryosphaeria rhodina* (Cooke) Von Arx.), has been reported from the Republic of Congo in association with rot of *E. grandis* roots (Roux *et al.* 2000a). The root collars and the stems above soil level exuded kino and developed small cankers. The disease spreads from the roots to the root collars and rest of the stem. The cankers associated with this disease caused a complete girdling, wilting and death of branches (Roux *et al.* 2000a).

In Kenya, **Armillaria** root rot has been reported on *E. microcorys* F. Mull and *E. saligna* (Mwangi *et al.* 1989, Onsando, Wargo & Waudo 1997). In South Africa, *Armillaria* root rot has been recorded on *Eucalyptus* sp. planted on a site cleared of indigenous forest (Bottomley 1937, Kotze 1935, Lückhoff 1964), while in Malawi Armillaria root rot was found associated with *E. saligna*, *E. microcorys* and *E. pilularis* Sm. (Lee 1970). Ivory (1987) reported that *Armillaria* spp. also infects *E. pilularis* Sm. in Zimbabwe.

***Ganoderma*** species cause root and butt rot on several woody plants including *Eucalyptus* spp., worldwide (Browne 1968). In Zimbabwe *E. grandis* trees were infected by this pathogen (Masuka 1990, Masuka & Nyoka 1995). The causative agent was identified as *G. sculptrutum* Llyod (Masuka & Nyoka 1995). Infected trees showed longitudinal bark splitting, stem swelling and gummosis, with dark to black lesions on the affected roots. Affected trees developed epicormic shoots and trees died from the crown downwards (Masuka & Nyoka 1995). Basidiocarps of the fungus may be found at the base of the stems or attached to lateral roots (Masuka & Nyoka 1995). Infection causes death of trees in patches or death of trees in a line, with the most recently killed trees at the edges of the patches (Masuka & Nyoka 1995).

***Polyporus baudonii*** Pat. Ryv., also known as *Pseudophaelus baudonii* (Pat.) Ryv. and *Phaeolus manihotis* Heim is found in several African countries on many woody plants (Browne 1968, Gibson 1979, Ivory 1987). This fungus is known to cause root rot on *Eucalyptus* spp. in South Africa and Ghana (Van der Westhuizen 1973, Ofosu-Asiedu 1975, Wingfield & Roux 2000a). *P. baudonii*

attacks roots and root collars of susceptible trees. Leaf chlorosis, unseasonal leaf shedding as well as die-back of small branches at one side of the crown are characteristic symptoms of infection. The bark of the infected trees also changes colour and becomes cracked and charred as if it has been burnt by fire (Ofosu-Asiedu 1975).

***Stem disease/canker***

***Cryphonectria*** canker caused by *Cryphonectria cubensis* (Burner) Hodges is considered to be one of the most serious canker diseases of *Eucalyptus* spp. in the tropics. It has been reported from different countries in Central and South America, Africa, Asia and Australia (Boerboom & Maas 1970, Hodges, Geary & Cordell 1979, Sharma, Mohanan & Florence 1985, Florence, Sharma & Mohanan 1986, Hodges, Alfenas & Ferreria 1986, Wingfield, Swart & Abear 1989, Conradie, Swart & Wingfield 1990). In Africa, Cryphonectria stem canker has been reported on *Eucalyptus* from Northern Africa (Gibson 1981), Cameroon (Sharma *et al.* 1985), South Africa (Wingfield *et al.* 1989, Conradie *et al.* 1990), and the Republic of Congo (Brazzavile) (Roux *et al.* 2000b).

Cryphonecria canker has been reported on many different *Eucalyptus* spp. In Africa these include *E. grandis* in South Africa, *E. grandis* and *E. urophylla* in the Congo (Sharma *et al.* 1985, Conradie *et al.* 1990, Roux *et al.* 2000b). It is not known which species were affected in Cameroon and North Africa. Typical symptoms of Cryphonectria canker in South Africa differ from those found in other parts of Africa and the world. In South Africa, the disease is characterised by the formation of swollen, cracked, basal cankers and root/collar rot (Wingfield *et al.* 1989, Conradie *et al.* 1990). Young trees die readily from root and root collar infections, while older trees tend to become stunted (Wingfield *et al.* 1989, Conradie *et al.* 1990). In the Congo, Cryphonectria canker is characterised by the more typical target shaped stem cankers found on above grounds parts of the trees such as those described from other parts of the world (Roux *et al.* 2000b). These stem cankers commonly coalesce to girdle and kill and can occur over the length of the stem, often around branch knots (Sharma *et al.* 1985, Wingfield & Roux 2000a). Long-necked fruiting bodies, with orange spore masses, form abundantly in the cracks and the basal cankers (Wingfield & Roux 2000a). In South Africa the disease has lead to the abandonment of some clones in subtropical areas of the country (Wingfield 1990).

*Cryphonectria eucalypti* Venter & M.J Wingfield, previously known as *Endothia gyrosa* (Schew) Fr. represents a newly described fungus, which is known to occur only in South Africa and

Australia (Venter *et al.* 2001). In South Africa it is commonly known as a pathogen of minor concern (Van der Westhuizen *et al.* 1993, Wingfield & Roux 2000a). Symptoms of infection are commonly characterised by the formation of superficial cracks in the bark of trees. These cankers commonly form in bands and may occur over the length of tree stems, although they are often most concentrated towards the bases of trees (Van der Westhuizen *et al.* 1993, Wingfield & Roux 2000a, Venter *et al.* 2001). Orange fruiting bodies are common between the cracks on infected stems. In some cases, these cracks provide entry sites for opportunistic pathogens such as *Botryosphaeria* spp. In some parts of South Africa, the pathogen has been associated with cankers and death of young stressed trees (Venter *et al.* 2001).

***Coniothyrium*** canker is a relatively newly discovered and important stem canker disease of *Eucalyptus* spp. This disease was first observed in 1988 in South Africa (Wingfield, Crous & Coutinho 1996). The causal agent was described as *Coniothyrium zuluense* Wingfield, Crous & Coutinho (Wingfield *et al.* 1996). The disease most typically damages *E. grandis* propagated from seed. It is also reported to affect several *E. grandis* clones and hybrids of *E. grandis* with *E. urophylla* and *E. camaldulensis* (Wingfield *et al.* 1996) in the warmer, humid areas of South Africa. The disease is characterised by discrete necrotic lesions on young green bark. At later stages, it forms large necrotic patches, which may spread over most of the stem. In severe cases, the trees produces epicormic shoots as a result of infection. In advanced stages of disease, infection may lead to top die-back and subsequent reduction in height growth (Wingfield *et al.* 1996, Wingfield & Roux 2000a).

***Botryosphaeria*** spp. and their anamorphs in the genera *Fusicoccum*, *Lasiodiplodia*, *Sphaeropsis* and *Microphomopsis* have one of the widest host ranges known for any group of pathogens. Amongst these hosts are many species of *Eucalytpus*. In Africa, confirmed reports of *B. rhodina* (anamorph: *Lasiodiplodia theobromae*) on *Eucalytpus* spp. have been made from South Africa (Smith, Kemp & Wingfield, 1994), the Republic of Congo (Roux *et al.* 2000b), Uganda (Roux *et al.* 2001a) and Malawi (Gibson 1964). Recently, a new *Botryosphaeria* sp., namely *B. eucalyptorum* Crous, H. Smith et M. J. Wingfield has been described on *Eucalyptus* in South Africa. This *Botryosphaeria* sp. is reported to be associated with cankers of the main stems of *E. grandis* and *E. nitens* (Deane Et maid.) Maid. (Smith *et al.* 2001).

Symptoms of infection by *Botryosphaeria* spp. range from leaf spots, to stem cankers, tip and shoot blight and root rot (Davison & Tay 1983, Barnard *et al.* 1987, Shearer, Tippett & Bartle 1987,

Smith *et al.* 1994). Stem cankers are characterized by cracking of the bark and the exudation of resin (Smith *et al.* 1994). When the bark is removed, extensive resin formation is observed in the cambium and wood (Smith *et al.* 1994). Trees often recover from current infections, resulting in dead, discoloured heart wood (Smith *et al.* 1994, Roux *et al.* 2000b), and such trees continue to grow until the next infection cycle.

Stress is known to be a major factor contributing to development of diseases caused by *Botryosphaeria* spp. on *Eucalyptus* spp. (Smith *et al.* 1994). According to Smith *et al.* (1994), plantations situated on marginal land are especially prone to infection by *B. dothidea* (Moug.) Ces. De Not. This pathogen is frequently associated with damage from hot and cold winds, late frost, drought, mechanical damage from hail, insect feeding or silvicultural practices (Schoeneweiss 1979, Ramos *et al.* 1991, Smith 1995, Wingfield & Roux 2000b).

*B. dothidea* occurs as latent endophytic infections in several *Eucalyptus* spp. (Bettucci & Saravay 1993, Fisher *et al.* 1993, Smith *et al.* 1996a, Smith, Wingfield & petrini 1996b ). In South Africa, *B. dothidea* was reported to be found as a latent pathogen in *E. camaldulensis*, *E. grandis*, *E. nitens* (Deane et Maid) Maid. and *E. smithii* R. T. Bak. (Smith *et al.* 1996a, 1996b). It causes asymptomatic endophytic infections in the leaves and in the xylem of *E. grandis* and *E. nitens*. Symptoms appear only when environmental conditions favour disease development (Bettucci & Saravay 1993).

***Cytospora*** spp. and their *Valsa* telomorphs are commonly isolated from *Eucalyptus* spp. in association with cankers. *Cytospora australiae* Speg, *C. eucalypticola* Van der Westhuizen and *C. eucalyptina* Speg., have been reported to cause cankers on *Eucalyptus* spp. in South Africa (Van der Westhuizen *et al.* 1993, Wingfield & Roux 2000a). *Cytospora* spp. were also reported in association with stem cankers of *Eucalyptus* spp. in Congo and Uganda (Roux *et al.* 2000b, Roux *et al.* 2001a). In Malawi, Cytospora cankers were observed on *E. saligna*, *E. citriodora* and *E. maculata* Hook (Lee 1970). These opportunistic pathogens are mainly isolated from trees under severe stress due to drought, or trees planted in wet swampy areas with poor drainage. Mechanical wounds are also common sites of infection (Shearer *et al.* 1987, Old, Yuan & Kobayashi 1991, Roux *et al.* 2001a). It is assumed that these fungi are endophytes (Smith 1995, Wingfield & Roux 2000b) although this matter has not been comprehensively investigated.

In tropical areas of the world, woody plants including *Eucalyptus* and *Acacia* spp. suffer from a canker disesase commonly known as **pink disease**. In South Africa, *E. macarthurrii* Deane & Maiden is attacked by the pink disease pathogen, *Erythricium salmonicolor* (Berk & Broome) Burds. [syn. *Corticium salmonicolor* Berk. & Br., *Phanerochaetea salmonicolor* (Berk. & Br.) Julich] (Wingfield & Roux 2000b, Roux *et al.* 2001c). Infection by *E. salmonicolor* causes the inner tissue of the bark, mainly the phloem and the cambium to become brown and eventually die. Later, epicormic shoots develop just below the cankered region. These epicormic shoots also die due to downward spread of infection and a wilting of young shoots (Sharma *et al.* 1988). Apart from South Africa, pink disease has been reported from *Eucalyptus* spp. in Nigeria and the Democratic Republic of Congo (Gibson 1964).

***Leaf diseases***

Leaf diseases can have a serious impact on the growth of trees. Many fungi have been recorded on *Eucalyptus* leaves (Gibson 1975, Lundquist & Baxter 1985, Crous, Knox-Davies & Wingfield 1989a). In severe cases these fungi may result in complete defoliation of trees and seriously impact on tree growth.

**Cylindrocladium** leaf blight, caused by several species of *Cylindrocladium* is one of the most devastating leaf diseases of *Eucalyptus* spp. *Cylindrocladium* leaf blight has been reported from several countries, especially in the tropics. Reports from Africa include those from South Africa (Crous, Phillips & Wingfield 1991) and the Republic of Congo (Brazzaville) (Roux *et al.* 2000b). Cylindrocladium leaf blight caused by *C. theae* (Petch) Subramanian was observed on *Eucalyptus* in the Congo in 1998 (Roux *et al.* 2000b). Lesions initially develop at the edges of leaves and gradually affect the entire leaf. The disease was also observed on twigs and branches. Leaves die on one or two branches and in severe cases the entire tree can be defoliated (Roux *et al.* 2000b). In South Africa, *C. colhounii* Peerally var. *macroconidialis* Crous, Wingfield & Alfenas var. nov. and *C. pauciramosum* C. L. Schoch et Crous have been recorded on *Eucalyptus* (Crous, Philips & Wingfield 1993, Schoch *et al.* 1999).

***Mycosphaerella*** leaf blotch disease (MLB) on *Eucalyptus* is associated with 30 species of *Mycosphaerella* Johnson (Crous 1998, Carnegie 2000, Hunter *et al.* 2002) and has a world-wide distribution including the tropics, subtropics, temperate and Mediterranean areas (Lundquist & Purnell 1987, Crous *et al.* 1989a, Carnegie & Keane 1994). Infections of *Eucalyptus* spp. by

*Mycosphaerella* spp. often show high host specificity (Crous & Wingfield 1996). For example, *M. cryptica* (Cooke) Hansf. is responsible for foliage damage on both juvenile and mature trees of *E. nitens* (Carnegie 1991), whereas *M. molleriana* Lindau. is the main cause of such foliage damage on *E. globulus* and *E. maidenii* F. Muel. (Lundquist & Purnell 1987).

Leaf spot fungi in the genus *Mycosphaerella* result in severe leaf necrosis, premature defoliation and loss of growth in *Eucalyptus* plantations (Crous *et al.* 1989a). In Africa, *M. molleriana* is responsible for leaf spot and defoliation on both juvenile and mature trees of *E. globulus* (Dungey *et al.* 1995, Lundquist & Purnell 1987). *M. molleriana* has resulted in an inability to establish *E. globulus* and *E. maidenii* in South Africa (Wingfield 1990). Similarly, planting of *E. nitens*, the most promising tree species for afforestation of frost prone areas is restricted because of attack by *Mycosphaerella* spp. (Lundquist 1985, Wingfield 1990). *Mycosphaerella* leaf blotch has also been reported from Uganda (Roux *et al.* 2001a), Zimbabwe and Kenya (Gibson 1964) as well as from Malawi (Lee 1970).

*Mycosphaerella nubilosa* (Cooke) Hansf. (Syn. *Sphaerella nubilosa* Cooke) is another *Mycosphaerella* sp. found in South Africa and Zambia. This fungus is the cause of leaf spot and blight of *E. globulus, E. maidenii, E. regnans* F. Mueller, *E. viminalis* L. and several other *Eucalyptus* spp. (Gibson 1975, Park & Keane 1982, Lundquist & Baxter 1985, Lundquist & Purnell 1987). Recently five new *Mycosphaerella* spp., namely *M. juvenis* Crous et M.J. Wingf. (Anamorph: *Uwebrauni juvenis* Crous et M.J. Wingf.), *M. africana* Crous et M.J. Wingf., *M. ellipsoidea* Crous et M.J. Wingf. (anamorph: *Uwebrauni ellipsoidea*), *M. crystallina* Crous & M.J. Wingf., and *M. lateralis* Crous et M.J. Wingf. (anamorph: *Uwebrauni lateralis* crous et M.J. Wingf.) were described in association with different *Eucalyptus* spp. in South Africa (Crous & Wingfield 1996). In South Africa, *M. marksii* Carnegie & Keane has also been reported from *E. grandis* and *E. nitens* (Crous & Wingfield 1996).

***Phaeoseptoria eucalypti*** Hansf. Emand [syn=*Kirramyces epicoccoides* (Cooke & Massee)] J. Walker, B. Sutton & Pascoe, which is now more correctly known as *Phaeophleospora epicoccoides* (Cooke & Massee) Crous, F.A. Ferreira & Sutton, (Crous, Ferreira & Sutton 1997), has been recorded in Malawi, Zambia and South Africa (Chipompha 1987, Wingfield 1987a, Crous, Knox-Davies & Wingfield 1988, Shakacite 1991). It causes discrete leaf spots on several *Eucalyptus* spp. (Crous, Knox-Davies & Wingfield 1989b). High levels of infection have been reported on *E. camaldulensis, E. globulus, E. saligna, E. grandis* and *E. tereticornis* Sm. (Chipompha 1987, Crous

*et al.* 1988), whereas the infection levels recorded on *E. grandis* have tended to be low. Infection is commonly observed on the older leaves and lower branches of trees (Crous *et al.* 1989b). Infected leaves initially develop numerous minute spots with purple margins. As infection develops the spots enlarge slightly. Black masses of spores are found on the under surfaces of the spots. In severe cases, infection leads to defoliation (Sharma *et al.* 1984, Chipompha 1987, Crous *et al.* 1988). According to these authors, the disease also affects seedlings in nurseries. Nichol, Wingfield & Swart (1992) indicated that plantation establishment conditions such as site preparation and fertilisation influence the susceptibility of *Eucalyptus* spp. to infection by *P. eucalypti.*

Two ***Pestalotiopsis*** spp. have been reported from *Eucalyptus* leaves in African countries. *Pestalotiopsis disseminata* (Thum.) Steyaert is reported to cause brown leaf blight on *E. citriodora*, while *P. funerea* (Desm.) Steyaert (syn.=*Pestalotia funerea* Desm.) has been reported to cause leaf spots on *E. globulus* (Doidge *et al.* 1953, Lundquist & Baxter 1985).

***Aulographina eucalypti*** (Cooke & Massee) Von Arx and Muller [anamorph: *Thyrinula eucalypti* (Cooke & Mass.) Swart] was recorded in South Africa on several *Eucalyptus* spp. including *E. globulus, E. grandis* and *E. nitens* (Crous *et al.* 1989b). It causes circular necrotic lesions on the upper or lower leaf surfaces (Doidge *et al.* 1953, Lundquist & Baxter 1985) and may result in extensive defoliation (Crous *et al.* 1989b). This disease is also known as corky leaf spot because of the distinct raised, corky spots, often with concentric rings.

***Pseudocercospora eucalyptorum*** Crous, Wingfield, Marasas and Sutton (Crous *et al.* 1989c), *Coniothyrium ovatum* Swart (Crous *et al.* 1988), *Fairmaniella leprosa* (Fairm.) Petrak and Syd. (Crous, Knox-Davis & Wingfield 1989d) and *Harknessia eucalypti* Cke. Apud Cke. & Hark. (Crous *et al.* 1989d) are among the other leaf pathogens recorded on several *Eucalyptus* spp. in South Africa. *Harknessia eucalypti, Pestalotiopsis* sp., *Botrytis* sp. and *Melanconium eucalypticola* Hansf. were reported from Zimbabwe in association with *Eucalyptus* spp. (Gibson 1964). *Harknessia* sp. and *Cryptosporiopsis* sp. have also been recorded on *Eucalyptus* spp. in Uganda (Roux *et al.* 2001a). *Conniella fragariae* (Oud.) Sutton was reported as the cause of leaf spot on *Eucalyptus* in Congo (Roux *et al.* 2000b). These diseases were not considered to be of great economic importance.

***Wilt diseases***

Wingfield (1990) indicated that the number of diseases affecting forest trees can be expected to increase significantly in the future. A large number of pathogens that have as yet, not appeared in a country, could result in devastation. For example Roux *et al.* (2000a) discovered an important new wilt and die-back disease of *Eucalyptus* in the Congo in 1998. The causal agent of this disease was identified as ***Ceratocystis fimbriata*** Ell. and Halst (Roux *et al.* 2000a). *Ceratocystis* spp. are well known causal agents of wilt disease and are amongst the most serious pathogens of woody plants in the world (Kile 1993). They range from weak pathogens to aggressive primary pathogens (Kile 1993).

The occurrence of *C. fimbriata* as a pathogen of *Eucalyptus* spp., in the Republic of the Congo, was the first record of a *Ceratocystis* sp. causing a vascular wilt on *Eucalyptus* spp. in the world. Infection by this pathogen led to a serious wilt disease of *E. urophylla* X *E. pellita* F. Muell (UP) and *E. tereticornis* Sm. X *E. grandis* (Roux *et al.* 2000a). Ceratocystis wilt has also recently been found in Uganda (Roux *et al.* 2001a). Plantations of *E. grandis* trees showed the development of epicormic shoots, dead tops and tree death (Roux *et al.* 2001a). Close examination of the main stems of the affected trees revealed extensive brown to blue streaking of the xylem. As was the case in the Congo, *Ceratocystis* wilt in Uganda resulted in high levels of mortality (Roux *et al.* 2001a). The most typical symptoms of this disease is the irregular (streaks) dark brown discoloration of the xylem (Roux *et al.* 2001a).

Bacterial wilt of *Eucalyptus* spp. caused by ***Ralstonia solanacearum*** Yabuuchi *et al.* (syn.: *Pseudomonas solanancearum*) was first described from Brazil (Hayward 1964, Ciesla, Diekmann & Putter 1996). The first report of bacterial wilt of *Eucalyptus* in Africa was in the mid 1990's, from South Africa (Coutinho *et al.* 2000). Its occurrence was also reported from the Republic of Congo and Uganda (Roux *et al.* 2000a, 2001a). The isolates found in Congo belong to Biovar 3, similar to the bacteria found in South Africa (Roux *et al.* 2000a, Coutinho *et al.* 2000). *Ralstonia solanacearum* survives in soil or on plant debris. This bacterial pathogen affects several different *Eucalyptus* spp., which include *E. urophylla, E. camaldulensis, E. grandis,* and *E. saligna. R. solanacearum* causes root disease and cracking around the root collars of infected trees (Roux *et al.* 2000a). The disease also causes extensive xylem discoloration and black streaks are present in the discoloured xylem (Hayward 1964, Roux *et al.* 2000a). A creamy to white bacterial ooze appears on the surface of cut stems (Roux *et al.* 2000a).

## DISEASES OF PLANTATION *CUPRESSUS* SPP. IN AFRICA

Trees in the *Cupressaceae* are widely planted in African countries as a source of sawn timber (Nsolomo, Madoffe & Maliondo 2000). In Africa, information on the diseases affecting the *Cupressaceae* is very limited, despite the fact that mortalities are commonly experienced.

The best described disease of the *Cupressaceae* in Africa is **Seiridium canker**. This destructive canker disease is caused by *Seiridium* spp. and is described world wide as a devastating disease in plantations and on ornamental cypresses (Graniti 1986). *S. cardinale* (Wagener) Sutton, *S. unicorni* (Cke and Ell.) Sutton & Gibson and *S. cupressi* (Guba) Boesewinkel are the three *Seiridium* spp. involved in causing stem canker on *Cupressaceae* (Boesewinkel 1983, Graniti 1986, Barnes *et al.* 2001). All three species have been found in Africa, although the taxonomy of these fungi has been a matter of substantial debate.

Seiridium canker has been reported from Kenya, North and South Africa (Rudd Jones 1953, Nattras Booth & Sutton 1963, Graniti 1986, Wingfield & du Toit 1986). The disease results in twig and branch cankers characterized by reddening or browning of the living bark. Infection results, in necrosis of tissue and gradually leads to girdling of the branches and stems of plants. This eventually results in death, first of the branches and then entire trees (Graniti 1986, 1998).

Seiridium canker, caused by *S. cupressi* (syn= *Rhynchosphaeria cupressi*) has resulted in serious losses in Kenya (Rudd Jones 1953, 1954a, 1954b, Gibson 1964). In this country, damage to fast growing *C. macrocarpa* Hartw. has resulted in termination of the planting of this tree. It has been substituted by slow growing, but less susceptible, *C. lusitanica* Mill. (Gibson 1964). Apart from Kenya, Seiridium canker has also been reported from Malawi, South Africa, Tanzania and Uganda (Gibson 1964, Graniti 1986, Wingfield & du Toit 1986).

Other diseases of *Cupressaceae* reported from Africa include stem gall, caused by *Agrobacterium tumefasciens* (Smith & Towsend 1907) Conn 1942 from Uganda and Kenya (Gibson 1964) and *Rhizoctonia lamellifera* Small. from *C. lusitanica* in Kenya (Gibson 1964). A serious stress-related disease caused by *Sphaeropsis sapinea f.* sp. *cupressi* Solel *et al.* has also been reported from South Africa (Linde, Kemp & Wingfield 1998). In Kenya, Armillaria root rot is found in association with *Cupressus lusitanica* (Mwangi *et al.* 1989, Onsando *et al.* 1997).

## DISEASES OF PLANTATION *ACACIA* SPP. IN AFRICA

A number of Australian *Acacias*, commonly known as wattles, have been introduced into Africa. *A. mearnsii* de Wild., *A. decurrens* Wendl., *A. mangium* Wild. and *A. auriculiformis* A. Cunn. Ex Benth are the major species planted in Africa (Anonymous 1978, Kihiyo & Kowero 1986, Khasa, Jallee & Bousquet 1994). Mostly, *Acacias* are used to extract tannin, for timber, pulp production, to promote biological nitrogen fixation and some are used for sand dune stabilization (Anonymous 1978, Kihiyo & Kowero 1986, Ngulube 1988, Khasa *et al.* 1994). Roux, Kemp & Wingfield (1995) provided an extensive review of the diseases of *A. mearnsii* in South Africa. The current treatment will, therefore, only briefly mention the major diseases of this tree in South Africa and will focus on diseases of other *Acacia* spp. and other African countries.

### *Root diseases*

***Phytophthora*** spp. are commonly associated with root diseases of Australian *Acacia* spp. *Phytophthora nicotianiae* (Dastur) Waterhouse [*P. parasitica* (Dastur)] was first reported from *A. mearnsii* in South Africa in the 1960's (Zeijlemaker 1971). The disease caused by *P. nicotianiae,* is commonly called black butt disease and results in collar rot of infected trees (Zeijlemaker 1971). The disease derives its common name from the resultant black discoloration and cracking of the bark at the bases of trees (Zeijlemaker 1971, Roux 2002). Infection may result in the death of trees, in a reduction of bark yield as well as a reduction in the quality of the thickest most valuable bark at the bases of trees (Sherry 1971, Haigh 1993, Roux & Wingfield 1997). More recently Roux & Wingfield (1997), isolated other *Phytophthora* spp. from black butt and root disease symptoms of *A. mearnsii* in South Africa. These included *P. boehmeriae* Sawada and *P. meadii.* McRae. In pathogenicity trials, both species were shown to be capable of causing lesions similar in size to those caused by *P. nicotianae* (Roux & Wingfield 1997).

It has been suggested that black butt is a complex of diseases not caused by a single organism (Zeijlemaker 1968). This hypothesis was supported by results of a survey conducted by Roux and Wingfield (1997). Several other pathogens were isolated from disease symptoms. It is currently thought that *Phytophthora* spp. are the primary pathogens resulting in cracks and other wounds that become infected by secondary and opportunistic pathogens. Reports also state that *P. nicotianae* can only be isolated from the basal part of infected trees (Wingfield & Roux 2000a, Roux 2002).

This has, however, been shown to be inaccurate, with *Phytophthora* spp. being isolated from areas at breast height and also from the xylem of wilting trees (Roux, personal communication).

In South Africa, three ***Ganoderma*** spp. are reported to be associated with root and collar rot of *A. mearnsii* (Gibson 1964, Lückhoff 1964). *Ganoderma lucidum* (Leyss.:Fr.) Karst causes white spongy rot (Bakshi 1976, Gorter 1977), *G. applanatum* (Pers. Wallr.) Pat. causes heart rot and *G. rugosum* Blume and Nees has been given as the cause of collar rot (Gibson 1964, Lückhoff 1964, Sherry 1971).

It has been reported that ***Lasiodiplodia theobromae*** (Synonym= *Botryodiplodia theobromae* Pat., *Diplodia natalensis* Pole Evans) caused collar rot of *A. mearnsii* in South Africa (Sherry 1971, Gibson 1975, Roux, Wingfield & Morris 1997). The disease is reported to affect the whole root system and infection spreads up the stems to form black cankers. In South Africa, several other root pathogens have also been recorded on *A. mearnsii*. These include *Macrophomina phaseolina* (Gibson 1975, Bakshi 1976), *Armillaria mellea* sensu lato and *Rhizoctonia* sp. (Kotze 1935, Laughton 1937). In Kenya, Armillaria root rot is found in association with *Acacia mearnsii, A. melanoxylon* R. Br. and *A. saligna* (Labill.) HL Wendl. (Mwangi *et al.* 1989, Onsando *et al.* 1997). Armillaria root rot, which is broadly ascribed to *A. mellea*, and collar rot caused by *Corticium* spp. have also been recorded on *A. mearnsii* in Malawi (Lee 1970).

***Stem diseases/canker***

In recent years, severe mortality of *A. mearnsii* has been reported from South Africa, caused by ***Botryosphaeria dothidea*** (Roux *et al.* 1995, Roux *et al.* 1997, Roux 2002). Infection causes stem cankers, tip die-back, wilt and death of infected trees. The internal symptoms of *Botryosphaeria* canker and wilt include discoloration and death of the cambium and xylem, which is manifested as a visible dark brown ring in cross sections of infected trees (Roux 2002). The disease is also associated with frost and drought stress.

***Fusarium*** spp. are known to be pathogens on a wide range of hosts. Roux & Wingfield (1997) reported several *Fusarium* spp. from die-back and canker symptoms on *A. mearnsii*. Included were *F. graminearium* Schwabe, which was isolated from stem and branch cankers (Roux *et al.* 2001b). *Fusarium* spp. have also been isolated from basal cankers associated with black butt disease (Zeijlemaker 1971), blister and mottle lesions associated with Ceratocystis wilt and mechanical

wounds on stems and branches (Zeijlemaker 1971, Roux *et al.* 2001b). *Fusarium graminearum* was shown to be pathogenic to *A. mearnsii* in inoculation experiments (Roux *et al.* 2001b).

Pink disease caused by ***Erythricium salmonicolor*** affects several different *Acacia* spp. In Africa its occurrence was reported on *A. mearnsii, A. auriculiformis* and other *Acacia* spp. from Mauritius and South Africa (Sherry 1971, Gibson 1975, Bakshi 1976). Infection by *E. salmonicolor* resulted in death of branches and leaf cast due to the girdling of the branches. As the infected bark dies, patches of pink mycelium appear on the surface of the dying bark (Gibson 1975, Wingfield & Roux 2000a, Roux 2002).

In South Africa ***Physalospora abdita*** (Berk & curt) N. E. Stevens (Bakshi 1976) and *Sphaeropsis* sp. (Roux & Wingfield 1997) have also been isolated from *A. mearnsii*. *P. abdita* caused stem and twig cankers on *A. mearnsii* and *A. decurrens* Wendl. (Browne 1968) while a *Sphaeropsis* sp. has been isolated from stem cankers (Roux & Wingfield 1997). In inoculation trials, the *Sphaeropsis* sp. showed high levels of pathogenicity (Roux & Wingfield 1997).

***Schizophyllum commune*** Fries is an opportunistic wound parasite that leads to the eventual death of trees. Pruning wounds are thought to be sites of infection for this pathogen (Ledeboer 1946). In Kenya, *Phoma herbarum* Westend. was reported to cause die-back on *A. mearnsii* and *A. deccurrens* (Olembo 1972). Infection by *P. herbarum* is also initiated through wounds (Olembo 1972, Gibson 1975).

***Leaf diseases***

Few foliage diseases have been reported on exotic Australian *Acacia* spp. They include leaf spot caused by *Camptomeris albiziae* (Petch) Mason (Sherry 1971, Wingfield & Roux 2000a) and *C. verruculosa* Syd. on *A. mearnsii* in South Africa (Bakshi 1976). The disease is associated with early leaf drop in autumn (Wingfield & Roux 2000a). A rust caused by *Uromycladium alpinum* McAlp. has also been reported from *A. mearnsii* in South Africa (Morris, Wingfield & Walker 1988). Apart from these two diseases on *A. mearnsii*, the only recorded leaf disease of Australian *Acacia* spp. grown in plantations in Africa is leaf spot of *A. longifolia* caused by *Cylindrocladium scoparium* Morgan (Hagemann & Rose 1988).

***Wilt disease***

One of the most devastating diseases of *A. mearnsii* is that caused by the wilt pathogen, ***Ceratocystis albofundus*** De Beer, Wingfield & Morris. This disease was first described from South Africa in 1988 (Morris, Wingfield & De Beer 1994). The most common symptom associated with *C. albofundus* infection is the rapid wilt and death of susceptible trees, the formation of swollen gum pockets in the stems, stem cankers, extensive oozing of gum and discoloration of the xylem (Morris *et al.* 1994, Roux *et al.* 1997, 2000a).

*Ceratocystis* wilt, caused by *C. albofundus,* has been reported only from South Africa (Morris *et al.* 1994) and Uganda (Roux *et al.* 2001a). Roux *et al.* (2001d) suggested that *C. albofundus* is native to South Africa. The only alternative hosts known for *C. albofundus* are South African *Protea* spp. (Gorter 1977) and *A. decurrens* (Roux *et al.* 2001d). *Ceratocystis* spp. require wounds to initiate infection. Thus, *C. albofundus* is especially damaging where trees are affected by hail, insect or silvicultural practices (Roux 2002) that cause wounding of trees.

## EXOTIC PLANTATION FORESTRY IN ETHIOPIA

In Ethiopia, wood plays a major role in meeting more than 85% of the energy requirements of the country. Mostly, this wood comes from the natural forests. For this reason, natural forest resources are diminishing rapidly. Estimates indicate that the natural forest cover has declined from 40% to 2.4% in the 1990's (Davidson 1988, Anonymous 1994). At present, the annual rate of forest exploitation is much higher than the annual replacement, both in terms of area and yield. If this trend continues, the remaining natural forests will not remain for long and it may not be possible to meet the demand for wood products. To overcome this problem, exotic tree planting was commenced and has been practiced for many years in different parts of Ethiopia. Generally speaking it is said that the planting of exotic tree species started with the introduction of *E. globulus* in the late 1890's.

Currently, in Ethiopia, fast growing exotic species such as *E. globulus, E. camaldulensis, E. saligna, E. grandis,* and *E. citriodora* are widely planted in different parts of the country (Persson 1995, Negash 1997). *C. lusitanica, P. patula, Grevillea robusta* Cunn., *A. mearnsii* and *A. decurrens* are among other genera planted, both in plantations and around homesteads. Plantations of these exotic species cover a total area of about 200 000 ha (Anonymous 1994, Vercoe 1995).

This figure indicates only the areas of the plantations in National Forest Priority Areas, Peri-urban plantations and community woodlots. It does not include those trees planted around homesteads, farmlands or those planted for rehabilitation of degraded land.

Many exotic plantation species die at the seedling stage or at maturity for various reasons. However, no study has been conducted to investigate the cause of this death and it has been usually arbitrarily associated with poor species site matching and inadequate tending practices. The role of biotic factors in tree death is underestimated, poorly understood and has not received much attention in Ethiopia.

Generally, little information is available on the damage pathogens cause to plantation trees in Ethiopia. A few records of tree diseases in Ethiopia can be found. Several of these deal with *Armillaria* spp. One such report mentions the infection of *Armillaria* spp. of pine trees (Mengistu 1992) and another indicates the occurrence of *A. mellea* in *Coffea arabica* L. plantations (Eshetu, Teame & Girma 2000). According to these reports *Armillaria* spp. were found on recently cleared and planted sites and where shade trees had been removed. Recently Ota, Intini & Hattori (2000) reported that the *Armillaria* sp. found on a hard wood species at Kerita and Jimma is *A. mellea sensu stricto*.

Some records of tree pathogens from Ethiopia can be found in herbarium and survey reports. For example, Gibson (1972) mentioned that T. Middleton had found *Dothistroma* needle blight on *P. radiata* trees around Addis Ababa. Simillarly, based on unpublished records, Crous *et al.* (1989c) mentioned that *Pseudocerospora eucalyptorum,* the cause of *Eucalyptus* leaf spot, has been recorded from Worota, North Ethiopia. Walker, Sutton & Pascoe (1992) also mentioned that they obtained a specimen of *Phaeoseptoria epicocoides* from Ethiopia that was collected from *E. saligna* and *E. globulus* at Gora and Gumuro. However, the importance of these fungi in causing leaf spot on *Eucalyptus* in Ethiopia is not clear.

There are some reports that deal with diseases of native tree species in Ethiopia. The results of one of these studies reported the occurrence of *Antrodia juniperina* (Murrill) Niemela & Ryvarden on *Juniperus exelsa* Hochest. Ex. Endl. (Niemela & Ryvarden 1975). *A. juniperina* is reported to be parasitic and saprophytic on stems of *J. exelsa* and to cause heart rot and necrosis of the butt. Infection causes intensive brown cubical rot of the wood (Niemela & Ryvarden 1975). In another report Niemela, Revenvall & Hjortstam (1998) recorded several decay fungi in natural stands of

*Hagenia abyssinica* (Bruce) J. F Gmel. in East Africa, including Ethiopia. This report only mentioned fungi involved in decaying *H. abyssinica.* They included *Hymenochaete ochromarginata* Talbot. collected from living trunks and stumps. This fungus is considered to be the main decayer of living *Hagenia* trees. Other wood rot fungi included *Phellinus ferruginosus* (Schrad. Fr:) Bourdot and Galizn and *Trametes socotrana* Cooke, collected from fallen branches and stems. Their role in disease of *Hagenia* can, however, be questioned as they are not generally considered to be primary pathogens. A number of *Corticioid* fungi have also recorded from *H. abyssinica* branches and stems. They include *Asterostroma medium* Bres., *Cystidiodontia isabellina* (Berk. & Broome) Hjortstam and *Dichostereum kenyense* Boidin & Lanq (Niemela *et al.* 1998).

Recently, tree deaths of unknown causes have been experienced in many plantations in Ethiopia. Many different disease symptoms are associated with the dying trees. This situation necessitates a study of the pathological problems in plantations in order to develop strategies to reduce losses to this important enerprise.

## CONCLUSIONS

In this review I have attempted to include information on as many of the diseases of exotic plantation trees in Africa as possible in the limited space available. However, the information available on forest diseases from African countries is scanty and often recorded in unpublished government reports. This limitation has meant that information has been gained form work done in only a few countries and often published only in the form of brief notes. Nevertheless, the information included in this review should help to provide insight into the impact of diseases in exotic plantations in Africa. Hopefully, it will also provide valuable foundation for future forest pathology studies in Africa.

The rapidly growing demand for forest products will necessitate the expansion of exotic plantations in Africa. The introduction of exotic tree species into Ethiopia commenced a century ago. Up to now, not less than 160 exotic trees and shrubs have been introduced to the country, for different purposes. In a situation where exotic plantations substitute the native forests, an outbreak of disease could severely damage plantations.

Diseases have had a serious impact on exotic plantations in various parts of Africa. Various of these have been discussed in this review. It must be expected that other diseases will negatively impact of exotic plantation forestry in the future. Thus, every effort must be made to reduce this situation.

It is clear from this review that the problems already experienced with tree diseases in Africa highlight the urgency of studying tree diseases in Ethiopia. In Ethiopia the significance of pathogens in tree health has had little attention in the past. Hence, to minimise the risk associated with diseases of exotic plantations it is essential to obtain adequate information on the prevalence of disease causing organisms in plantations. It is also equally important to understand the risks of diseases to various tree species suited to planting in the country. This knowledge will provide a firm base on which to develop appropriate disease management strategies.

## REFERENCES

Abomo-Ndongo, S. & Guillaumin, J.-J. (1997) Somatic incompatibility among African *Armillaria* isolates. *European Journal of Forest Pathology* **27**: 201-206.

Allen, E. A. & Humble, L. M. (2002) Non-indigenous introductions: A threat to Canada's forests and forest economy. *Canadian Journal of Plant Pathology* **24**: 103-110.

Anagnostakis, S. L. (1987) Chestnut blight: the classical problem of an introduced pathogen. *Mycologia* **79**: 23-37.

Anagnostakis, S. L. (1988) *Cryphonectria parasitica*, cause of chestnut blight. *Advances in Plant Pathology* **6**: 123-136.

Anderson, R. L., Belcher, E. & Miller, T. (1984) Occurrence of seed fungi inside slash Pine seed produced in seed orchards in the United States. *Seed Science and Technology* **12**: 795-799.

Anonymous (1978) Biological control of Australian *Acacias*. *News No. 62*. Pietermaritzburg. South Africa.

Anonymous (1994) *Ethiopian Forestry Action Plan. Final report.*, Vol. I. pp 14 and Vol. II, pp 34. Addis Ababa, Ethiopia.

Anonymous (1998). *Forestry facts for the year 1996/97*. Forestry Owners Association. Rivonia, South Africa.

Bakshi, B. K. (1976) Wattles-*Acacia* spp. In *Forest pathology: Principles and practice in forestry:* pp191-194. F. K. I. Press. Forest Research Institute and Colleges, Dehra Dun, India.

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

Barnes, I., Roux, J., Wingfield, M. J., Coetzee, M. P. A. & Wingfield, B. D. (2001) Characterization of *Seiridium* spp. associated with cypress canker, based on β-Tubulin and Histone sequence. *Plant Disease* **85**: 317-321.

Baxter, A. P., Van der Westhuizen, G. C. A. & Eicker, A. (1983) Morphology and taxonomy of South African isolates of *Colletotrichum*. *South African Journal of Botany* **2**: 259-289.

Bettucci, L. & Saravay, M. (1993) Endophytic fungi of *Eucalyptus globulus*: a preliminary study. *Mycological Research* **97**: 679-682.

Bloomberg, W. J. (1981) Disease caused by *Fusarium* in forest nurseries. In *Fusarium*: *Disease, biology and taxonomy* (P. E. Nelson, T. A. Toussoun, & R. J. Cook eds): pp. 178-187. Pennsylvania State University Press: University Park, USA.

Boerboom, J. H. & Maas, P. W. T. (1970) Canker of *Eucalyptus grandis* and *E. saligna* in Surinam caused by *Endothia havanensis*. *Turrialba* **20**: 94-99.

Boesewinkel, H. J. (1983) New records of the three fungi causing cypress canker in New Zealand, *Seiridium cupressi* (Guba) comb. nov and *S. cardinale* on *Cupressocyparis* and *S. unicorne* on *Cryptomeria* and *Cupressus*. *Transactions of the British Mycological Society* **80**: 544-547.

Bottomley, A. M. (1937) Some of the more important diseases affecting timber planting in the Transvaal. *South African Journal of Science* **33**: 373-376.

Brasier, C. M. (1990) China and the origins of Dutch elm disease: an appraisal. *Plant Pathology* **39**: 5-16.

Bright, C. (1998) *Life out of bounds. Bio-invasion in a borderless world.* New York: W. W. Norton.

Browne, F. G. (1968) *Pest and diseases of forest plantation trees.* Clarendon Press: Oxford, UK.

Carnegie, A. J. (1991) *The susceptibility of certain Eucalyptus species and provenances to infection by Mycosphaerella spp. and other leaf parasites*. B. Sc. Honours Thesis. La Trobe University, Australia.

Carnegie, A. J. (2000) *A study of the species of Mycosphaerella on Eucalyptus in Australia and the impact of the Mycosphaerella leaf disease on Eucalyptus globules Labill.* Ph. D Thesis. The University of Melbourne, Australia.

Carnegie, A. J. & Keane, P. J. (1994) Variation in susceptibility of *Eucalyptus globulus* provenances to *Mycosphaerella* leaf disease. *Mycological Research* **98**: 413-418.

Chipompha, N. W. S. (1987) *Phaeoseptoria eucalypti*: A new pathogen of *Eucalyptus* in Malawi. *South African Journal of Forestry* **142**: 10-12.

Chou, C. K. S. (1981) Monoculture, species diversifications, and disease hazards in forestry. *New Zealand Journal of Forestry* **26**: 20-36.

Christensen, P. S. & Gibson, I. A. S. (1964) Further observations in Kenya on a foliage disease of Pines caused by *Dothistroma pini* Hulbary, effect of disease on height and diameter increment in three and four year old *Pinus radiata*. *Commonwealth Forestry Review* **43**:326-331.

Ciesla, W. M., Diekmann, M. & Putter, C. A. J. (1996) *FAO/IPGRI Technical guidelines for the safe movement of germpalasm*, No. 17. *Eucalyptus* spp. FAO/IPGRI: Rome, Italy.

Ciesla, W. M., Mbugua, D. K. & Ward, J. D. (1995) Ensuring forest health and productivity; A perspective from Kenya. *Journal of Forestry* **93**: 36-39.

Coetzee, M. P. A. (1997) *Characterisation of Armillaria in South Africa*. M. Sc. Thesis, Faculty of Science, Department of Microbiology and Biochemistry, University of Orange Free State, South Africa.

Coetzee, M. P. A., Wingfield, B. D., Coutinho, T. A. & Wingfield, M. J. (2000) Identification of the causal agent of *Armillaria* root rot of *Pinus* species in South Africa. *Mycologia* **92**: 777-785.

Conradie, E., Swart, W. J. & Wingfield, M. J. (1990) *Cryphonectria* canker of *Eucalyptus*, an important disease in plantation forestry in South Africa. *South African Forestry Journal* **152**: 43-49.

Coutinho, T. A., Wingfield, M. J., Alfenas, A. C. & Crous, P. W. (1998) *Eucalyptus* rust: a disease with the potential for serious international implications. *Plant Disease* **82**: 819-825.

Coutinho, T. A., Roux, J., Riedel, K. H., Terblanche, J. & Wingfield, M. J. (2000) First report of bacterial wilt caused by *Ralstonia solanacearum* on *Eucalyptus* in South Africa. *Forest Pathology* **30**:1-8.

Coutinho, T. A., Preisig, O., Mergaert, J., Cnockaert, M. C., Riedel, K-H., Swings, J. & Wingfield, M. J. (2001) Bacterial blight and die-back of *Eucalyptus* species hybrids and clones in South Africa. *Plant Disease* **86**: 20-25.

Crous, P. W., Knox-Davies, P. S. & Wingfield, M. J. (1988) *Phaeoseptoria eucalypti* and *Coniothyrium ovatum* on *Eucalyptus* spp. in South Africa. *Phytophylactica* **20**: 337-340.

Crous, P. W., Knox-Davies, P. S. & Wingfield, M. J. (1989a) A list of *Eucalyptus* leaf fungi and their potential importance to South African forestry. *South African Forestry Journal* **149:**16-29.

Crous, P. W., Knox-Davies, P. S. & Wingfield, M. J. (1989b) A summary of fungal leaf pathogens of *Eucalyptus* and the diseases they cause in South Africa. *South African Forestry Journal* **149**: 9-16.

Crous, P. W., Wingfield, M. J., Marasas, W. F. O. & Sutton, B. C. (1989c) *Pseudocercospora eucalyptorum* sp. nov. on *Eucalyptus* leaves. *Mycological Research* **93**: 394-398.

Crous, P. W., Knox-Davies, P. S., & Wingfield, M. J. (1989d) Newly recorded foliage fungi of *Eucalyptus* spp. in South Africa. *Phytophylactica* **21**: 85-88.

Crous, P. W., Knox-Davies, P. S. & Wingfield, M. J. (1989e) Infection studies with *Phaeoseptoria eucalypti* and *Coniothyrium ovatum* on *Eucalyptus* spp. *South African Forestry Journal* **149**: 31-35.

Crous, P. W., Phillips, A. J. L. & Wingfield, M. J. (1991) The genera *Cylindrocladium* and *Cylindrocladiella* in South Africa, with special reference to forest nurseries. *South African Forestry Journal* **41**: 11-14.

Crous, P. W., Phillips, A. J. L. & Wingfield, M. J. (1993) New records of *Cylindrocladium* and *Cylindrocladiella* spp. in South Africa. *Plant Pathology* **42**: 302-305.

Crous, P. W. & Wingfield, M. J. (1996) Species of *Mycosphaerella* and their anamorphs associated with leaf blotch disease of *Eucalyptus* in South Africa. *Mycologia* **88**: 441-458.

Crous, P. W., Ferreira, F. A. & Sutton, B. C. (1997) A comparison of the fungal genera *Phaeophleospora* and *Kirramyces* (Coelomycetes). *South African Journal of Botany* **63**: 111-115.

Crous, P. W. (1998) *Mycosphaerella* spp. and their anamorphs associated with leaf spot diseases of *Eucalyptus*. *Mycologia Memior* **21**: 1-170.

Currie, D. & Toes, E. (1978) Stem volume loss due to severe *Diplodia* infection in a young *Pinus radiata* stand. *New Zealand Journal of Forestry* **23**:143-148.

Da Costa, E. B. (1955) Effects of blue stain on the strength of *Pinus radiata*. *CSIRO Forest Products Newsletter* **209**, Australia.

Darvas, J. M., Scott, D. B. & Kotze, D. B. (1978) Fungi associated with damping-off in coniferous seedlings in South African nurseries. *South African Forestry Journal* **104**:15-19.

Davidson, J. (1988) Preparatory assistance of research for afforestation and conservation, in Ethiopia. Research management of the Forestry Research Centre. FAO/UNDP. Addis Ababa, Ethiopia.

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

Denison, N. P. & Kietzka, J. E. (1993) The use and importance of hybrid intensive forestry in South Africa. *South African Forestry Journal* **165**: 55-60.

Doidge, E. M. (1950) The South African fungi and lichens to the end of 1945. *Bothalia* **5**: 1-1094.

Doidge, E. M., Bottomley, A. M., Van Der Plank, J. E. & Pauer, G. D. C. (1953) A revised list of plant diseases in South Africa. South African Department of Agriculture. *Science Bulletin* **346**.

Dungey, H. S., Carnegie, A. J., Ades, P. K. & Potts, B. M. (1995) Genetic variation in *Eucalyptus nitens, E. globulus* and their $F_1$ hybrid to damage by *Mycosphaerella* leaf disease. In *Proceedings of CRCTHF-IUFRO Conference on Eucalyptus Plantations: Improving Fibre Yield and Quality*: pp 235-236. CRCTHF-IUFRO, Hobart, Australia.

Dwinell, L. D., Barrows-Broaddus, J. B. & Kuhlman, E. G. (1985) Pitch canker: A disease complex of Southern pines. *Plant Disease* **69**: 270-276.

Eldridge, K. G. (1961) Significance of *Diplodia pinea* in plantations. *Review of Applied Mycology* **41**: 339

Eldridge, K., Davison, J., Harwood, C. & Van Wyk, G. (1997) *Eucalyptus* domestication and breeding. Oxford Science Publications.

Erwin, D. L. & Ribeiro, O. K. (1996) *Phytophthora, diseases world wide*. APS Press, St. Paul, Minnesota.

Eshetu Derso, Teame Gebrezgi & Girma Adugna (2000) Significance of minor diseases of *Coffea arabica* L. in Ethiopia. A review. In *Proceedings of the workshop on control of Coffee berry Disease (CBD) in Ethiopia*. p. 58-65. Ethiopian Agricultural Research Organisation: Addis Ababa, Ethiopia.

Evans, J. (1984) *Plantation forestry in the tropics*. Clarendon Press: Oxford, UK.

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

Florence, E. J. M., Sharma, J. K. & Mohanan, C. (1986) A Stem canker disease of *Eucalyptus* caused by *Cryphonectria cubensis* in Kerala, Kerala Forest Research Institute. *Scientific Paper* **66**: 384-387.

Germishuizen, P. J. (1979) *The re-establishment of Pinus patula Schlecht et Cham. on a pulp wood rotation in Swaziland*. MSc. Thesis. University of Stellenbosch. South Africa.

Germishuizen, P. J. (1984) *Rhizina undulata* –A Pine seedling pathogen in South Africa. In *IUFRO symposium on site and productivity of fast growing plantations* (D. C. Grey, A. P. G. Schonau, C. J. Shuutz, A. Van Laar eds): pp 968. South African Forestry Research Institute. Pretoria, South Africa.

Gibbs, J. N. (1978) Intercontinental epidemiology of Dutch Elm Disease. *Annual Review of Phytopathology* **16**: 287-307.

Gibson, I. A. S. (1964) The impact of disease on forest product in Africa. In *FAO/ IUFRO symposium on internationally dangerous forest diseases and insect*: pp 1-13. Oxford, UK.

Gibson, I. A. S., Christensen, P. S. & Munga, F. N. (1964) First observations in Kenya on a foliage disease of pines, caused by *Dothistroma pini* Hulbary. *Commonwealth Forestry Review* **43**: 31-48.

Gibson, I. A. S. & Hudson, J. C. (1969) Pelleting of pine seeds with Rhizoctol and other fungicides for control of damping-off in Kenya highland nurseries. *East African Agriculture and Forestry Journal* **35**: 99-103.

Gibson, I. A. S. & Munga, F. M. (1969) A note on terminal crook disease of pines in Kenya. *East African Agriculture and Forestry Journal* **35**: 135-140.

Gibson, I. A. S. (1970) Diseases of *Pinus patula*- A review. *Commonwealth Forestry Review* **49**: 267-274.

Gibson, I. A. S. (1972) Dothistroma blight of *Pinus radiata*. *Annual Review of Phytopathology* **10**: 51-72.

Gibson, I. A. S. (1975) *Diseases of forest trees widely planted as exotics in the tropics and Southern Hemisphere. Part I. Important members of the Myrtaceae, Leguminosae, and Meliace*: pp. 21-34. Commonwealth Forestry Institute. University of Oxford, UK.

Gibson, I. A. S. (1979) *Diseases of forest trees widely planted as exotics in the tropics and southern hemisphere. Part II, The genus pinus*: Commonwealth Forestry Institute. University of Oxford, Oxford, UK.

Gibson, I. A. S. (1981) A canker diseas of *Eucalyptus* new to Africa. *Forest Genetics Resource Information* **10**: 23-24.

Gorter, G. J. A. (1977) Index of plant pathogens and the diseases they cause in cultivated plants in South Africa. Deparment of Agricultural Technical Srvices, South Africa. *Science Bulletin* **392.**

Graniti, A. (1986) *Seiridium cardinale* and other cypress cankers. Bulletin OEPP/EPPO. *Bulletin* **16**: 479-486.

Graniti, A. (1998) Cypress canker: a pandemic in progress. *Annual Review of Pahtology* **36**: 91-114.

Hagemann, G. D. & Rose, P. D. (1988) Leaf spot and blight on *Acacia longifolia* caused by *Cylindrocladium scoparium*: A new host record. *Phytophylactica* **20**: 311-316.

Haigh, H. (1993) *Growing black wattle*. Forestry Development, Department of Water Affairs and Forestry, Pretoria, *Extension Leaflet* **1/93** (15).

Hayward, A. C. (1964) Characteristics of *Pseudomonas solanacearum*. *Journal of Applied Bacteriology* **27**: 265-277.

Heather, W. A., Pratt, B. H. & Chin, T. Y. (1977) Pre- and post-emergence damping-off on seedlings of *Pinus* species by *Phytophthora cinnamomi* and *Ph. drechsleri*. *Australian Journal of Botany* **25**: 358-359.

Hepting, G. H. (1974) Death of the American Chestnut. *Journal of Forest History* **18**: 60-67.

Heybroek, H. M. (1980) Monoculture versus mixture: Interactions between susceptible and resistant trees in mixed stands. In *Proceedings of The Third International Workshop on the Genetics of Host-Parasite Interactions in Forestry* (H. M. Heyboek, B. R. Stephan and K. Von Weissenberg eds): pp 326-341. Centre for Agricultural Publishing and Documentation, Wageningen, Netherlands.

Hocking, D. (1968) Fungi associated with damped-off and healthy pine seedlings and with the seed in East African pine nurseries. *Transactions of the British Mycological Society* **51**: 227-232.

Hodges, C. S. (1979) The implications of diseases and insects in managing tropical forests. In *Simposio Internacional Sobre Las Ciencias Forestales y su contribucion al desarrollo de la America tropical*. Editado por Manuel Chavarria: pp 75-80. San Jose, Costa Rica.

Hodges, C. S., Geary, T. F. & Cordell, C. E. (1979) The occurrence of *Diaporthe cubensis* on *Eucalyptus* in Florida, Hawaii and Puerto Rico. *Plant Disease Reporter* **63**: 216-220.

Hodges, C. S., Alfenas, A. C. & Ferreria, F. A. (1986) The conspecificity of *Cryphonetria cubensis* and *Endothia eugeniae*. *Mycologia* **78**: 343-350.

Hunter, G. C., Crous, P. W., Roux, J., Coutinho, T. A., Wingfield, B. D. & Wingfield, M. J. (2002) Identification of *Mycosphaerella* spp. associated with leaf disease of *Eucalyptus nitens* in South Africa. *Australian Journal of Pathology* (Submitted).

Ivory, M. H. (1968) Reaction of pines in Kenya to atacks by *Dothistroma pini* var. *Keniensis*. *East African Agricultural and Forestry Journal* **33**: 236-244.

Ivory, M. H. & Wingfield, M. J. (1986) First report of *Mycosphaerella gibsonii* in South Africa. *Phytophylactica* **18**: 51-53.

Ivory, M. H. (1987) *Diseases and disorders of Pines in the tropics*. Overseas Research publication. No 31. Oxford Forestry Institute: Oxford, UK.

Ivory, M. H. (1994) Records of foliage pathogens of *Pinus* species in tropical countries. *Plant Pathology* **43**: 511-518.

Khasa, P. D., Jallee, G. & Bousquet, J. (1994) Biological studies in the utilization of *Racosperma auriculiformis* and *Racosperma mangium* in tropical countries. *Journal of Tropical Forest Science* **6**: 442-443.

Kihiyo, V. B. & Kowero, G. S. (1986) Some economic aspects of the wattle industry in Tanzania. *Journal of World Forest Resouce Management* **2**: 57-62.

Kile, G. A. (1993) Plant disease caused by species of *Ceratocystis sensu stricto* and *Chalara*. In *Ceratocystis and Ophiostoma: Taxonomy, Ecology, and Pathogenicity*. (M. J. Wingfield, K. A. Seifert, J. A. Webber, M. N. eds): pp 173-183. APS Press. St Paul, Minnesota.

Kotze, J. J. (1935) Forest fungi: The position in South Africa. *In the British Empire Forestry Conference*. Government printer: Pretoria.

Laughton, E. M. (1937) The incidence of fungal disease on timber trees in South Africa. *South African Journal of Science* **33**: 377-382.

Leakey, R. R. B. (1987) Clonal forestry in the tropics-A review of development strategies and opportunities. *Commonwealth Forestry Review* **66**: 61-75.

Ledeboer, M. S. J. (1946) Development in pathological research on Wattle. *South African Forestry Journal* **4**: 30-45.

Lee, R. F. (1970) *A first checklist of tree diseases in Malawi*. Malawi Research Institute. Research records No 43.

Libby, W. J. (1982) What is a safe number of clones per plantation? In: resistance to disease and pests in forest trees. In *Proceeding of the 3rd international workshop on the genetics of host parasite interactions in forestry*: pp 342-360. Wageningen, The Netherlands.

Linde, C., Kemp, G. H. J. & Wingfield, M. J. (1994a) Disease of *Pines* and *Eucalyptus* in South Africa associated with *Pythium* and *Phytophthora* species. *South African Forestry Journal* **169**: 25-32.

Linde, C., Kemp, G. H. J. & Wingfield, M. J. (1994b) *Pythium irregulare* associated with *Pinus* seedling death on previously cultivated lands. *Plant Disease* **78**: 1002-1005.

Linde, C., Kemp, G. H. J. & Wingfield, M. J. (1994c) *Pythium* and *Phytophthora* spp. associated with *Eucalyptus* and pines in South Africa. *European Journal of Forest Pathology* **24**: 345-356.

Linde, C., Wingfield, M. J. & Kemp, G. H. J. (1994d) Root and root collar disease of *Eucalyptus grandis* caused by *Pythium splendens*. *Plant Disease* **78**:1006-1009.

Linde, C., Kemp, G. H. J. & Wingfield, M. J. (1998) First report of *Sphaeropsis sapinea* canker on cypress in South Africa. *European Journal of Forest Pathology* **27**: 173-177.

Lückhoff, H. A. (1955) Two hitherto unrecorded fungal diseases attacking pine and *Eucalyptus* in South Africa. *Journal of the South African Forest Association* **26**: 147-161.

Lückhoff, H. A. (1964) Diseases of exotic plantation trees in the Republic of South Africa. In IV *FAO/IUFR Symposium on international dangerous forest diseases and insects*: pp 1-11, Oxford, UK

Lundquist, J. E. (1984a) The occurrence and distribution of Rhizina root rot in South Africa and Swaziland. *South African Forestry Journal* **78**: 22-24.

Lundquist, J. E. (1984b) First report of terminal crook disease caused by *Colletotrichum acutatum* on *Pinus radiata* seeldings in South Africa. *Plant Disease* **68:** 732 (abstract).

Lundquist, J. E. & Roux, C. (1984) Dothistroma needle blight of *Pinus patula, P. radiata* and *P. canariensis* in South Africa. *Plant Disease* **68**: 918 (abstract).

Lundquist, J. E. (1985) Reduced growth rates of *Eucalyptus nitens* caused by *Mycosphaerella molleriana*. *Phytophylactica* **17**: 55 (Abstract).

Lundquist, J. E. & Baxter, A. P. (1985) Fungi associated with *Eucalyptus* in South Africa. *South African Forestry Journal* **138**: 9-19.

Lundquist, J. E. (1986) Fungi associated with *Pinus* in South Africa. Part I. The Transvaal. *South African Forestry Journal* **130**:1-13.

Lundquist, J. E. & Foreman, T. C. (1986) A leaf blight of *Eucalyptus* seedlings in South African nurseries caused by *Hainesia lythri* . *South African Forestry Journal* **136**: 22-25.

Lundquist, J. E. (1987) Fungi associated with *Pinus* in South Africa. Part II. The Cape. *South African Forestry Journal* **140**: 5-15.

Lundquist, J. E. & Purnell, R. C. (1987) Effects of Mycosphaerella leaf spot on growth of *Eucalyptus nitens*. *Plant Disease* **71**: 1025-1029.

Maiteki, G. A., Hakiza, G. J., Kiwuso, P. & Opiro, K. (1999) Pests and diseases of tree seedlings. A survey report. Report submitted to the strengthening of Forestry Department Project (SFD), Uganda Forestry Department, Kampala, Uganda.

Manion, P. D. (1981) *Tree disease concepts*. Prentice Hall, New Jersey.

Marks, G. C. & Kassaby, F. Y. (1974) The Pathogenicity of *Pythium* spp. and *Phytophthora drechsleri* to *Eucalyptus* spp. *Australian Journal of Botany* **22**: 661-668.

Marks, G. C. & Minko, G. (1969) The pathogenicity of *Diplodia pinea* to *Pinus radiata* D. Don. *Australian Journal of Botany* **17**: 1-12.

Maseko, B., Burgess, T., Coutinho, T. A. & Wingfield, M. J. (2001) First report of *Phythophthora nicotianae* associated with *Eucalyptus* die-back in South Africa. *Plant Pathology* **50**: 413.

Masuka, A. (1989) The incidence of Armillaria root and butt diseases in *Pinus* spp. plantations in Zimbabwe. *Commonwealth Forestry Review* **68**:121-125.

Masuka, A. J. (1990) A new canker of *Eucalyptus grandis* Hill ex Maid in Zimbabwe. *Commonwealth Forestry Review* **69**: 195-200.

Masuka, A. J. & Nyoka, B. I. (1995) Susceptibility of *Eucalyptus grandis* provenances to a root rot associated with *Ganoderma sculptrutum* in Zimbabwe. *European Journal of Forest Pathology* **25**: 65-72.

Mengistu Huluka (1992) Some aspects of forest tree disease in Ethiopia. In *National workshop on setting Forestry Research Priorities*: pp 98-120. Forestry Research Centre. Addis Ababa, Ethiopia.

Mohammed, C. (1994) The detection and species identification of African *Armillaria*. In *Modern assays for plant pathogenic fungi*: *Identification, detection and quantification* (A. Schots, F. M. Dewey, & R. P. Oliver eds): pp 141-147. CAB International. Oxford, UK.

Morgan, P. D. & Driver, C. H. (1972) Rhizina root rot of Douglas-fir seedlings planted on burned sites in Washington. *Plant Disease Reporter* **56**: 407-409.

Morris, M. J., Wingfield, M. J. & Walker, J. (1988) First record of a rust on *Acacia mearnsii* in South Africa. *Transactions of the British Mycological Society* **90**: 324-327.

Morris, M. J., Wingfield, M. J. & De Beer, C. (1994) Gummosis and wilt of *Acacia mearnsii* in South Africa caused by *Ceratocystis fimbriata*. *Plant Pathology* **42**: 814-817.

Mwangi, L. M., Lin, D. & Hubbes, M. (1989) Identification of Kenyan *Armillaria* isolates by cultural morphology, intersterility tests and analysis of isozyme profiles. *European Journal of Forest Pathology* **19**: 399-406.

Mwangi, L., Mwenje, E., Makambila, C., Chanakira-Nyahwa, F., Guillaumin, J-J. & Mohammed, C. (1994) Ecology and pathogenecity of *Armillaria* in Kenya, Zimbabwe and the Congo. In *Proceedings of the 8th IUFRO Conference on Root and Butt Rots* (Johanson and J. Stenlid eds): pp 34-44. Upsala, Sweden.

Mwenje, E. & Ride, J. P. (1996) Morphological and biochemical characterization of *Armillaria* isolates from Zimbabwe. *Plant Pathology* **45**: 1036-1051.

Nattrass, R. M., Booth, C. & Sutton, B. C. (1963) *Rhynchosphaeria cupressi* sp. nov. the causal organsim of Cupressus canker in Kenya. *Transactions of the British Mycological Society* **46**: 102-106.

Negash Mammo (1997) *Performance of Pinus patula and Pinus patula ssp. tecunumanii provenances at Bonga*. M.Sc. Thesis. Swedish University of Agricultural Sciences, Faculty of Forestry. Sweden.

Newhook, F. J. & Podger, F. D. (1972) The role of *Phytophthora cinnamomi* in Australian and New Zealand forests. *Annual Review of Phytopathology* **10**: 299-326.

Ngulube, M. R. (1988) Survival and growth of seedlings of 14 Australian dry-zone *Acacias* under nursery conditions in Zoma, Malawi. *Forest Ecology and Management* **25**: 291-297.

Nichol, N. S., Wingfield, M. J. & Swart, W. J. (1992) Differences in susceptibility of *Eucalyptus* species to *Phaeoseptoria eucalypti*. *European Journal of Forest Pathology* **22**: 418-423.

Niemela, T., & Ryvarden, L. (1975) Studies in the *Aphyllophorales* of Africa IV: *Antrodia juniperina*, new for East Africa. *Transactions of the British Mycological Society* **65**: 427-432.

Niemela, T., Renvall, P. & Hjortstam, K. (1998) *Hagenia abyssinica* and its fungal decayers in natural stands. *Journal of Botany* **55**: 473-484.

Nsolomo, V. R., Madoffe, S. S. & Maliondo, S. M. S. (2000) Prospects for control of disease and insect pests of exotic trees in East Africa. Department of Forest Biology, Sokoine University of Agriculture, Morogoro, Tanzania. Unpublished.

Ofosu-Asiedu, A. (1975) A new disease of *Eucalyptus* in Ghana. *Transactions of the British Mycological Society* **65**: 285-289.

Old, K. M., Yuan, Z. Q., and Kobayashi, T. (1991) A *Valsa* teleomorph for *Cytospora eucalypticola*. *Mycological Research* **95**: 1253-1256.

Olembo, T. W. (1972) *Phoma herbarum* Westend.: A pathogen of *Acacia mearnsii* De Wild. in Kenya. *East African Agricultural and Forestry Journal* **October 1972**: 201-206.

Onsando, J. M., Wargo, P. M. & Waudo, S. W. (1997) Distribution, severity and spread of *Armillaria* root disease in Kenya tea plantations. *Plant Disease* **81**: 133-137.

Ota, Y., Intini, M. & Hattori, T. (2000) Genetic characterization of heterothallic and non-heterothallic *Armillaria mellea* sensu stricto. *Mycological Research* **104**: 1046-1054.

Palm, M. E. (1999) Mycology and world trade: a review from the front line. *Mycologia* **91**: 1-12.

Palmer, M. A. & Nicholls, T. H. (1985) Shoot blight and collar rot of *Pinus resinosa* caused by *Sphaeropsis sapinea* in forest nurseries. *Plant Disease* **69**: 739-740.

Palmer, M. A., McRoberts, R. E. & Nicholls, T. H. (1988) Source of inoculum of *Sphaeropsis sapinea* in forest tree nurseries. *Phytopathology* **78**: 831-835.

Park, R. F. & Keane, P. J. (1982) Leaf diseases of *Eucalyptus* associated with *Mycosphaerella* species. *Transactions of the British Mycological Society* **79**: 101-115.

Pegler, D. N. (1977) A preliminary agaric flora of East Africa. *Kew Bulletin, Additional Series* **6**: 91-95.

Persson, A. (1995) Exotic-prospects and risks from European and African viewpoint. *Buvisindi Agricultural Science* **9**: 47-62.

Peterson, G. W. (1977) Infection, epidemiology and control of Diplodia blight of Austrian Ponderosa and Scots pines. *Phytopathology* **67**: 511-514.

Punithalingam, E. & Waterston, J. M. (1970) *Diplodia pinea*. C.M.I. Descriptions of pathogenic fungi and bacteria. No 273. Mycological Institute: Kew, Surrey, England.

Raabe, R. D. (1962) Host list of the root rot fungus, *Armillaria mellea*. *Hilgardia* **33**: 25-88.

Ramos, L. J., Lara, S. P., MacMillan, R. T. & Narayanana, K. R. (1991) Tip dieback of Mango (*Mangifera indica*) caused by *Botryosphaeria ribis*. *Plant Disease* **75**: 315-318.

Rattan, P. S. & Pawsey, R. G. (1981) Death of tea in Malawi caused by *Pseudophaeolus baudonii*. *Tropical Pest Managment* **27**: 225-229.

Roberds, J. H., Namkoong, G. & Skrøppa, T. (1990) Genetic analysis of risk in clonal populations of forest trees. *Theoretical and Applied Genetics* **79**: 841-848.

Roux, J., Kemp, G. H. J & Wingfield, M. J. (1995) Diseases of black wattle in South Africa. A review. *South African Forestry Journal* **174**: 35-40.

Roux, J. & Wingfield, M. J. (1997) Survey and virulence of fungi occurring on diseased *Acacia mearnsii* in South Africa. *Forest Ecology and Management* **99**: 327-336.

Roux, J., Wingfield, M. J. & Morris, M. J. (1997) *Botryosphaeria dothidea*, a pathogen of *Acacia mearnsii* in South Africa. *South African Journal of Science* **93**: XII.

Roux, J., Wingfield, M. J., Bouillett, J-P., Wingfield, B. D. & Alfenas, A. C. (2000a) A serious new wilt disease of *Eucalyptus* caused by *Ceratocystis fimbriata* in Central Africa. *European Journal of Forest Pathology* **30**: 175-184.

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

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

Roux, J., Steenkamp, E. T., Marasas, W. F. O., Wingfield, M. J. & Wingfield, B. D. (2001b) Characterization of *Fusarium graminearum* from *Acacia* and *Eucalyptus* using β-tubilin and histone gene sequences. *Mycologia* **11**: 704-711.

Roux, J., Harrington, T. C., Steimel, J. P. & Wingfield, M. J. (2001c) Genetic variation in the Wattle wilt pathogen *Ceratocystis albofundus*. *Mycoscience* **42**: 327-332.

Roux, J., Heath, R. N., Van der Hoef, A. & Wingfield, M. J. (2001d) First report of pink disease on *Eucalyptus* and *Podocarpus* in South Africa. *Phytopathology* **S78**. (Abstract).

Roux, J. (2002) Diseases of *Acacia mearnsii* in South Africa. In *Black wattle the South African research experience* (R. W. Dunlop and L. A Maclennan eds): pp 121-126. Institute of Commercial Forestry Research. Pietermaritzburg, South Africa.

Rudd Jones, D. (1953) Studies of canker disease of Cypresses in East Africa, caused by *Monochaetia unicornis* (Cook & Ellis) Sacc. I. Observations on pathology and possible origin of the disease. *Annals of Applied Biology* **40**: 323-343.

Rudd Jones, D. (1954a) Studies on canker disease of Cypresses in East Africa, caused by *Monochaetia unicornis* (Cooke & Ellis) Sacc. II Variation in the morphology and physiology of the pathogen. *Transactions of the British Mycological Society* **37**: 286-305.

Rudd Jones, D. (1954b) Studies on a canker disease of Cypresses in East Africa caused by *Monochaetia unicornis* (Cooke & Ellis) Sacc. III Resistance and susceptibility of species of *Cupressus* and allied genera. *Annals of Applied Biology* **41**: 325-335.

Schoch, C. L., Crous, P. W., Wingfield, B. D. & Wingfield, M. J. (1999) The *Cylindrocladium candelabrum* species complex includes four distinct mating populations. *Mycologia* **91**: 286-298.

Schoeneweiss, D. F. (1979) Protection against stress predisposition to *Botryosphaeria* cankers in containerised *Cornus stolonifera* by soil injection with Benomyl. *Plant Disease Reporter* **63:** 896-900.

Shakacite, O. (1991) *A review on the pathology of Eucalyptus in Zambia*. Research note No. 51. Divison of Forest Research. Kitwe, Zambia.

Sharma, J. K., Mohanan, C. & Florence, E. J. M. (1984) Nursery diseases of *Eucalyptus* in Kerala. *European Journal of Forest Pathology* **14**: 77-89.

Sharma, J. K., Mohanan, C. & Florence, E. J. M. (1985) Occurrence of *Cryphonectria* canker disease of *Eucalyptus* in Kerala, India. *Annals of Applied Biology* **106**: 265-276.

Sharma, J. K., Florence, E. J. M., Sankaran, K. V. & Mohanan, C. (1988) Differential Phytotoxic response of cut shoots of *Eucalyptus* to culture filtrates of the pink disease fungus. *Corticium salmonicolor*. *Forest Ecology and Management* **24**: 97-111.

Shaw, C. G. and Kile, G. A. (1991) *Armillaria root disease*. Agriculture Handbook, No. 691. U.S.D.A. Forest Service, United States of Agriculture, Washington D.C.

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

Sherry, S. P. (1971) *The Black Wattle (Acacia mearnsii* de Wild.). University of Natal Press: Pietermaritzburg, South Africa.

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

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

Smith, H., Wingfield, M. J., Crous, P. W. & Coutinho, T. A. (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, M. J. & Petrini, O. (1996b) *Botryosphaeria dothidea* endophytic in *Eucalyptus grandis* and *Eucalyptus nitens* in South Africa. *Forest Ecology and Management* **89**: 189-95.

Smith, H., Crous, P. W., Wingfield, M. J., Coutinho, T. A. & Wingfield, B. D. (2001) *Botryosphaeria eucalyptorum* sp. nov. a new species in the *B. dothidea*-complex on *Eucalyptus* in South Africa. *Mycologia* **93**: 277-285.

Strouts, R. G. & Winter, T. G. (1994) *Diagnosis of ill-health in trees, Research for amenity trees* no. 2. Forestry Commission, UK.

Swart, W. J., Knox-Davies, P. S. & Wingfield, M. J. (1985) *Sphaeropsis sapinea* with special reference to its occurrence in *Pinus* spp. in South Africa. *South African Forestry Journal* **35**: 1-8.

Swart, W. J., Wingfield, M. J. & Knox-Davies, P. S. (1987) Factors associated with *Sphaeropsis sapinea* infection of pine trees in South Africa. *Phytophylactica* **19**: 505-510.

Tidball, C. J. & Linderman, R. G. (1990) *Phytophthora* root and stem rot of apple rootstocks from stool beds. *Plant Disease* **74**: 141-146.

Turnbull, J. W. (1991) Future use of *Eucalyptus*: Opportunities and problems. In *Proceedings of IUFRO symposium on Intensive Forestry: The role of Eucalyptus* (A. P. G. Schonau eds): pp 2-27. South African Institute of Forestry. Pretoria, South Africa.

Van der Westhuizen, G. C. A. (1973) *Polyporus baudonii* Pat. on *Eucalyptus* spp. in South Africa. *Bothalia* **11**: 143-151.

Van der Westhuizen, I. P., Wingfield, M. J., Kemp. G. H. J. & Swart, W. J. (1993) First report of the canker pathogen *Endothia gyrosa* on *Eucalyptus* in South Africa. *Plant Pathology* **42**: 661-663.

Venter, M., Myburg, H., Wingfield, B. D., Coutinho, T. A. & Wingfield, M. J. (2001) A new species of *Cryphonectria* from South Africa and Australia pathogenic to *Eucalyptus*. *Sydowia* **54**: 98-117.

Vercoe, T. K. (1995) International, social and economic importance of Australian *Eucalyptus*. In: *Eucalyptus pests and diseases* (M. Diekmann and J. B. Ball eds): pp. 14-20. International Plant Genetic Resource Institute (IPGRI). Bangkok, Thailand.

Viljoen, A., Wingfield, M. J. & Crous, P. W. (1992) Fungal pathogens in *Pinus* and *Eucalyptus* seedling nurseries in South Africa: A review. *South African Forestry Journal* **161**: 45-51.

Viljoen, A., Wingfield, M. J. & Marasas, W. F. O. (1994) First report of *Fusarium subglutinans f.* sp. *pini* on pine seedlings in South Africa. *Plant Disease* **78**: 309-312.

Viljoen, A., Wingfield, M. J., Kemp, G. H. J. & Marasas, W. F. O. (1995) Suceptibility of pines in South Africa to the pitch canker fungus *Fusarium subglutinans* f. sp. *pini*. *Plant Pathology* **44**: 877-882.

Viljoen, A., Marasas, W. F. O., Wingfield, M. J. & Viljoen, C. D. (1997a) Characterization of *Fusarium subglutinans f.* sp. *pini* causing root disease of *Pinus patula* seedlings in South Africa. *Mycological Research* **101**: 437-445.

Viljoen, A., Wingfield, M. J., Marasas, W. F. O. & Coutinho, T. A. (1997b) Pitch canker of pines-A contemporary review. *South African Journal of Science* **93**: 411- 417

Von Broembsen, S. L. (1984) Occurence of *Phytophthora cinnamomi* on indigenous and exotic hosts in South Africa, with special reference to the South Western Cape Province. *Phytophylactica* **16**: 221-225.

Von Broembsen, S. L. & Donald, D. G. M. (1981) Control of *Phytophthora* root rot and other soil borne disease of forest nurseries. *South African Forestry Journal* **117**: 37-40.

Walker, J., Sutton, B. C. & Pascoe, I. G. (1992) *Phaeoseptoria eucalypti* and similar fungi on *Eucalyptus*, with description of *Kirramyces* gen. nov. (Coelomycetes). *Mycological Research* **96**: 911-924.

Wargo, P. M. & Shaw, C. G. (1985) *Armillaria* root rot: The puzzle is being solved. *Plant Disease* **69**: 826-832.

Wingfield, M. J. & Knox-Davies, P. S. (1980a) Association of *Diplodia pinea* with a root disease of pines in South Africa. *Plant Disease* **64**: 221-223.

Wingfield, M. J. & Knox-Davies, P. S. (1980b) Observations on diseases in *Pine* and *Eucalyptus* plantations in South Africa. *Phytophylatica* **12**: 57-60.

Wingfield, M. J. & du Toit, I. (1986) Cypress canker caused by two *Seiridium* species in South Africa. *Phytophylactica* **18**: 43 (Abstract).

Wingfield, M. J. (1987a) Foliar pathogens of *Eucalyptus* in South Africa. *Phytophylactica* **19**: 123.

Wingfield, M. J. (1987b) Diseases in South African forest plantations. In *Forestry Hand Book* (2). (K. Von Gadow, D. W. Van der Zel, A. Van Laar, A. P. G. Schonau, H. W. Kassier, P. W. Warkotch, H. F. Vermaas, D. L. Own & J. V. Jordan eds): pp 153-164. The South African Association of Forestry. Pretoria, South Africa.

Wingfield, M. J., Swart, W. J. & Von Broembsen, S. L. (1988) Root diseases of pines and *Eucalyptus* in South Africa. In *Proceedings of the Seventh IUFRO Conference on Root and Butt rots* (D. J. Morrison ed): pp 563-5569. Canada Pacific Forestry Centre: Canada.

Wingfield, M. J., Swart, W. J. & Abear, B. J. (1989) First record of *Cryphonectria* canker of *Eucalyptus* in South Africa. *Phytophylactica* **21**: 311-313.

Wingfield, M. J. (1990) Current status and future prospects of forest pathology in South Africa. *South African Journal of Science* **86**: 60-62.

Wingfield, M. J., Swart, W. J. & Kemp, G. H. J. (1991) Pathology considerations in clonal propagation of *Eucalyptus* with special reference to the South African situation. In *Proceedings of the IUFRO symposium on Intensive Forestry: The Role of Eucalyptus* (A. P. G. Schonau ed): pp 811-820. South African Institute of Forestry. Pretoria, South Africa.

Wingfield, M. J., Crous, P. W. & Swart, W. J. (1993) *Sporothrix eucalypti* (sp. nov.), a shoot and leaf pathogen of *Eucalyptus* in South Africa. *Mycopathologia* **123**: 159-164.

Wingfield, M. J., Crous, P. W. & Coutinho, T. A. (1996) A serious canker disease of *Eucalyptus* in South Africa caused by a new species of *Coniothyrium*. *Mycopathologia* **136**: 139-145.

Wingfield, M. J. & Roux, J. (2000a) Forest pathogens, forests and society. *In Proceedings of XXI IUFRO World Congress on Forest and society. The Role of Research* (Vol. I): pp 174-180. Kuala Lumpur, Malaysia.

Wingfield, M. J. & Roux, J. (2000b) Plant diseases of South African forest plantations. In *South African Forestry Handbook* (D. L. Owen ed): pp 241-252. The South African Forestry Institute. Pretoria, South Africa.

Wingfield, M. J., Slippers, B., Roux, J. & Wingfield, B. D. (2001) World-wide movement of exotic forest fungi, especially in the tropics and the Southern Hemisphere. *Bioscience* **51**: 134-140.

Wright, J. P. & Marks, G. C. (1970) Loss of merchantable wood in radiata pine associated with infection by *Diplodia pinea*. *Australian Forestry* **34**: 107-119.

Zeijlemaker, F. C. J. (1968) *The gummosis of black wattle: a complex of diseases.* Report for 1967-1968: pp 40-43. Wattle Research Institute, University of Natal: South Africa.

Zeijlemaker, F. C. J. (1971) Black butt disease of black wattle caused by *Phytophthora nicotianae* var *parasitica*. *Phytopathology* **61**: 144-145.

Zentmyer, G. A. (1980) *Phytophthora cinnamomi and the disease it causes. Monograph 10.* pp 96. American Phytopathological Society, St. Paul, Minnesota.

Ziller, W. G. (1974) The tree rusts of Western Canada. Canadian Forestry Service publication No. 1329. Department of Environment, Victoria, British Colombia.

Zwolinski, J. B., Swart, W. J. & Wingfield, M. J. (1990a) Intensity of dieback induced by *Sphaeropsis sapinea* in relation to site conditions. *European Journal of Forest Pathology* **20**: 167-174

Zwolinski, J. B., Swart, W. J., & Wingfield, M. J. (1990b) Economic impact of post hail outbreak of dieback induced by *Sphaeropsis sapinea*. *European Journal of Forestry Pathology* **20**: 405-411.

**Table 1**. Diseases reported in plantation forestry nurseries in African countries

| Pathogen | Host | Symptoms | Distribution | References |
|---|---|---|---|---|
| *Botrytis cinerea* (Pers.) (Anam=*Sclerotinia fuckeliana* de Bary) | *E. globulus, P. patula* | Grey mould, Seed damage, Seedling die-back | S. Africa, Kenya, Zimbabwe, Tanzania | Viljoen, Wingfield & Crous 1992, Gibson 1964, Browne 1968 |
| *Colletotrichum acutatum* Simmonds | *P. radiata* | Necrosis (Shoot tip, terminal bud, & needles), stunting & stem malformation | S. Africa, Kenya | Gibson & Munga 1969, Lundquist 1984b |
| *C. gloeosporioides* (Penz.) Penz. & Sacc. | *E. dives, E. grandis, E. globulus, E. saligna* | Leaf spot, stem canker | S. Africa | Viljoen *et al.* 1992, Baxter, Van Westhuizen & Eicker 1983 |
| *Coniella castaneicola* (Ell. Ev.) Sutton | *E. camaldulensis, E. globulus* | Leaf spot | S. Africa | Viljoen *et al.* 1992 |
| *Cylindrocladiella* spp. | *Eucalyptus* spp., *Acacia* spp., *Pinus* spp. | Damping-off, seedling blight | S. Africa | Crous *et al.* 1993 |
| *C. camelliae* Venkataramani & Venkata Ram | *Eucalyptus* spp., *A. mearnsii P. radiata, Eucalyptus* spp. | Root rot, leaf spot | S. Africa | Crous *et al.* 1993 |
| *C. parva* Anderson | | Root infection, seedling death | S. Africa, Malawi | Crous *et al.* 1991 |

| Pathogen | Host | Symptoms | Distribution | References |
|---|---|---|---|---|
| *Cylindrocarpon destructans* (Zins) Scholten | *P. radiata, P. roxburghii* | Root rot, Damping-off | South Africa | Darvas *et al.* 1978 |
| *Cylindrocladium pauciramosum* C.L. Schoch et Crous | *Eucalyptus* spp., *Acacia* spp., Conifers | Root rot, damping-off, leaf blight, stem canker | S. Africa, Kenya | Lundquist & Baxter 1985, Crous *et al.* 1991, Crous *et al.* 1993, Schoch *et al.* 1999, Roux 2001* |
| *C. colhouni* Peeraly | *E. grandis* | Leaf spot, root rot, wilt | S. Africa | Crous *et al.* 1993 |
| *C. clavatum* Hodges & May | *Eucalyptus* spp., *P. radiata* | Damping-off, seedling blight | S. Africa | Crous *et al.* 1993 |
| *Fusarium* spp. | *Pinus* spp., *Eucalyptus* spp., *Acacia* spp. | Damping-off | S. Africa, E. Africa, Uganda | Browne 1968, Hocking 1968, Maiteki *et al.* 1999 |
| *F. oxysporum* (Schlecht. Ex Fr. | *P. palustris, P. roxburghii, P. taeda, P. caribaea, P. khasya A. mearnsii* | Damping-off | S. Africa, E. Africa, Uganda | Darvas *et al.* 1978, Viljoen, Wingfield & Crous 1992, 1994 |
| *F. solani* (Mart.) Sacc. | *P. patula, P. caribaea, P. khasya A. mearnsii* | Damping-off | S. Africa, E. Africa, Uganda | Bakshi 1976, Viljoen *et al.* 1995, Roux *et al.* 2001b |
| *F. moniliformis* Sheld. | *P. radiata, P. roxburghii, P. caribaea, P. khasya* | Damping-off | S. Africa, E. Africa | Zeijlemaker 1968 |
| *F. equiseti* (Corda) Sacc. | *P. caribaea, P. khasya, P. radiata, P. roxburghii* | Damping-off | E. Africa, S. Africa | Hocking 1968, Darvas *et al.* 1978 |

Roux et al 2001* personal communication

| Pathogen | Host | Symptoms | Distribution | References |
|---|---|---|---|---|
| *F. semitectum* Berk. & Rav | *P. caribaea, P. khasya* | Damping-off | E. Africa, S. Africa | Hocking 1968, Darvas *et al.* 1978 |
| *F. subglutinans* | *P. patula* | Root rot, damping-off | S. Africa | Viljoen *et al.* 1997a, 1997b |
| *Hainesia lythri* (Desm.) Hohn | *E. globulus, E. robusta, E. saligna, E. grandis* | Leaf spotting, stunting, multiple stems | S. Africa, Zambia | Baxter *et al.* 1983, Lundquist 1986, Lundquist & Foreman 1986, Crous *et al.* 1993 |
| *Harknessia hawaiiensis* (Stevens & Young) | *E. grandis, E. nitens* | Leaf spot | S. Africa | Crous *et al.* 1989b |
| *Helicobasidium compactum* (Boedijn) | *P. patula* | Root infection | Tanzania | Browne 1968 |
| *Oidium* spp. | *A. mearnsii* | Leaf deformation, leaf drop, stunting | S. Africa, Uganda | Roux *et al.* 2001a, Roux 2002 |
| *Macrophomina phaseolina* (Tassi.) G. | *A. mearnsii, A. decurrens, Eucalyptus* spp. & *Pinus* spp. | Stunting, chlorosis, foliage death, necrotic lesions on roots | Malawi, Zimbabwe, Tanzania, S. Africa | Gibson 1975, Bakshi 1976 Darvas *et al.* 1978 |
| *Pantoea ananatis* Corrig. (syn= *Erwinia ananas, E. uredovora* | *E. grandis, E. saligna, E. dunii, E. nitens, E. smithii* | Leaf blight, shoot die-back | S. Africa | Coutinho *et al.* 2001 |

| Pathogen | Host | Symptoms | Distribution | References |
|---|---|---|---|---|
| *Phaeoseptoria eucalypti* Hansf. (*Kirramyces epicoccoides* ) | *E. bicostata, E. camaldulensis, E. cladocalyx, E. dunnii, E. globulus* spp., *E. saligna* | Leaf spot, defoliation | S. Africa, Malawi, Uganda | Crous *et al.* 1989e, Chipompha, 1987, Roux *et al.* 2001a, Viljoen *et al.* 1992 |
| *Phytophthora* spp. | *Eucalyptus* spp., *Pinus* spp. | Damping-off, root rot | S. Africa, Uganda | Darvas *et al.* 1978, Maiteki *et al.* 1999 |
| *P. cinnamomi* Rand | *E. citriodora, P. elliottii, P. halepensis, P. patula, P. pinaster, P.radiata* | Damping-off, root rot | S. Africa | Darvas *et al.* 1978 |
| *Pythium* spp. | *P. patula* & *Pinus* spp. | Damping-off, root rot | S. Africa, E. Africa, Uganda | Doidge 1950, Hocking 1968, Maiteki *et al.* 1999 |
| *P. ultimum* Trow | *E. grandis* | Root rot | S. Africa, E. Africa, | Gibson 1970, Darvas *et al.* 1978 |
| *P. irregularae* Buisman | *Pinus* spp., *Eucalyptus* spp. | Damping-off | S. Africa | Hocking 1968, Viljoen *et al.* 1992 |
| *P. pyrilobum* Vaartaja | *Pinus* spp., *E. grandis* | Damping-off | S. Africa | Linde *et al.* 1994a |
| *P. splendens* H.Braun | *E. grandis* | Damping-off | S. Africa | Linde *et al.* 1994b |
| *Pseudocercospora pinidensiflorae* (Horri & Nambu) Deighton | *P. halepensis, P. patula, P. radiata* | Needle blight | S. Africa | Viljoen *et al.* 1992 |

| Pathogen | Host | Symptoms | Distribution | References |
|---|---|---|---|---|
| *Rhizoctonia solani* Kuhn [anam.=*Thanetophorus cucumeries* (Frank) Don =*Corticium solani* (Prill & Delacr.) Bourd. & Galz.] | *Pinus* spp., *E. grandis* | Damping-off, root rot, collar rot, seedling blight | S. Africa, E. Africa, Uganda | Darvas *et al.* 1978, Viljoen *et al.* 1992, Maiteki *et al.* 1999, Hocking 1968, Gibson & Hudson 1969, Gibson 1970 |
| *Rosellinia necatrix* (Hartig) Prill. [anam= *Dematophora necatrix* Hartig] | *Pinus* spp. | Root infection | Kenya, Tanzania | Gibson 1964, Browne 1968 |
| *Sphaerotheca pannosa* (Wallr.: Fr.) Lev. *(syn= Aephitomorpha pannosa, Erysiphe pannosa, Oidium leucocnium, Oidium eucalypti*) | *E. camaldulensis, E. globulus, E. maidenii* | Leaf spot, malformation of leaves & shoots | S. Africa, Uganda, Kenya | Crous *et al.* 1989e, 1989c, Chipompha 1987, Roux *et al.* 2001a, Roux 2001* |
| *Sphaeropsis sapinea* | *P. patula* | Needle & shoot blight | S. Africa | Darvas *et al.* 1978 |
| *Sporothrix eucalypti* Wingfield, Crous & Swart sp. nov. | *E. grandis* | Leaf spot, defoliation, shoot die-back | S. Africa | Wingfield, Crous & Swart 1993 |

* Personal communication.


# Chapter Two
# Diseases of Exotic Plantation *Eucalyptus* and *Pinus* Species in Ethiopia

Published as: Alemu Gezahgne, J. Roux & M. J. Wingfield (2003) Diseases of exotic plantation *Eucalyptus* and *Pinus* species in Ethiopia. *South African Journal of Science* **99**:29-33

**ABSTRACT**

A survey of diseases in exotic plantations was undertaken in Southern and South Western Ethiopia during 2000 and 2001. The aim was to consider the occurrence and distribution of diseases of major plantation species in this country and to provide a foundation for further research. Samples were collected from plantations and trees planted around farms and homesteads in and around Wondo Genet, Munessa Shashemene, Jima, Bedele, Mizan and Menagesha and included those from roots, stems and leaves. Armillaria root rot was the most common disease, mainly associated with *Pinus patula* but was also found on *Acacia abyssinica*, *Cordia alliodora* and *Cedrela odorata* trees. Stem cankers associated with *Botryosphaeria* spp. were common on *Eucalyptus globulus, E. saligna* and *E. citriodora.* Stem canker disease associated with a *Coniothyrium* sp. was frequently observed on *E. camaldulensis*. Leaf blotch associated with *Mycosphaerella* spp. was common on *E. globulus* in most areas where this species is planted. In addition, *Sphaeropsis sapinea* on *Pinus* spp., cankers associated with *Cytospora* spp. and pink disease caused by *Erythricium salmonicolor* on *Eucalyptus* were also recorded in some plantations. This is the first general evaluation of plantation diseases in Ethiopia and it will provide a foundation for developing planting and disease management strategies, to ensure optimum production in plantations.

## INTRODUCTION

Establishment of exotic plantation forestry has been successful and profitable in many tropical and subtropical countries (Gibson 1979, Evans 1984). The timber derived from these plantations is commonly used to produce pulp and paper, viscose and rayon. It also provides a resource for construction and in developing countries, is an important source of fuelwood (Evans 1984, Turnbull 1991).

In Ethiopia, wood provides 85% of the country's energy requirements and is used for construction purposes. However, the natural forest resource is diminishing rapidly (Anonymous 1994). This is while the demand for forest products is rapidly increasing, necessitating the establishment of plantations of rapidly growing trees. In Ethiopia, the introduction of fast growing exotic tree species took place a century ago, with the introduction of *Eucalyptus globulus* Labill. in the late 1890's (Persson 1995). Since then, several other *Eucalyptus* spp., as well as *Cupressus, Pinus, Grevillea* and *Acacia* species have been widely planted in plantations and around farms and homesteads (Evans 1984, Persson 1995). Plantations of these exotic species now occupy approximately 200 000 ha (Anonymous 1994).

Plantations of exotic species have been highly successful in many countries (Wingfield 1990, Persson 1995). This is partially attributed to the separation of the trees from their natural enemies. However, these trees are established as monocultures in new environments and they are exposed to unique suites of pests and diseases. Thus, serious disease problems have emerged in most countries where they have been planted (Wingfield 1990).

Diseases have had serious impacts on exotic plantation forestry and in some cases, have resulted in the abandonment, or restriction of species to specific localities. For example, the fast-growing *Pinus radiata* D. Don. has been abandoned in several Eastern, Central and Southern African countries due to Dothistroma needle blight caused by *Dothistroma septospora* (Dorog.) Morelet (Gibson 1972, Ciesla, Mbugua & Ward 1995). In these

countries, *P. radiata* has been substituted by slower growing *P. patula* Schl. & Cham. but, in South Africa, severe losses to *P. patula* have subsequently occurred due to an interaction between hail damage and *Sphaeropsis sapinea* (Fr.:Fr.) Dyko & Sutton (Swart, Wingfield & Knox-Davies 1987). Similarly, planting *Cupressus macrocarpa* Hartw., which showed remarkable growth in Kenya and other East African countries, has been abandoned due to cypress stem canker caused by *Seiridium cupressi* (Guba) Boesew (Rudd Jones 1953, Gibson 1964). As a result, the slow growing *C. lusitanica* Mill. has been introduced as an alternative species (Rudd Jones 1953, Gibson 1964).

Diseases have negatively affected the planting of *Eucalyptus* spp. for example, Mycosphaerella leaf blotch on *E. globulus, E. nitens* (Deane et Maid.) Maid. and *E. maidenii* F. Muell. has resulted in reduction of planting these species (Lundquist & Purnell 1987). Likewise, *E. fastigata* Deane et Maid. and *E. fraxinoides* Deane et Maid., which initially performed well in frost prone areas of South Africa, have been abandoned due to root disease caused by *Phytophthora cinnamomi* Rands (Linde, Kemp & Wingfield 1994).

Several diseases new to *Eucalyptus*, for example stem canker caused by *Cryphonectria cubensis* (Bruner) Hodges (Conradie, Swart & Wingfield 1990), *Coniothyrium zuluense* Wingfield, Crous & Coutinho (Wingfield, Crous & Coutinho 1996), and wilt caused by *Ceratocystis fimbriata* Ellis & Halst. (Roux *et al.* 2000), have appeared in recent years. These pathogens not only damage the trees in their exotic habitat, but now also threaten *Eucalyptus* in their areas of origin (Wingfield 1990, 1999).

Knowledge of plantation diseases in Ethiopia is limited. This is despite the fact that tree death is common in the country. These deaths are typically attributed to poor site-species matching, poor management and adverse climatic conditions. The role of biotic factors in tree death is underestimated, poorly understood and has received little attention. Thus, a survey of plantation tree species was conducted in some parts of South and South Western Ethiopia in 2000 and 2001. The objective was specifically to determine the occurrence of diseases of exotic plantation species, to provide a basis for further study

and to establish a foundation for disease avoidance. This study provides the first detailed overview of plantation tree pathogens in Ethiopia.

## MATERIALS AND METHODS

### *Survey areas and sample collection*

Surveys were conducted in April 2000 and in June-September 2001 in Southern and South Western Ethiopia. Collections were made in plantations and small-holdings around Munessa Shashemene, Wondo Genet, Jima, Mizan Teferi, Bedele, Menagesha and Addis Ababa (Figure 1, Table 1). Samples were primarily collected from *Eucalyptus* and *Pinus* plantations and included samples from roots, bark, stems, twigs and leaves.

### *Isolation techniques*

Samples were collected from all trees showing symptoms of disease. Diseased plant tissue was collected and kept in paper bags for transport to the laboratory. Growth media used to isolate the fungi included 2% malt extract agar (MEA, Biolab) and MEA amended with 100 ppm streptomycin (MEAS) for the isolation of Ascomycetes and Coelomycetes. A selective medium containing benomyl was used for the isolation of Basidiomycetes (Harrington, Worall & Baker 1992).

In the laboratory, several different techniques were used to isolate disease-causing organisms. These included the transfer of pieces of mycelium or fruiting bodies from diseased plant tissue directly onto the growth medium; incubating symptomatic plant material in moist chambers; as well as inoculating segments of plant parts with disease symptoms onto growth media. All plates were incubated at 25 °C to induce fungal growth. For the isolation of *Mycosphaerella* spp., discs of leaves with disease symptoms were attached beneath the cover of petri dishes with the pseudothecia facing downward, so that spores were released onto MEAS (Crous, Phillips & Wingfield 1991). After 24 hr, ascospore germination was checked under the microscope and single germinating

ascospores were transferred to MEA. Microscope slides were prepared of each isolate to determine the germination pattern of ascospores.

Fungi isolated in this study were identified and representative isolates of the pathogens are maintained in the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Specimens have also been deposited in the herbarium of the South African National Fungus Collection, Pretoria (PREM).

## RESULTS

The results of this survey clearly demonstrate the prevalence of some important tree diseases in the exotic plantations examined. Diseases recorded during this survey included root diseases, stem cankers and leaf diseases.

### *Root diseases*

#### *Armillaria Root Rot*

Armillaria root rot was commonly found associated with the death of *P. patula* in Wondo Genet, Belete and Bedele. The causal fungus (PREM 57377 and 57378) was also isolated from dying *Acacia abyssinica* Hochst. trees growing in *Pinus* plantations. In addition, Armillaria root rot was found in association with dead and dying *Cordia alliodora* (Ruiz & Pav.) Oken. and *Cedrella odorata* L. trees in a research plot near Aman. Symptoms were typical of those known for the disease and included death of trees in groups, wilting and yellowing of the crowns (Figure 2a), the occurrence of white mycelial fans between the bark and the wood of symptomatic trees (Figure 2b) as well as the occurrence of rhizomorphs on the bark of infected trees. Armillaria root rot was the major cause for the death of *P. patula* at Wondo Genet. The damage caused at other plantation sites appeared to be mild at the time of this survey.

***Stem cankers***

Stem cankers were observed on several *Eucalyptus* spp. Disease symptoms included bark cracking, production of copious amounts of kino, stem discoloration and malformation, as well as the production of kino pockets in the xylem (Figures 3, 4).

***Stem Canker associated with Botryosphaeria***

The most common disease observed on *Eucalyptus* spp. in Ethiopia was canker, from which a *Botryosphaeria* sp. (PREM 57379, 57380 and 57381) was isolated. At Wondo Genet these cankers were found on *E. saligna* Sm., *E. grandis* Hill ex Maid., *E. citriodora* Hook and *E. globulus*. At Munessa Shashemene, they were observed on *E. globulus*, both on coppice and first generation stands, as well as on mature *E. saligna*. At these two sites, stem cracking and kino exudation was observed over the entire length of stems of affected trees. When the bark was removed from these trees, well developed kino pockets were visible in the cambium and xylem (Figure 3a).

In the Jima area, similar disease symptoms were observed on *E. citriodora* and *E. saligna*. Here the damage was most severe on *E. citriodora* and not less than 50% of the trees in the plantation near Jima were symptomatic, but no death of trees were observed at the time of the survey. On this species, large basal cankers were observed. The disease was characterised by black discoloration and cracking of stems, starting at ground level up to approximately one metre height (Figure 3b). When the bark was removed, the cambium was completely discoloured and soaked with kino. Two or three layers of black lines were observed in the wood indicating different seasons of infection (Figure 3c). At Menagesha symptoms of stem canker were commonly found on coppice stems of *E. globulus*. At this site, several coppice stems were dead and wilting. A *Botryosphaeria* sp. was frequently isolated from symptomatic plant material collected from all sites.

***Coniothyrium Stem Canker***

Stems of *E. camaldulensis* Dehnh. trees in Jiren plantation at Jima and on trees in wood lots between Jima and Woliso, as well as between Wolkite and Sodo, were seriously affected by a stem canker disease. The disease resulted in extensive stem malformation. Initial symptoms of the disease include small discrete lesions on young green bark. Patches of large necrotic lesions developed from these on the stems, branches and twigs (Figure 4a). A *Coniothyrium* sp. (PREM 57382) was consistently found sporulating on the surface of the lesions. Stems often showed a reddish colour due to the exudation of kino from the cracks. The wood of the affected stems showed the formation of pitted kino pockets (Figure 4b). Several of the infected trees produced epicormic shoots. It was estimated that at least 50% of the trees in a stand were affected by this pathogen.

***Pink disease***

Stem samples of diseased *E. camaldulensis* obtained from Pawe in the Benshangul Gumuz region, yielded structures typical of the pink disease pathogen, *Erythricium salmonicolor* (Berk. & Broome) Burds. (Syn. *Corticium salmonicolor* Berk. & Br.). Branch die-back, stem cankers, branch and stem girdling, production of epicormic shoots on the stems, death of trees as well as the production of pink mycelial growth on symptomatic plant parts are characteristic symptoms of pink disease. The fungus produced typical flat/resupinate fruiting structures on the surface of affected stems.

***Leaf disease***

***Mycosphaerella leaf blotch***

Leaf spot and blight was commonly observed on *E. globulus*, wherever this species is grown. The symptoms observed on *E. globulus* are characteristic of those caused by *Mycosphaerella* spp. and in many cases, resulted in defoliation of young trees (Figure 5a, 5b). Isolations from leaves with leaf blotch symptoms consistently yielded

*Mycosphaerella* spp. (PREM 57386). From the examination of the germination patterns of ascospores, it is clear that more than one *Mycosphaerella* sp. is involved in causing leaf blotch on *E. globulus* leaves in Ethiopia.

***Other fungi***

Several other fungi, known to be associated with tree disease elsewhere in the world, were found in *Pinus* and *Eucalyptus* plantations, although they appeared to be relatively unimportant. For example, *Sphaeropsis sapinea* was isolated from pine cones collected from Wondo Genet and Munessa Shashemene. Species of *Cytospora, Fusarium graminearum* Schwabe and *Cylindrocladium* Morgan were also isolated from *Eucalyptus* branches collected from Wondo Genet, Wolkite and Menagesha. *Phaeophleospora eucalypti* (Cooke & Massee) Crous, F. A. Ferreira & B. Sutton was common on *E. camaldulensis* and *E. grandis* leaves, in all areas examined.

## DISCUSSION

Planting exotic species in plantations has been practised for more than a century in Ethiopia. The impact of diseases on plantation development has, however, received minimal attention. In recent years, tree deaths have been frequent but usually attributed to extreme climatic and poor site conditions. The results of this study have shown that several well-known fungal pathogens are involved in causing considerable damage in exotic plantations. This study thus provides the first comprehensive documentation of plantation diseases in Ethiopia and provides a firm foundation for future study.

Root rot caused by an *Armillaria* sp. was frequently found in *P. patula* plantations. *Armillaria* spp. are known to cause root rot on a wide range of tree species including both exotic as well as native trees and are known world-wide (Shaw & Kile 1991). The identification of Armillaria root rot from native *A. abyssinica* as well as from *C. odorata* and *C. alliodora* suggest that this disease could be important not only in *P. patula*, but also on other trees, including native species. Further study is essential to determine its

role in causing root rot in other localities not included in this survey. Previous studies have attributed Armillaria root rot in Ethiopia to *A. mellea* (Mengistu 1992, Eshetu, Teame & Girma 2000, Otta, Intini & Hattori 2000). Fruiting bodies recovered from this survey, however, do not match the macro-morphological characteristics of *A. mellea*. We are currently conducting further studies to identify the species isolated during the present surveys.

*Botryosphaeria* spp. have a cosmopolitan distribution and are found on many different hosts including *Eucalyptus* spp. (Barnard *et al.* 1987, Smith, Kemp & Wingfield 1994) and it was not surprising to find them in this study. They are considered to be opportunistic wound and stress related pathogens (Pusey 1989, Smith *et al.* 1994). Environmental stress such as drought (Pusey 1989) and frost (Wene & Schoenesweiss 1980) especially, provide conducive conditions for disease development. *Botryosphaeria* spp. are also known as endophytes and are found in healthy plant tissues (Smith, Wingfield & Petrini 1999). In some areas the presence of this pathogen seems to have resulted in poor growth of the coppice sprouts of *E. globulus* and it most likely contributed to the failure of coppice development. Regenerating *Eucalyptus* spp. by coppicing is widely practised in Ethiopia, and further investigation is needed to determine the association of the stem canker with poor growth and coppice failure. Currently studies are underway to determine which *Botryosphaeria* spp. are involved in causing stem canker on *Eucalyptus* spp. in Ethiopia and thus to evaluate their relative importance.

Stem canker associated with a fungus that closely resembles *C. zuluense* was the most common stem canker found affecting *E. camaldulensis*. This is the most widely planted *Eucalyptus* sp. in Ethiopia and given the importance of the disease on *Eucalyptus* spp., clones and hybrids in South Africa, Thailand and Mexico (Wingfield *et al.*1996, Van Zyl *et al.* 2002, Roux, Wingfield & Cibrán 2002), this disease is of considerable concern. Coniothyrium canker is considered to be one of the most important threats to *Eucalyptus* plantation forestry in the world. This disease not only complicates debarking but it also affects the quality of sawn timber, growth and in severe cases may also result in death of trees (Wingfield *et al.* 1996, Roux *et al.* 2002, Van Zyl *et al.* 2002). At present little is

known regarding its occurrence in other *E. camaldulensis* growing areas of Ethiopia or whether it infects other *Eucalyptus* spp. It will, therefore, be important to conduct further surveys for this disease. A study is currently also in progress to confirm the identity of the *Coniothyrium* sp. found in Ethiopia and to determine whether it is the same fungus found in South Africa and elsewhere in the world.

Pink disease caused by *E. salmonicolor* is common in the tropics, affecting a wide range of hosts including *Eucalyptus* spp., coffee, rubber, cacao, tea, *Acacia* and *Podocarpus* spp. (Gibson 1975, Roux *et al.* 2001a) and its discovery on *Eucalyptus* in Ethiopia is considered important. In South Africa, pink disease has been reported on *E. macarthurii* Deane et Maid. and *E. cloeziana* F. Mueller in temperate areas of the country (Roux *et al.* 2001a). The damage caused by this disease is of concern for the development of *E. camaldulensis* in Ethiopia and studies including those relating to distribution and host range are required in Ethiopia.

Leaf blotch caused by *Mycosphaerella* spp. is widely distributed and important on *Eucalyptus* spp. world-wide. It is especially well-known for the defoliation it causes on *E. globulus* and *E. nitens* (Park & Keane 1982, Lundquist & Purnell 1987), and its occurrence on *E. globulus* in Ethiopia is significant. Elsewhere in Africa, MLB has been reported from South Africa (Crous & Wingfield 1996), Uganda (Roux *et al.* 2001b), Malawi (Lee 1970), Zimbabwe and Kenya (Gibson 1964). Thirty *Mycosphaerella* spp. have been described in association with leaf blotch of *Eucalyptus*, of which 11 have been recorded on the African continent (Hunter *et al.* 2003). Nothing is known regarding the diversity, distribution or importance of *Mycosphaerella* spp. in Ethiopia and these questions deserve investigation.

Results of this study include many new records of diseases of *Pinus* and *Eucalyptus* spp. in Ethiopia. They also provide a foundation on which to base future studies and to develop management strategies. In the past, tree deaths have been ascribed to factors such as adverse climatic conditions, poor species selection and inadequate post-planting management. This study has shown that the situation is more complicated and that

diseases play an important role. These findings suggest that management strategies to reduce the impact of diseases, and facilities to diagnose and monitor these problems should be instituted. In addition, most of the pathogens require more detailed taxonomic study and pathogenicity tests should be conducted to better understand their role in tree death.

## REFERENCES

Anonymous (1994) *Ethiopian Forestry Action Plan* (EFAP). Final report, vol. I , pp 14 and vol. II, pp 34. Addis Ababa, Ethiopia.

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

Ciesla, W. M., Mbugua, D. K. & Ward, J. D. (1995) Ensuring forest health and productivity; A perspective from Kenya. *Journal of Forestry* **93**: 36-39.

Conradie, E., Swart, W. J. & Wingfield, M. J. (1990) Cryphonectria canker of *Eucalyptus,* an important disease in plantation forestry in South Africa. *South African Forestry Journal* **152**: 43-49.

Crous, P. W., Phillips, A. J. L. & Wingfield, M. J. (1991) The genera *Cylindrocladium* and *Cylindrocladiella* in South Africa, with special reference to forest nurseries. *South African Forestry Journal* **157**: 69-85.

Crous, P. W. & Wingfield, M. J. (1996) Species of *Mycosphaerella* and their anamorphs associated with leaf blotch disease of *Eucalyptus* in South Africa. *Mycologia* **88**: 441-458.

Crous, P. W. (1998) *Mycosphaerella* spp. and their anamorphs associated with leaf spot diseases of *Eucalyptus*. *Mycologia Memoir* **21**: 1-170.

Eshetu Derso, Teame G. Ezgi & Girma, A. (2000) Significance of minor diseases of *Coffea arabica* L. in Ethiopia. A review. In *Proceedings of the workshop on control of Coffee berry Disease (CBD) in Ethiopia*. Ethiopian Agricultural Research Organization. pp 58-65. Addis Ababa, Ethiopia.

Evans, J. (1984) *Plantation forestry in the tropics*. Clarendon Press, Oxford. UK.

Gibson, I. A. S. (1964) The impact of disease on forest product in Africa. FAO/ Forpest. 64. In *Proceedings of the FAO/ IUFRO symposium on internationally dangerous forest diseases and insects*. pp 1-13. Oxford.

Gibson, I. A. S. (1972) Dothistroma blight of *Pinus radiata*. *Annual Review of Phytopathology* **10**: 51-72.

Gibson, I. A. S. (1975) *Diseases of forest trees widely planted as exotics in the tropics and Southern Hemisphere, Part I. Important members of the Myrtaceae, Leguminosae and Meliace*. Commonwealth Forestry Institute: University of Oxford, UK.

Gibson, I. A. S. (1979) *Diseases of forest trees widely planted as exotics in the tropics and southern hemisphere, part II. The genus Pinus*. Commonwealth Forestry Institute, University of Oxford.

Hunter, G. C., Crous, P. W., Roux, J., Coutinho, T. A., Wingfield, B. D. & Wingfield. M. J. (2003) Identification of *Mycosphaerella* spp. Associated with leaf diseases of Eucalyptus nitens in South Africa. *Australian Plant Pathology* (Submitted).

Harrington, T. C., Worrall, J. J. & Baker, F. A. (1992) Armillaria. In *Methods for research on soilborne phytopathogenic fungi* (L. L. Singleton, J. D. Mihail and C. M. Rush eds.): pp 81-85. The American Phytopathological Society, St. Paul, Minnesota.

Hunter, G. C., Crous, P. W., Roux, J., Coutinho, T. A., Wingfield, B. D. & Wingfield, M. J. (2003) Identification of Mycosphaerella spp. Associated with leaf disease of Eucalyptus nitens in South Africa. *Australian Journal of Pathology*, (submitted).

Lee, R. F. (1970) *A first check list of tree diseases in Malawi*. Malawi Research Institute. Research Record No. 43.

Linde, C., Kemp, G. H. J. & Wingfield, M. J. (1994) Diseases of Pines and *Eucalypts* in South Africa associated with *Pythium* and *Phytophthora* species. *South African Forestry Journal* **169**: 25-32.

Lundquist, J. E. & Purnell, R. C. (1987) Effects of Mycosphaerella leaf spot on growth of *Eucalyptus nitens*. *Plant Disease* **71**: 1025-1029.

Mengistu Huluka (1992) Some aspects of forest tree disease in Ethiopia. In *National workshop on setting Forestry Research Priorities*. Forestry Research Centre. Addis Ababa, Ethiopia.

Ota, Y., Intini M. & Hattori, T. (2000) Genetic characterisation of heterothallic and non-heterothallic *Armillaria mellea sensu sticto*. *Mycological Research* **104**: 1046-1054.

Park, R. F. & Keane, P. J. (1982) Leaf diseases of *Eucalyptus* associated with *Mycosphaerella* species. *Transactions of the British Mycological Society* **79**: 101-115.

Persson, A. (1995) Exotics-prospects and risks from European and African viewpoints. *Buvisindi Agricultural Science* **9**: 47-62.

Pusey, P. L. (1989) Influence of water stress on susceptibility of nonwounded peach bark to *Botryosphaeria dothidea*. *Plant Disease* **73**: 1000-1003.

Roux, J., Wingfield, M. J., Bouillet J-P., Wingfield, B. D. & Alfenas, A. C. (2000) A serious new wilt disease of *Eucalyptus* caused by *Ceratocystis fimbriata* in Central Africa. *European Journal of Forest Pathology* **30**: 175-184.

Roux, J., Heath, R. N., van der Hoef, A. & Wingfield, M. J. (2001a) First report of pink disease on *Eucalyptus* and *Podocarpus* in South Africa. *Phytopathology* **91**: S78.

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

Roux, J., Wingfield, M. J. & Cibrián, D. (2002) First report of Coniothyrium canker of *Eucalyptus* in Mexico. *Plant Pathology* **51**: 382.

Rudd Jones, D. (1953) Studies on a canker disease of Cypresses in East Africa, caused by *Monochaetia unicornis* (Cook and Ellis) Sacc. I. Observations on pathology, spread and possible origins of the disease. *Annals of Applied Biology* **40**: 323-343.

Shaw, C. G. & Kile, G. A. (1991) Armillaria root disease. Agriculture Hand book No. 691. Forest Service, United States Department of Agriculture. Washington D.C.

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

Smith, H., Wingfield, M. J. & Petrini, O. (1999) *Botryosphaeria dothidea* endophytic in *Eucalyptus grandis* and *Eucalyptus nitens* in South Africa. *Forest Ecology and Management* **89**: 189-195.

Swart, W. J., Wingfield, M. J. & Knox-Davies, P. S. (1987) Factors associated with *Sphaeropsis sapinea* infection of pine trees in South Africa. *Phytophylactica* **19**: 505-510.

Turnbull, J. W. (1991) Future use of *Eucalyptus*. Opportunities and problems. In *Proceedings of the IUFRO Symposium on Intensive Forestry: The role of Eucalyptus* (A.P. G. Schonau ed): pp. 2-27. South African Institute of Forestry. Pretoria, South Africa.

Van Zyl, L. M., Coutinho, T. A., Wingfield, M. J., Pongpanich, K. & Wingfield, B. D. (2002) Morphological and molecular relatedness of geographically diverse isolates of *Coniothyrium zuluense* from South Africa and Thailand. *Mycological Research* **106**: 51-59.

Wene, E. G. & Schoeneweiss, D. F. (1980) Localised freezing predisposition to Botryosphaeria canker in differentially frozen wood stems. *Canadian Journal of Botany* **58**: 1455-1458.

Wingfield, M. J. (1990) Current status and future prospects of forest pathology in South Africa. *South African Journal of Science* **86**: 60-62.

Wingfield, M. J. (1999) Pathogens in exotic plantation forestry. *International Forestry Review* **1**: 163-168.

Wingfield, M. J., Crous, P. W. & Coutinho, T. A. (1996) A serious canker disease of *Eucalyptus* in South Africa caused by a new species of *Coniothyrium*. *Mycopathologia* **136**: 139-145.

Table 1. Climatic conditions and altitude of survey areas

| Locality | Mean Annual Temperature $^0$C | Mean Annual Rainfall (mm) | Altitude (masl) |
|---|---|---|---|
| Bedele | 19 | 1 800 | 2 010 |
| Hossana | 16 | 1 300 | 2 320 |
| Jima | 20 | 1 500 | 1 750 |
| Menagesha | 14 | 1 017 | 2 400 |
| Mizan/Aman | 24 | 2 200 | 1 350 |
| Munessa Shashemene | 19 | 1 200 | 2 140-2 600 |
| Woliso | 17 | 1 100 | 2 150 |
| Wondo Genet | 19 | 1 200 | 1 800-2 200 |


**Figure 1**. Map of Ethiopia showing the plantation areas where samples were conducted.


**Figure 2**. Symptoms of Armillaria root rot on *Pinus* spp. (A) Group death and crown die-back of *P. patula*, (B) White mycelial fan expanding up the stem of an infected tree.


**Figure 3**. Disease symptoms associated with *Botryosphaeria* on *Eucalyptus* spp. (A) Wood discoloration on *E. saligna* associated with stem canker, (B) Basal canker resulting in the cracking of the stem of *E. citriodora*, (C) Internal resin zones associated with external cankers on *E. citriodora*


**Figure 4**. Coniothyrium canker of *Eucalyptus camaldulensis*. (A) Infected twig, (B) Kino Pocket.

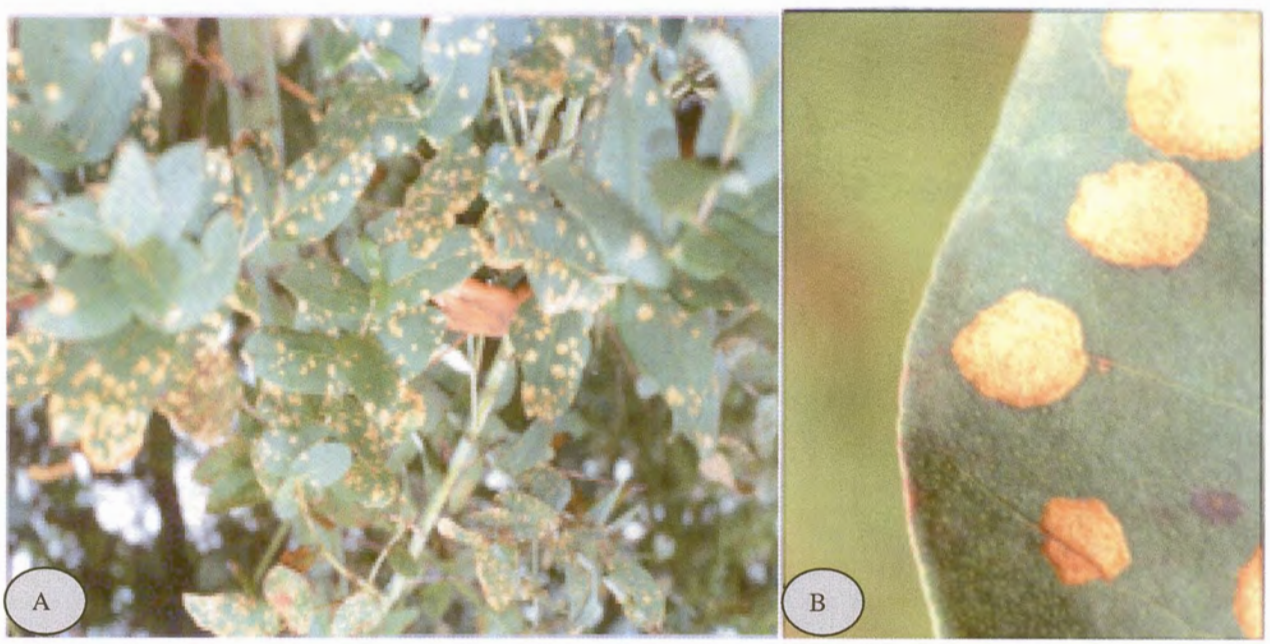
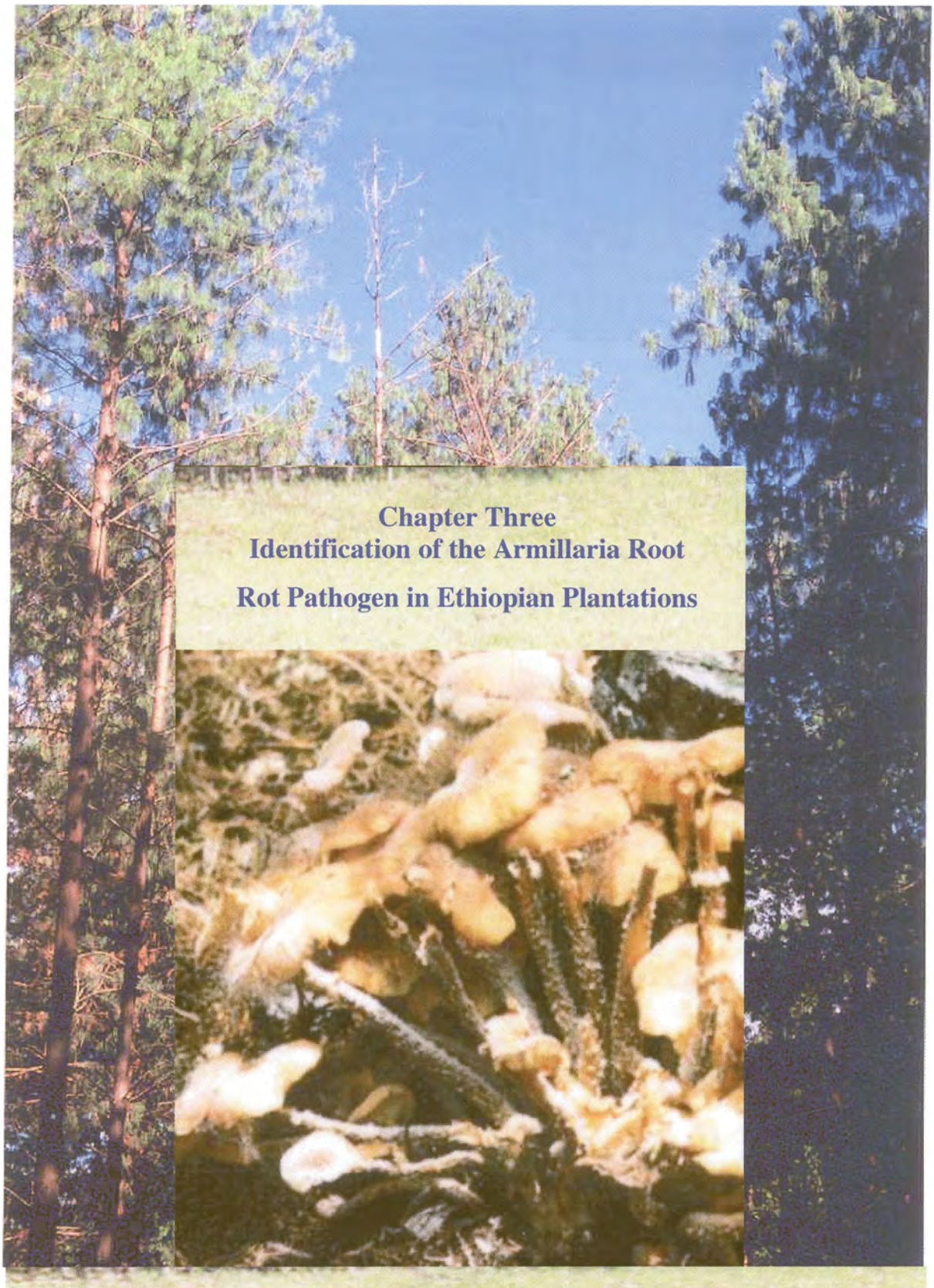



**Figure 5**. Leaf spots on *E. globulus* associated with *Mycosphaerella* spp. (A) multiple leaf infections, (B) single spots with black pseudothecia.



# Chapter Three
# Identification of the Armillaria Root Rot Pathogen in Ethiopian Plantations



Abstract published as Alemu Gezahgne, M.P.A. Coetzee, J. Roux & M. J. Wingfield (2002) Armillaria root rot Ethiopia. South African Journal of Science 98:IV.

## ABSTRACT

Armillaria root rot is a well-known disease on a wide range of plants, world-wide. In Ethiopia, the disease has previously been reported on *Pinus* spp., *Coffea arabica* and on various native hardwoods. The causal agent of the disease has been attributed to *Armillaria mellea*, a species now known to represent a complex of many different taxa. The aim of this study was to determine the extent of Armillaria root rot and the identity of the *Armillaria* sp. in Ethiopian plantations. As part of a plantation disease survey in 2000 and 2001, samples were collected in plantations at and around Munessa Shashemene, Wondo Genet, Jima, Mizan and Bedele, in South and South Western Ethiopia. Basidiocarps were collected and their morphology studied. Morphological identification was confirmed by sequencing the IGS-1 region of the ribosomal rRNA operon and comparing data with published sequences of *Armillaria* spp. *Armillaria* isolates were collected from *Acacia abyssinica, Pinus patula, Cederela odorata* and *Cordia alliodora* trees. Sporocarps were found on stumps of native *Juniperus exelsa*. Basidiocarp morphology suggested that the *Armillaria* sp. collected from *J. exelsa* is *A. fuscipes*. This identification was confirmed for all isolates, based on sequence data. *A. fuscipes* is known to be common in Southern Africa. Its widespread occurrence in Ethiopia suggests that it is also the major cause of Armillaria root rot in that country.

## INTRODUCTION

*Armillaria* species cause root rot on a wide range of hosts, world-wide. These include many species such as *Eucalyptus, Pinus, Acacia* and *Cupressus* that are utilized in plantations (Wargo & Shaw 1985, Hood, Redfern & Kile 1991, Kile, McDonald & Byler 1991). *Armillaria* spp. have been regarded as primary pathogens, stress induced secondary invaders and saprophytes (Wargo & Shaw 1985, Shaw & Kile 1991). Group death, wilting and yellowing of tree tops, resin exudation, as well as the occurrence of white mycelial fans under the bark of infected trees are common symptoms of *Armillaria* infections. In many cases, rhizomorphs are also found associated with Armillaria root rot and these structures facilitate spread of *Armillaria* through the soil (Morrison, Williams & Whitney 1991).

The morphological characteristics of *Armillaria* spp. including the mycelium, rhizomorphs and basidiocarps have traditionally been the most important criteria for identification. The mycelium and the rhizomorphs of many species of these fungi, however, exhibit limited variation, restricting their use in species identification (Watling, Kile & Gregory 1982, Garraway, Hüttermann & Wargo 1991). In contrast, morphological characteristics of the basidiocarps, have provided useful taxonomic characters for species delimitation (Shaw & Kile 1991). However, the seasonal and irregular production of these structures, coupled with their scarcity, has complicated identification of *Armillaria* spp., based on morphology (Watling *et al.* 1982, Wargo & Shaw 1985, Mohammed *et al.* 1994). It is largely due to these limitations that *Armillaria mellea* (Vahl:Fr.) Kumer *sensu lato* was long considered to be a single variable species causing root rot, world-wide (Singer 1956).

In recent years, mating studies (Korhonen 1978, Ullrich & Anderson 1978, Anderson & Ullrich 1979), biochemical comparisons (Morrison *et al.* 1985, Mwangi, Lin & Hubbes 1989, Mwenje & Ride 1996) and DNA based techniques (Anderson & Stasovski 1992, Coetzee *et al.* 2000) have been used to study the biology, taxonomy, and phylogeny of *Armillaria* spp. Currently, it is known that the *Armillaria* species complex, originally

treated as *A. mellea sensu lato*, consists of at least 36 different species (Wargo & Shaw 1985, Volk & Burdsall 1995).

DNA-based characterisation provides a useful tool to identify *Armillaria* spp. The intergenic spacer region (IGS-1) of the rDNA operon is most commonly used to identify and study the relationship of *Armillaria* isolates (Anderson & Stasovski 1992, Coetzee *et al.* 2000). Restriction fragment length polymorphism (RFLP) patterns of this rDNA region are also commonly used to discriminate between *Armillaria* isolates (Harrington & Wingfield 1995).

Armillaria root rot has been reported from several countries in South, East and Western Africa. In Africa, this disease has been found associated with both cash crop plants such as coffee and tea as well as on forest plantation species including those of *Pinus*, *Eucalyptus*, *Acacia* and *Grevillea* (Mwangi *et al.* 1989, Onsando, Wargo & Waud 1997). The disease has generally been ascribed to *Armillaria mellea* (Vahl.:Fr.) P. Kumm. and *A. heimii* Pegler (Pegler 1977, Ivory 1987, Mohammed, Guillaumin & Berthelay 1989). However, recent studies conducted on *Armillaria* isolates from Africa reported the occurrence of *A. heimii*, *A. mellea sensu stricto* (Mwangi *et al.* 1989, Augustian *et al.* 1994, Guillaumin, Mohammed & Abomo-Ndongo 1994, Mohammed *et al.* 1994, Mwangi *et al.* 1994, Mwenje & Ride 1996, Abomo-Ndongo & Guillaumin 1997), *A. camerunensis* (Henn.) Volk & Burdsall [=*A. camerunensis* (Henn) = *A. mellea* (Vahl.:Fr.) P. Kumer var *camerunsis* Henn] (Singer 1986, Mohammed *et al.* 1989, Volk & Burdsall 1995), *A. mellea* (Vahl.:Fr.) P. Kumm. sub species *Africana* (Mohammed *et al.* 1994, Volk & Burdsall 1995) and *A. fuscipes* Petch (Coetzee *et al.* 2000).

In Ethiopia, damage due to Armillaria root rot has been reported from *Pinus patula* Schiede & Deppe plantations at various sites (Mengistu 1992, Dagne 1998, Alemu, Roux & Wingfield 2003). Tree death in plantations due to this disease has been estimated to be between 5-20 % (Dagne 1998). Eshetu, Teame & Girma (2000) also noted that Armillaria root rot caused minor damage in coffee (*Coffea arabica* L.) plantations. Despite this, little attention has been given to the disease. It has generally been assumed

that Armillaria root rot is caused by *A. mellea* (Mengistu 1992, Eshetu *et al.* 2000) and no detailed study has been conducted to identify the *Armillaria* spp. found in Ethiopia. However, a recent study using somatic incompatibility, isozyme comparisons and Random Amplified Polymorphic DNA (RAPD) analyses has suggested the presence of *A. mellea* on hard woods in the Kerita and Jima areas of Ethiopia (Ota, Intini & Hattori 2000).

During a survey of plantation forestry diseases in Ethiopia, conducted in 2000 and 2001, Armillaria root rot was identified as a common cause of tree mortality (Alemu *et al.* 2003). The species identity of the causal agent was, however, not known. The aim of this study was thus to identify the *Armillaria* isolates obtained from the surveys and to consider their phylogenetic relationships with other *Armillaria* species. To accomplish these objectives morphological characteristics of the basidiocarps and DNA-based comparisons including RFLP and DNA sequencing of the IGS-1 region of the rRNA operon, were used.

## MATERIALS AND METHODS

### *Sample collection and isolation*

Surveys were conducted in forestry plantations at Munessa Shashemene, Jima, Bedele, Aman/Mizan and Wondo Genet (Figure 1). Typical symptoms of Armillaria root rot were used to recognise centres of infection. Samples were collected from roots, stumps and stems of dead and dying trees. Small pieces of mycelium from the white mycelial fans, between the bark and the wood were transferred to a selective medium, containing benomyl and streptomycin (Harrington, Worall & Baker 1992). Cultures were incubated at 25 °C in the dark for three weeks. Pieces of mycelium from the tips of the cultures were then transferred to 2% MEA (2% Biolab Malt Extract, 1.5% Biolab Agar) plates to multiply them for further use. Stock cultures of all the isolates used in this study are maintained on 2% MEA slants at 5 °C in the culture collection (CMW) of the Forestry

and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa (Table 1).

***Basidiocarp morphology***

Basidiocarps collected from stumps of felled *Juniperus exelsa* Hochest. Ex. Endl. trees were used to study their morphology. Morphological characteristics of these structures were compared with those published for other species. Characters examined included the colour of the basidiocarps and size of the pileus and stipes. Rayner's (1970) colour chart was used to determine colors.

***DNA extraction***

Representative isolates (CMW5837, 5844, 5846, 8967, 8969, 8971) (Table 1) from different sites and hosts were grown in liquid MY medium (2% Biolab malt extract, 0.3% Biolab yeast extract agar) in the dark at 25 °C, for approximately three weeks. Mycelium was harvested from cultures by centrifugation (8000 *g*, 30 min) and freeze dried. The dried mycelial samples were ground to a fine powder in liquid nitrogen using a mortar and pestle. DNA was extracted using a modified version of the DNA extraction method of Raeder and Broda (1985). Extraction buffer (200 mM Tris-HCl pH 8; 25 mM EDTA; 250 mM NaCl) (1000 µl) was added to about 0.5 g of powdered mycelium and incubated at 60 °C for 30 min. This was followed by a phenol:chlorophorm extraction step. Cell debris was removed by centrifugation at 13000 *g* for one hour. Further phenol:chlorophorm extractions were done on the aqueous phase until a clean interphase was obtained. Chlorophorm extractions were done to remove the traces of phenol. Sodium Acetate (3M NaAc) and absolute ethanol were added to precipitate the nucleic acids and they were collected by centrifugation at 13000 *g*. The nucleic acid pellet was washed with 70% ethanol, vacuum dried and dissolved in 50 µl sterile water. RNase A (0.01 mg/ml) (Roche) was added to the DNA and water suspensions to remove RNA and incubated overnight at 37 °C in a water bath. The resulting DNA was visualised under

UV illumination after electrophoresis on a 1% agarose gel (Promega, Madison, Wisconsin) stained with ethidium bromide.

***DNA amplification***

The IGS-1 region of the ribosomal RNA (rRNA) operon was amplified using the polymerase chain reaction (PCR). This region was amplified with Primers P-1 (5'-TTG CAG ACG ACT TGA ATG G- 3') (Hsiau 1996) and 5S-2B (5' CAC CGC ATC CCG TCT GAT CTG CG 3') (Coetzee *et al.* 2000). The PCR mixtures used included dNTPs (200µM of each), $MgCl_2$ (2.66mM), 10 x buffer containing $MgCl_2$ (supplied with enzyme), 0.375 µM of each primer, *Taq* polymerase (2.6 U) (Roche) and approximately 80 ng template DNA. The final reaction volume was adjusted to 50 µl with $H_2O$. The PCR programme consisted of an initial denaturation step at 96 °C for 2 min. This was followed by 35 cycles of annealing at 58 °C for 30 s, elongation at 72 °C for 2 min., a ramp time of 1.5 s and another denaturation at 94 °C for 30s. A final elongation step was allowed for 7 min at 72 °C. Prior to sequencing, the PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN, Germany). The fragment sizes of the PCR products were determined after electrophoresis on a 1% agarose gel stained with ethidium bromide and visualised under UV illumination. A 100 bp molecular marker was used to determine the size of the PCR fragments.

***Restriction enzyme digestion***

Restriction Fragment Length Polymorphism (RFLP) profiles of isolates included in this study were obtained by digesting the IGS-1 amplicons with the restriction endonuclease *Alu* I (Harrington & Wingfield 1995). IGS-1 amplicons were digested by adding 3 units of *Alu* I restriction endonuclease to 18 µl of unpurified PCR product. Digestion was allowed to occur overnight at 37 °C. DNA fragments were separated on a 3% (w/v) agarose gel (Promega, Madison, Wisconsin) stained with ethidium bromide and profiles were visualised under UV illumination. A 100 base molecular weight marker was used to determine the fragment sizes. The absolute fragment sizes were determined using the

programme Gelreader 20.5 (National Center, Supercomputing Applications, University of Illinois at Urbana-Champaign, 1991). RFLP patterns and sizes of IGS-1 amplicons for the Ethiopian *Armillaria* isolates were compared with those of *Armillaria* spp. published by Coetzee *et al.* (2000).

***Cloning***

It was not possible to sequence the IGS-1 amplicons directly and they were subsequently cloned to resolve this problem. Ligation of the PCR products was conducted using the PGEM® T Easy Vector System (Promega Corporation), 2X Rapid Ligation Buffer, T4 DNA Ligase, PCR products and deionized water according to the protocols outlined by the manufacturer. This reaction was incubated for one hour at room temperature. For transformation, JM109 High Efficiency Competent cells provided with the PGEM® T EASY Vector System II were used. Two µl of the ligation reactions were transferred to 1.5 ml Eppendorf tubes and 25 µl competent cell solution added to each Eppendorf tube. Isolation of recombinant plasmid DNA was accomplished using a standard plasmid miniprep procedure, using the instructions provided by the company.

***DNA sequencing***

Plasmid DNA was used as template to sequence the inserted IGS-1 region of the *Armillaria* samples. DNA sequences were determined using an automated (ABI PRISM™ 3100) DNA sequencer. The inserted region was sequenced in both directions using primers T7 (5'-ATT ATG CTG AGT GAT ATC CC- 3') and SP6 (5'- ATT TAG GTG ACA CTA TAG AA-3') (Promega 1999). The sequencing reactions were prepared using the Big Dye sequence system (ABI Advanced Biotechnology Institute, Perkin-Elmer) as recommended in the manufacturer's protocols.

### *Analysis of DNA sequence data*

Sequence Navigator version 1.01 (ABI PRISM™, Perkin Elmer) was used to manually align the sequence data by inserting gaps. Analysis of the sequence data was done using PAUP* version 4.0b2 (Swofford 1998). In the sequence data analysis, indels of more than 1 base were excluded and substituted with multi-state characters and gaps treated as a 5$^{th}$ character. IGS-1 DNA sequences obtained in this study were aligned against the data matrix published by Coetzee *et al.* (2000) and available on Tree Base (Table 1). Phylogenetic trees generated were rooted to *A. heimii* as the monophyletic sister group to the taxa. Analyses were done using Neighbor-Joining distance analysis and the total character difference was used to generate the tree. The confidence levels of the branching points were determined by 1000 bootstrap replicates.

## RESULTS

### *Sample collection and isolation*

Symptoms of Armillaria root rot were found in plantations at Wondo Genet, Munessa Shashemene, Belete/Jima, Bedele and Aman/Mizan. Armillaria root rot was identified on 10-13 year old *P. patula*, *Acacia abyssinica* Hochest, *Cordia alliodora* (Ruiz & Pav) Oken and *Cedrela odorata* L. trees (Table 1, Figure 1). The characteristic symptoms of infection included groups of dead trees (Figure 2a), wilting and chlorosis, as well as the occurrence of white mycelial fans (Figure 2b) under the bark of diseased trees. Masses of light brown rhizomorphs were found on diseased *C. alliodora* trees, in a research plot at Aman (Figure 2c). The causal fungus was successfully isolated from symptomatic trees and grown on the selective medium. A total of 32 isolates were collected from the different hosts. In culture, the *Armillaria* isolates produced a flat whitish mycelium. Brown, cylindrical rhizomorphs were produced abundantly in cultures (Figure 2d). At the time of sample collection, the incidence of Armillaria root rot damage was most pronounced on *P. patula* at Wondo Genet.

***Basidiocarp morphology***

Ten basidiocarps were collected from stumps of *J. exelsa* trees, in a plantation at Wondo Genet (Figure 2e). These basidiocarps were used to partially identify the *Armillaria* sp. in this study. When the colour, the size of the stipe and the pileus of the basidiocarps were considered, the basidiocarps from Ethiopia showed close similarities with the basidiocarp morphology of *A. fuscipes* (Coetzee *et al.* 2000) and differed from those of the much smaller *A. heimii*. The pileus of the fungus had an average diameter of 45 mm and the length of the stipes varied between 60-87 mm. These measurements are more similar to those of the basidiocarps of *A. fuscipes* (Pileus = 51 mm, Stipe = 64-84 mm) than of *A. heimii* (Pileus = 30 mm, Stipe = 25-45 mm) [Figure 2e].

***DNA amplification***

The IGS regions of all *Armillaria* isolates from Ethiopia were successfully amplified with primers P-1 and 5S-2B. The PCR products of all *Armillaria* isolates used in this study yielded fragments of approximately 1200 base pairs (bp). This PCR fragment size is similar to that published for *A. fuscipes* (Coetzee *et al.* 2000).

***Restriction enzyme digestion***

*Alu* I restriction digestion of PCR amplicons generated identical fragment patterns for all isolates. Three distinct bands with sizes of approximately 370, 250 and 95 bp were obtained (Figure 3). Comparison of RFLP profiles of the Ethiopian *Armillaria* isolates with published profiles for *A. fuscipes* and *A. heimii* (Coetzee *et al.* 2000) showed that the RFLP patterns of *Armillaria* isolates from Ethiopia are different to both *A. fuscipes* and *A. heimii* (Table 2). Furthermore, the Ethiopian RFLP profile did not match that of any other *Armillaria* sp. for which such profiles are available.

***DNA sequencing***

The IGS sequence of the *Armillaria* isolates from Ethiopia, before alignment, varied between 1056 and 1100 bp. A Blast search using the IGS-1 and 5S gene sequences for these isolates against sequences in GenBank [National Centre for Biotechnology information (NCBI), US National Institute of Health Bethesad, (http:/ www.ncbi.nlm. Nih.gov/BLAST)], indicated that the DNA sequences of *Armillaria* isolates from Ethiopia most closely match with the sequences of *A. fuscipes* and *A. heimii*. Therefore, the DNA sequences of the Ethiopian *Armillaria* isolates were aligned with these two species (Coetzee *et al.* 2000). A total of 1247 characters were obtained for analysis after manual alignment.

***Analysis of DNA sequence data***

The *Armillaria* isolates used in this study formed two main groups in a neighbour-Joining tree (Figure 4). Sequences of *Armillaria* isolates from South Africa and La Reunion, which were previously identified as *A. fuscipes* (Coetzee *et al.* 2000) grouped together with a bootstrap support of 90%. The *Armillaria* isolates from Ethiopia resided in a separate cluster with 74% bootstrap support. The Ethiopian *Armillaria* isolates grouped separately from *A. heimii,* showing the closest affinity to *A. fuscipes,* although with some differences. The Ethiopian isolates differed from *A. fuscipes* in having several indels. Isolate CMW8971 differed from *A. fuscipes* with only 11 bp indels (of which 7 bps are deletions), while other Ethiopian isolates showed more variation. The most notable of these are isolates CMW5838 and CMW5846, which have 16 bp deletions, whereas isolates CMW5844, CMW8967 and CMW8969 have 33 bp deletions and contain one restriction site at position nine. Despite these differences, the Ethiopian isolates group with the *A. fuscipes* clade with a bootstrap of 100 % and separately from *A. heimii*.

**DISCUSSION**

Recently, the importance of plantation forestry diseases in Ethiopia has been afforded considerable attention. Results from this study thus, form part of the first comprehensive

plantation disease survey conducted in the country (Alemu *et al.* 2003). This study furthermore presents results of the first extensive survey of Armillaria root rot in Ethiopian forest plantations. Our data clearly show that the dominant *Armillaria* sp. causing root rot and death in plantations is *A. fuscipes*. This is the first report of *A. fuscipes* from Ethiopia and also greatly extends its host range.

Damage from Armillaria root rot has been observed in several African countries, where it has been attributed mainly to *A. mellea* and *A. heimii* (Pegler 1986, Ivory 1987). *Armillaria fuscipes* was recently reported to be common in Southern Africa (Coetzee *et al.* 2000). Outside Africa, *A. fuscipes* is known only from Sir Lanka, where it was first described and where Pegler (1986) suggested that it could have been introduced from Africa. The taxonomic status of *A. heimii* and *A. fuscipes* has, however, been confused for many years. It has thus been suggested that *A. heimii* is conspecific with *A. fuscipes* and the latter name was retained (Pegler 1986, Kile & Watling 1988, Watling, Kile & Burdsall 1991). Recent studies have shown the existence of significant variation between *A. heimii* isolates from various African countries (Augustain *et al.* 1994, Mohammed *et al.* 1994, Mwenje & Ride 1996). A DNA based study conducted on Southern African *Armillaria* isolates, thought to represent *A. heimii* showed that they are dissimilar to *A. heimii* from Zambia, Zimbabwe and Cameroon (Coetzee *et al.* 2000). In the study of Coetzee *et al.* (2000), *Armillaria* isolates from South Africa were shown to represent *A. fuscipes*, and not *A. heimii*. Similarly, *Armillaria* isolates from La Reunion, believed to represent *A. heimii* were found to be identical to the South African *Armillaria* isolates and identified as *A. fuscipes* (Coetzee *et al.* 2000). This study provided clear evidence that these two species represent distinct taxa. The results of the present study show that the Ethiopian *Armillaria* isolates represent *A. fuscipes,* although some differences were observed in RFLP and IGS sequence data.

Basidiocarp morphology has commonly been used to determine the relationships of *Armillaria* spp. (Bérubé & Dessureault 1989, Watling *et al.* 1991). The macro-morphological characters including colour and structures of the pileus, veil, annulus and stipe are reliable characters for this purpose (Bérubé & Dessureault 1989). Seasonal

availability of the basidiocarps, however, limits the use of basidiocarp morphology for species identification. In this study, very few fruiting structures were obtained and these were only from Wondo Genet. The macro-morphological characters of these basidiocarps were different from those of *A. heimii*, having larger pileus and stipes, compared to the small basidiocarps of *A. heimii* (Kile & Watling 1988). The basidiocarps from Ethiopia were very similar to those from South Africa, known to represent *A. fuscipes*. It was not possible to collect a culture linked to these basidiocarps but the proximity of the dying trees to others from which cultures and DNA sequences were obtained provides strong circumstantial evidence that the fungus is the same.

Coetzee *et al.* (2000), showed that the 5S ribosomal rRNA gene of African *A. fuscipes* and *A. heimii* isolates are in opposite orientation in comparison to other *Armillaria* spp. Because of this, primers used to amplify the IGS-1 region of non-African isolates failed to amplify the IGS-1 region of African *Armillaria* isolates (Coetzee *et al.* 2000). Primer 5S-2B was, therefore, used to amplify the IGS-1 region of African *Armillaria* spp. The IGS-1 region of the *Armillaria* isolates from Ethiopia was successfully amplified with primers P-1 and 5S-2B indicating that the 5S gene of Ethiopian *Armillaria* isolates has the same orientation as that of other African *A. fuscipes* and *A. heimii* isolates. This provides further support for our belief that the Ethiopian isolates represent *A. fuscipes*.

A recent population study on *Armillaria* spp. in Ethiopia reported that *A. mellea* is responsible for root rot on hard-wood trees in the Jima and Kerita areas (Ota *et al.* 2000). An isolate from symptomatic *P. patula* trees near Jima in our study, produced the same RFLP profile as those of other *Armillaria* isolates that we have identified as *A. fuscipes*. This suggests that the causative agent of Armillaria root rot of *P. patula* around Jima is identical to other isolates included in our study and that it also represents *A. fuscipes*. The results of Ota *et al.* (2000) and this study, thus suggest that more than one *Armillaria* spp. might be involved in causing Armillaria root rot in Ethiopia. This emphasises the importance of conducting further and more comprehensive studies on the diversity, distribution, and host range of Armillaria root rot in Ethiopia.

RFLP patterns of all Ethiopian *Armillaria* isolates differed from those of *A. fuscipes* and all other *Armillaria* spp. This difference in RFLP pattern was supported by DNA sequence data, which showed the deletion of indels within one of the restriction sites. Although the Ethiopian isolates grouped closely to *A. fuscipes,* they formed a separate sub-clade. This suggests that the *Armillaria* samples from Ethiopia could represent a distinct species, closely related to *A. fuscipes*. Macro- and micro-morphological comparison of the basidiocarps will be essential to understand the significance of this variation.

Results of this study have shown that *Armillaria* root rot not only affects *P. patula,* but that it also kills *Co. alliodora* and *C. odorata* trees planted in research plots at Aman, near Mizan. The fungus was also found on *A. abyssinica* and *J. excelsa,* species native to Ethiopia and growing in the *Pinus* plantations at Bedele and Wondo Genet. Most plantations in Ethiopia are made up of exotic species and these are planted on sites previously occupied by indigenous hardwoods. This suggests that stumps of the native hardwoods could be sources of the initial inoculum needed to infect exotic species. Planting of *Pinus* spp. in these areas should be avoided. The occurrence of the same *Armillaria* sp. on these different tree species implies that this pathogen could be damaging to a wider range of trees in the country. In order to better understand the distribution, diversity and host range of *Armillaria* spp. as well as to investigate its importance in other plantation areas, this study should be extended to other parts of Ethiopia.

## REFERENCES

Abomo-Ndongo, S. & Guillaumin, J.-J. (1997) Somatic incompatibility among African *Armillaria* isolates. *European Journal of Forest Pathology* **27**: 201-206.

Alemu Gezahgne, Roux, J., & Wingfield, M. J. (2003) Diseases of exotic plantation *Eucalyptus* and *Pinus* species in Ethiopia. *South African Journal of Science* **99**:29-33.

Anderson, J. B. & Ullrich, R. C. (1979) Biological species of *Armillaria mellea* in North America. *Mycologia* **71**: 402-414.

Anderson, J. B. & Stasovski, E. (1992) Molecular phylogeny of Northern Hemisphere species of *Armillaria*. *Mycologia* **84**: 505-516.

Agustain, A., Mohammed, C., Guillaumin, J-J. & Botton, B. (1994) Discrimination of some African *Armillaria* species by isozyme electrophoretic analysis. *New Phytologist* **128**: 135-143.

Bérubé, J. A. & Dessureault, M. (1989) Morphological studies of the *Armillaria mellea* complex: Two new species, *A. gemina* and *A. calvescens*. *Mycologia* **81**: 216-225.

Coetzee, M. P. A., Wingfield, B. D., Coutinho, T. A. & Wingfield, M. J. (2000) Identification of the causal agent of Armillaria root rot of *Pinus* species in South Africa. *Mycologia* **92**: 777-785.

Coetzee, M. P. A., Wingfield, B. D., Harrington, T. C., Steimel, J., Coutinho, T. A. & Wingfield, M. J. (2001) The root rot fungus *Armillaria mellea* introduced into South Africa by early Dutch settlers. *Molecular Ecology* **10**: 387-396.

Dagne Duguma (1998) *Incidence and Prevalence of Armillaria root rot in some plantations of Wondo Genet*. BSc. Thesis. Wondo Genet Collage of Forestry. Wondo Genet, Ethiopia.

Eshetu Derso, Teame Gebrezgi & Girma Adugna (2000) Significance of minor diseases of *Coffea arabica* L. in Ethiopia: A review. In *Proceedings of the workshop on control of Coffee berry disease (CBD) in Ethiopia*. Ethiopian Agricultural Research Organization: 58-65. Addis Ababa, Ethiopia.

Garraway, M. O., Hüttermann, A. & Wargo, P. M. (1991) Ontogeny and physiology. In *Armillaria root rot disease* (C. G. Shaw & G. A. Kile eds): pp 21-47. Agriculture Handbook No. 691. Forest Service, United States Department of Agriculture, Washington DC.

Guillaumin, J-J., Mohammed, C. & Abomo-Ndongo, S. (1994) Vegetative incompatibility and sexual systems of *Armillaria* isolates from tropical Africa. In *Proceedings of the 8$^{th}$ IUFRO Conference on Root and Butt Rot* (M. Johansson and J. Stenlid Eds): pp. 349-354. Uppsala, Swedish University of Agricultural Sciences. Uppsala, Sweden.

Harrington, T. C. & Wingfield, B. D. (1995) A PCR-based identification method for species of *Armillaria*. *Mycologia* **87**: 280-288.

Harrington, T. C., Worrall, J. J. & Baker, F. A. (1992) Armillaria. In: *Methods for research on soilborne phytopathogenic fungi*. ( L. L. Singleton, J. D., Mihail & C. M. Rush eds): pp 81-85. APS press, The American Phytopathological Society, St. Paul, Minnesota.

Hood, I. A., Redfern, D. B. & Kile, G. A. (1991) *Armillaria* in planted hosts. In *Armillaria root disease* (C. G. Shaw & G. A. Kile eds): pp 122-149. Agriculture Handbook No. 691. Forest Service, United States Department of Agriculture, Washington DC.

Hsiau, P. T-W. (1996) The taxonomy and phylogeny of the mycangial fungi from *Dendroctonus brevicomis* and *D. frontalis* (Coleoptera: Scolytidae). Ph.D Thesis, Ames, Iowa, Iowa State University. U.S.A

Ivory, M. H. (1987) *Diseases and disorders of pines in the tropics. A field and laboratory manual*. Overseas Research Publications Number 31. Burgess & Son Ltd. Abingdon, Oxfordshire.

Kile, G. A., McDonald, G. I. & Byler, J. W. (1991) Ecology and disease in natural forests. In *Armillaria root disease* (C. G. Shaw & G. A. Kile eds): pp 102-121. Agriculture Hand book No. 691. Forest Service, United States Department of Agriculture, Washington DC.

Kile, G. A. & Watling, R. (1988) Identification and occurrence of Australian *Armillaria* species, including *A. pallidula* sp. nov. and comparative studies between them and non-Australian tropical and Indian *Armillaria*. *Transactions of the British Mycological Society* **91**: 305-315.

Korhonen, K. (1978) Interfertility and clonal size in the *Armillariella mellea* complex. *Karstenia* **18**: 31-42.

Mengistu Huluka (1992). Some aspects of forest tree disease in Ethiopia. In *National workshop on setting forestry research priorities*. Forestry Research Centre, Addis Ababa, Ethiopia.

Mohammed, C., Guillaumin, J.-J & Berthelay, S. (1989) Preliminary investigations about the taxonomy and genetics of African *Armillaria* species. In *Proceedings of the*

*7th IUFRO Conference on Root & Butt Rots* (D. Morrison Ed): pp. 447-457. Vernon & Victoria. British Colombia, Canada.

Mohammed, C., Guillaumin, J.J., Botton, B. & Intini, M. (1994) Species of *Armillaria* in tropical Africa. In *Proceedings of the 8th IUFRO Conference on Root and Butt Rot* ( M. Johansson and J. Stenlid Ed): pp. 402-410. Uppsala, Swedish University of Agricultural Sciences. Uppsala, Sweden.

Morrison, D. J., Thomson, A. J., Chu, D., Peet, F. G., & Sahota, T. S. (1985) Isozyme patterns of *Armillaria* intersterility groups occurring in British Colombia. *Canadian Journal of Microbiology* **31**: 651-653.

Morrison, D. J., Williams, R. E., & Whitney, R. D. (1991) Infection, disease development, diagnosis and detection. In *Armillaria root disease.* Agriculture Handbook No. 691 (C.G. Shaw & G.A. Kile Eds): pp 62-75. Forest Service, United States Department of Agriculture, Washington DC.

Mwangi, L., Lin, D. & Hubbes, M. (1989) Identification of Kenyan *Armillaria* isolates by cultural morphology, intersterility tests and analysis of isozyme profiles. *European Journal of Forest Pathology* **19**: 399-406.

Mwangi, L. M., Mwenje, E., Makambila, C., Chanakira-Nyahwa, F. Guillaumin, J-J. & Mohammed, C. (1994) Ecology and pathogenicity of *Armillaria* in Kenya, Zimbabwe and the Congo. In *Proceedings of the 8th IUFRO Conference on Root and Butt Rots* (M. Johansson & J. Stenlid ed)**:** pp 34-44. Uppsala, Swedish University of Agricultural Sciences.

Mwenje, E. & Ride, J. P. (1996) Morphological and biochemical characterization of *Armillaria* isolates from Zimbabwe. *Plant Pathology* **45**: 1036-1051.

Onsando, J. M., Wargo, P. M. & Waud, S. W. (1997) Distribution, severity, and spread of Armillaria root disease in Kenya tea plantations. *Plant Disease* **81**: 133-137.

Ota, Y., Intini, M., & Hattori, T. (2000) Genetic characterization of heterothallic and non-heterothallic *Armillaria mellea sensu stricto*. *Mycological Research* **104**: 1046-1054.

Pegler, D. N. (1977) A preliminary agaric flora of East Africa. *Kew Bulletin, Additional Series* **6**: 91-95.

Pegler, D. N. (1986) Agaric flora of Sir Lanka. *Kew Bulletin Additional Series XII*. Royal Botanical Gardens, Kew, London.

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

Rayner, R. W. (1970) *A mycological colour chart*. Commonwealth Mycological Institute, Kew, Botanic Gardens.

Shaw, C. G. & Kile, G. A.( 1991) *Armillaria root disease*. Agriculture Handbook No. 691. Forest Service, United States Department of Agriculture, Washington D.C.

Singer, R. (1956) The *Armillariella mellea* group. *Lloydia* **19**: 176-178.

Singer, R. (1986). *The Agaricales in modern taxonomy* (4th edition). Koeltz Scientific Books, Koenigestein, Germany.

Swofford D. L. (1998) PAUP*: Phylogenetic Analysis Using Parismony (* and other methods). Vers:4. Sunderland, Masschusetts:Sinauer Associates.

Ullrich, R. C. & Anderson, J. B. (1978) Sex and diploidy in *Armillaria mellea*. *Experimental Mycology* **2**: 119-129.

Volk, T. J. & Burdsall, H. H. (1995) A nomenclatural study of *Armillaria* and *Armillariella* species. *Synopsis Fungorum* **8**: 1-121.

Wargo, P. M. & Shaw, C. G. (1985) Armillaria root rot: the puzzle is being resolved. *Plant Disease* **69**: 826-832

Watling, R., Kile, G. A. & Gregory, N. M. (1982) The genus *Armillaria*-nomenclature, typification, the identity of *Armillaria mellea* and species differentiation. *Transactions of the British Mycological Society* **78**: 271-285.

Watling, R., Kile, G. A. & Burdsall, H. Jr. (1991) Nomenclature, taxonomy and identification. In *Armillaria root disease* (C. G. Shaw & G. A. Kile eds). pp. 1-9. Agriculture Handbook No. 691. Forest Service, United States Department of Agriculture, Washington DC.

**Table 1.** *Armillaria* isolates used in this study

| Identity | Culture number [a] | Host | Origin | Collector | Accession No. |
|---|---|---|---|---|---|
| *A. fuscipes* [b] | CMW5838 | *Pinus patula* | Wondo Genet, Ethiopia | Alemu Gezahgne & Roux, J. | AY172029 |
| *A. fuscipes* [b] | CMW5844 | *P. patula* | Wondo Genet, Ethiopia | Alemu Gezahgne & Roux, J. | AY172032 |
| *A. fuscipes* [b] | CMW5846 | *P. patula* | Wondo Genet, Ethiopia | Alemu Gezahgne & Roux, J. | AY172030 |
| *A. fuscipes* [b] | CMW8967 | *Cordia alliodora* | Aman/Mizan, Ethiopia | Alemu Gezahgne & Roux, J. | AY172031 |
| *A. fuscipes* [b] | CMW8969 | *Acacia abyssinica* | Bedele, Ethiopia | Alemu Gezahgne & Roux, J. | AY172034 |
| *A. fuscipes* [b] | CMW8971 | *P. patula* | Belete/Jima, Ethiopia | Alemu Gezahgne & Roux, J. | AY172033 |
| *A. fuscipes* [c] | CMW2717 | *P. elliottii* | Sabie, South Africa | Wingfield, M. J. | AF204821 |
| *A. fuscipes* [c] | CMW2740 | *P. patula* | Entabeni, South Africa | Wingfield, M. J. | AF204822 |
| *A. fuscipes* [c] | CMW3167 | *P. elliottii* | Sabie, South Africa | Ivory, M. | AF204823 |
| *A. heimii* [c] | CMW3152 | Unknown | Western Province, Cameroon | Watling, R. | AF204826 |
| *A. heimii* [c] | CMW3164 | *Pelargonium asperum* | Saint-Denis, La Reunion | Fabergue, C. | AF204824 |
| *A. heimii* [c] | CMW3173 | *Tectona grandis* | Dola Hill, Zambia | Ivory, M. | AF204825 |
| *A. heimii* [c] | CMW3955 | *Acacia xanthophloea* | Harare, Zimbabwe | Wingfield, M. J. & Coetzee, M. P. A. | AF204827 |

[a] CMW numbers refer to the culture collection numbers of the Tree Pathology Co-operative Programme (TPCP), FABI, University of Pretoria. Pretoria, South Africa.
[b] Isolates sequenced in this study.
[c] Sequence of *Armillaria*, in FABI database, identical to those submitted to GenBank (Coetzee *et al.* 2000).

**Table 2.** Comparison of RFLP sizes of *Armillaria* isolates

| *Armillaria* from Ethiopia | *A. fuscipes*[a] | *A. heimii*[a] | *A. mellea*[b] |
|---|---|---|---|
| 370 | 365 | 530 | 215 |
| 250 | 245 | 220 | 175 |
| 95 | 135 | 175 | 150 |

[a] Data obtained from Coetzee *et al.* 2000

[b] Data obtained from Coetzee *et al.* 2001


**Figure 1**. Map of Ethiopia showing the plantation areas where surveys were conducted.


**Figure 2.** Symptoms and signs of *Armillaria* infection. (A) Group death of infected trees in a *Pinus patula* stand in Wondo Genet, Ethiopia, (B) White mycelial fan, (C) Rhizomorphs found between bark and wood on *Cordia alliodora*, (D) Culture of *Armillaria* growing on MEA, (E) Basidiocarps of *Armillaria* on a *Juniperus exelsa* stump.

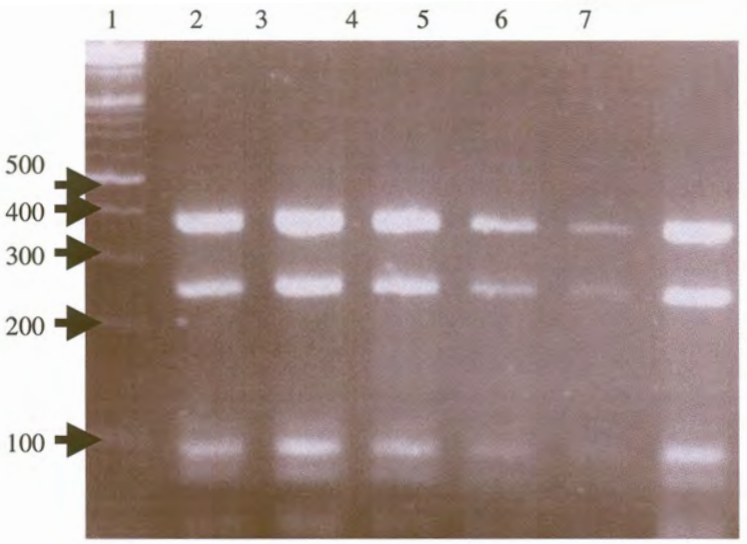



**Figure 3**. *Alu I* restriction fragment patterns of *Armillaria* isolates from Ethiopia on 3% agarose gel stained with ethidium bromide. Lane 1=Molecular marker, 2=CMW 5844, 3=CMW 5846, 4=CMW 5838, 5=CMW 8969, 6=CMW 8967 and 7=CMW 8971.

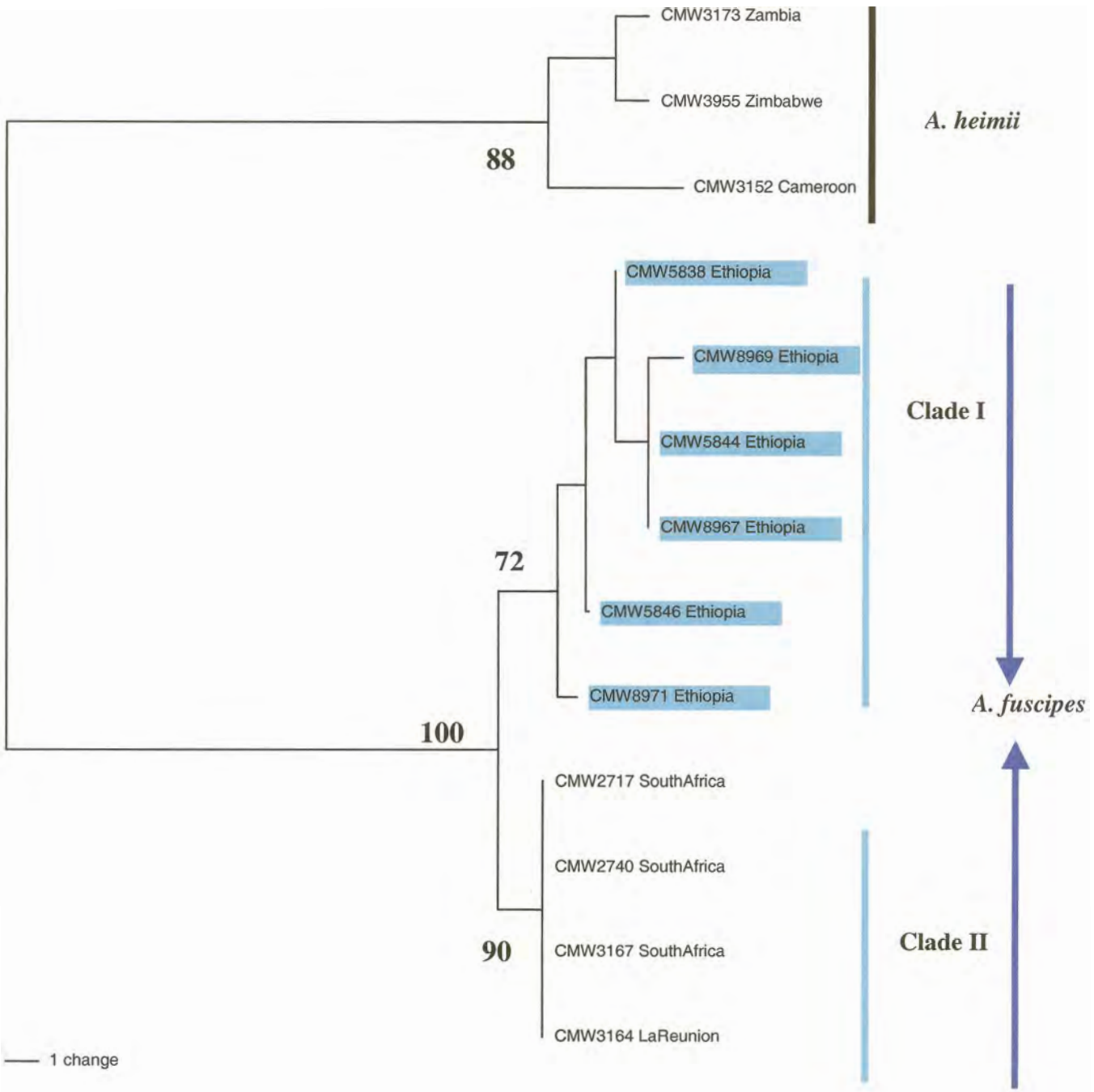



**Figure 4**. Phylogram generated from Neighbor Joining analysis of the IGS-1 sequence data used in this study. Bootstrap values are shown on the branches.

```
                          150        160        170        180        190        200        210
CMW3173_ZAMBIA        AACAGCATGT TTAATGGA-- ---------- --AGCCTATT GTGTATAATA TTGGTATATA CGGTGTACGG
CMW3152_CAMEROON      .......... ........-- ---------- --.......G .......... .......... ..........
CMW3955_ZIMBABWE      .......... ........-- ---------- --........ .......... .......... ..........
CMW5837_ETHIOPIA      .......... .G......GG GGTATGGATC CA...G.... ..-------- -----..... .......T..
CMW5846_ETHIOPIA      .......... .G......GG GGTATGGATC CA...G.... ..-------- -----..... .......T..
CMW8968_ETHIOPIA      .......... .G......GG GGTATGGATC CA...G.... ..-------- -----..... .......T..
CMW5844_ETHIOPIA      .......... .G......GG GGTATGGATC CA...G.... ..-------- -----..... .......T..
CMW8967_ETHIOPIA      .......... .G......GG GGTATGGATC CA...G.... ..-------- -----..... .......T..
CMW8971_ETHIOPIA      .......... .G......GG GGTATGGATC CA...G.... ..-------- -----..... .......T..
CMW2717_SOUTH_AFRICA  .......... .G......GG GGTATGGATC CA...G.... ..-------- -----..... .......T..
CMW2740_SOUTH_AFRICA  .......... .G......GG GGTATGGATC CA...G.... ..-------- -----..... .......T..
CMW3167_SOUTH_AFRICA  .......... .G......GG GGTATGGATC CA...G.... ..-------- -----..... .......T..
CMW3164_LA_REUNION    .......... .G......GG GGTATGGATC CA...G.... ..-------- -----..... .......T..

                          220        230        240        250        260        270        280
CMW3173_ZAMBIA        AGTACGGGTA TACAGAAGAG -----TATAC AGTACAGTAG ACAGTATATA TATATATA-- --TTATAT-A
CMW3152_CAMEROON      .......... .......... -----..... .......... .......... ........-- --.......-.
CMW3955_ZIMBABWE      .......... .......... -----..... .......... .......... ........-- --.......-.
CMW5837_ETHIOPIA      ...G...... .......... AAGAG..... ...G.....C .......... ........TA TA....G.A.
CMW5846_ETHIOPIA      ...G...... .......... AAGAG..... ...G.....C .......... ........TA --....G.A.
CMW8968_ETHIOPIA      ...G...... .......... AAGAG..... ...G.....C .......... ........TA TA....G.A.
CMW5844_ETHIOPIA      ...G...... .......... AAGAG..... ...G.....C .......... ........TA TA....G.A.
CMW8967_ETHIOPIA      ...G...... .......... AAGAG..... ...G.....C .......... ........TA TA....G.A.
CMW8971_ETHIOPIA      ...G...... .......... AAGAG..... ...G.....C .......... ........TA TA....G.A.
CMW2717_SOUTH_AFRICA  ...G...... .......... AAGAG..... ...G.....C .......... ........TA --....G.A.
CMW2740_SOUTH_AFRICA  ...G...... .......... AAGAG..... ...G.....C .......... ........TA --....G.A.
CMW3167_SOUTH_AFRICA  ...G...... .......... AAGAG..... ...G.....C .......... ........TA --....G.A.
CMW3164_LA_REUNION    ...G...... .......... AAGAG..... ...G.....C .......... ........TA --....G.A.
```

```
                                290        300        310        320        330        340        350
CMW3173_ZAMBIA          TCTAT--GAC TTGGACTTGG ACTTGTACTT GGACTTGGAT CTTGGATCAC AATGCAAGTA AGGTAGTAGG
CMW3152_CAMEROON        .....--... .C........ .......... .......... .......... .......... ..........
CMW3955_ZIMBABWE        .....--... .......... .......... .......... .......... .......... ..........
CMW5837_ETHIOPIA        ....CAT... .......... .......... .......... .....G---- ---------- ---.T.A.AT
CMW5846_ETHIOPIA        ....CAT... .......... .......... .......... .....G---- ---------- ---.T.A.AT
CMW8968_ETHIOPIA        ....CAT... .......... .......... .......... .....G---- ---------- ---.T.A.AT
CMW5844_ETHIOPIA        ....CAT... .......... .......... .......... .....G---- ---------- ---.T.A.AT
CMW8967_ETHIOPIA        ....CAT... .......... .......... .......... .....G---- ---------- ---.T.A.AT
CMW8971_ETHIOPIA        ....CAT... .......... .......... .......... .....G---- ---------- ---.T.A.AT
CMW2717_SOUTH_AFRICA    ....CAT... .......... .......... .......... .....G..-- --------.T G...T.A.AT
CMW2740_SOUTH_AFRICA    ....CAT... .......... .......... .......... .....G..-- --------.T G...T.A.AT
CMW3167_SOUTH_AFRICA    ....CAT... .......... .......... .......... .....G..-- --------.T G...T.A.AT
CMW3164_LA_REUNION      ....CAT... .......... .......... .......... .....G..-- --------.T G...T.A.AT


                                360        370        380        390        400        410        420
CMW3173_ZAMBIA          CAATGCAACG CAAGGCTAGT AGACAACGCA AGGCAATGCA AGGATAGTAG ACAATGCAAG GCAATGCAAG
CMW3152_CAMEROON        .......... .......... .A..G..... .......... .......... .......... .......---
CMW3955_ZIMBABWE        .......... .......... .......... .......... .......... .......... ..........
CMW5837_ETHIOPIA        ..-------- ---------- ------.CA. ..A....... ....A----- ---------- ----------
CMW5846_ETHIOPIA        ..-------- ---------- ------.CA. ..A....... ....A----- ---------- ----------
CMW8968_ETHIOPIA        ..-------- ---------- ------.CA. ..A....... ....A----- ---------- ----------
CMW5844_ETHIOPIA        ..-------- ---------- ------.CA. ..A....... ....A----- ---------- ----------
CMW8967_ETHIOPIA        ..-------- ---------- ------.CA. ..A....... ....A----- ---------- ----------
CMW8971_ETHIOPIA        ..-------- ---------- ------.CA. ..A....... ....A----- ---------- ----------
CMW2717_SOUTH_AFRICA    ..-------- ---------- ------.CA. ..A....... ....A----- ---------- ----------
CMW2740_SOUTH_AFRICA    ..-------- ---------- ------.CA. ..A....... ....A----- ---------- ----------
CMW3167_SOUTH_AFRICA    ..-------- ---------- ------.CA. ..A....... ....A----- ---------- ----------
CMW3164_LA_REUNION      ..-------- ---------- ------.CA. ..A....... ....A----- ---------- ----------
```

```
                          430        440        450        460        470        480        490
CMW3173_ZAMBIA        GCTAGTAGAC AACGCAACGC AATGCAA-GG CTAGTAGACA ACGCAAGGC- -AAGTAAGCT AGCAGGCAGA
CMW3152_CAMEROON      ---------- ---------- -----..-.. .......... .........- -......... ..........
CMW3955_ZIMBABWE      .......... .......... .......-.. .......... .........- G......... ..........
CMW5837_ETHIOPIA      .......... ..T....G.- -----..-.- .......... .GA.G.C--- -......... .C.......T
CMW5846_ETHIOPIA      .......... ..T....G.- -----..-.- .......... .GA.G.C--- -......... .C.......T
CMW8968_ETHIOPIA      .......... ..T....G.- -----..-.- .......... .GA.G.C--- -......... .C.G......
CMW5844_ETHIOPIA      .......... ..T....G.- -----..-.- .......... .GA.G.C--- -......... .C........
CMW8967_ETHIOPIA      .......... ..T....G.- -----..-.- .......... .GA.G.C--- -......... .C........
CMW8971_ETHIOPIA      .......... ..T....G.- -----..-.- .......... .GA.G.C--- -......... .C........
CMW2717_SOUTH_AFRICA  ---------- ---------- --------.- .......... .GA.G.C--- -......... .C........
CMW2740_SOUTH_AFRICA  ---------- ---------- --------.- ..T....... .GA.G.C--- -......... .C........
CMW3167_SOUTH_AFRICA  ---------- ---------- --------.- ..T....... .GA.G.C--- -......... .C........
CMW3164_LA_REUNION    ---------- ---------- --------.- ..N....... .GA.G.C--- -......... .C........

                          500        510        520        530        540        550        560
CMW3173_ZAMBIA        CTTGTGAG-- TTGAGAGCTT GTACGCATGT CTTAGTTGGT GTGCA----- ---------- ----------
CMW3152_CAMEROON      ........-- .......... .......... .......... .....----- ---------- ----------
CMW3955_ZIMBABWE      ........-- .......... .......... .......... .....----- ---------- ----------
CMW5837_ETHIOPIA      ....A...-- -------... .......... T........C .C...TAGAG TCTTTGGACT TGGGACTTGG
CMW5846_ETHIOPIA      ....A...-- -------... .......... .........C .C...TAGAG TCTTTGGACT TGGGACTTGG
CMW8968_ETHIOPIA      ........-- ---------- ---------- ----.....C .C...TAGAG TCTTTGGACT TGGGACTTGG
CMW5844_ETHIOPIA      ........-- ---------- ---------- ----.....C .C...TAGAG TCTTTGGACT TGGGACTTGG
CMW8967_ETHIOPIA      ........-- ---------- ---------- ----.....C .C...TAGAG TCTTTGGACT TGGGACTTGG
CMW8971_ETHIOPIA      ........TC .......... .......... .........C .C...TAGAG TCTTTGGACT TGGGACTTGG
CMW2717_SOUTH_AFRICA  ........TC .......... ........C. .........C .C...TAGAG TCTTTGGACT TGGGACTTGG
CMW2740_SOUTH_AFRICA  ........TC .......... ........C. .........C .C...TAGAG TCTTTGGACT TGGGACTTGG
CMW3167_SOUTH_AFRICA  ........TC .......... ........C. .........C .C...TAGAG TCTTTGGACT TGGGACTTGG
CMW3164_LA_REUNION    ........TC .......... ........C. .........C .C...TAGAG TCTTTGGACT TGGGACTTGG
```

```
                          570        580        590        600        610        620        630
CMW3173_ZAMBIA            ---------- --TTGCGGAC TTGG------ ---------G CATTGA-GGG CTTGTATGCA -CGCA--CCT
CMW3152_CAMEROON          ---------- --........ ....------ ---------. ......-... .......... T...--...
CMW3955_ZIMBABWE          ---------- --........ ....------ ---------. ......-... .......... -...--...
CMW5837_ETHIOPIA          ACAGCCAACG GA........ ....ACAGAA TTGCAAGCT. ......-.C. ..C...C... T-...TG...
CMW5846_ETHIOPIA          ACAGCCAACG GA........ ....ACAGAA TTGCAAGCT. ......-.C. ..C...C... T-...TG...
CMW8968_ETHIOPIA          ACAGCCAACG GA........ ....ACAGAA TTGCAAGCT. ......-.C. ..C...C... T-...TG...
CMW5844_ETHIOPIA          ACAGCCAACG GA........ ....ACAGAA TTGCAAGCT. ......-.C. ..C...C... T-...TG...
CMW8967_ETHIOPIA          ACAGCCAACG GA........ ....ACAGAA TTGCAAGCT. ......-.C. ..C...C... T-...TG...
CMW8971_ETHIOPIA          ACAGCCAACG GA........ ....ACAGAA TTGCAAGCT. ......-.C. ..C...C... T-...TG...
CMW2717_SOUTH_AFRICA      ACACCCAATG GA........ ....ACAGAA TTGCAAGCT. ......-.C. ..C...C... T-...TG...
CMW2740_SOUTH_AFRICA      ACACCCAATG GA........ ....ACAGAA TTGCAAGCT. ......-.C. ..C...C... T-...TG...
CMW3167_SOUTH_AFRICA      ACACCCAATG GA........ ....ACAGAA TTGCAAGCT. ......A.C. ..C...C... T-...TG...
CMW3164_LA_REUNION        ACACCCAATG GA........ ....ACAGAA TTGCAAGCT. ......-.C. ..C...C... T-...TG...

                          640        650        660        670        680        690        700
CMW3173_ZAMBIA            TAACGGACTT GGACATTGAG GTGTATGCAC G---CTT--- ---------- -GGACATTGA G---------
CMW3152_CAMEROON          ..-....... .......... .......... .GACA..GAG GTGTATGCAC -......... .---------
CMW3955_ZIMBABWE          ..-....... .......... .......... .CAC...ACG GACTT----- -......... .---------
CMW5837_ETHIOPIA          ..CTT.T... .C.-----.. C..C..C..T .--A...GCC CTCAAGCAAA T.--....C. ATGCGTCGAC
CMW5846_ETHIOPIA          ..CTT.T... .C.-----.. C..C..C..T .--A...GCC CTCAAGCAAA T.--....C. ATGCGTCGAC
CMW8968_ETHIOPIA          ..CTT.T... .C.-----.. C..C..C..T .--A...GCC CTCAAGCAAA T.--....C. ATGCGTCGAC
CMW5844_ETHIOPIA          ..CTT.T... .C.-----.. C..C..C..T .--A...GCC CTCAAGCAAA T.--....C. ATGCGTCGAC
CMW8967_ETHIOPIA          ..CTT.T... .C.-----.. C..C..C..T .--A...GCC CTCAAGCAAA T.--....C. ATGCGTCGAC
CMW8971_ETHIOPIA          ..CTT.T... .C.-----.. C..C..C..T .--A...GCC CTCAAGCAAA T.--....C. ATGCGTCGAC
CMW2717_SOUTH_AFRICA      ..CTT.T... .C.-----.. C..C..C..T .--A...GCC CTCAAGCAAA T.--..... ATGCGTCGAC
CMW2740_SOUTH_AFRICA      ..CTT.T... .C.-----.. C..C..C..T .--A...GCC CTCAAGCAAA T.--..... ATGCGTCGAC
CMW3167_SOUTH_AFRICA      ..CTT.T... .C.-----.. C..C..C..T .--A...GCC CTCAAGCAAA T.--..... ATGCGTCGAC
CMW3164_LA_REUNION        ..CTT.T... .C.-----.. C..C..C..T .--A...GCC CTCAAGCAAA T.--..... ATGCGTCGAC
```

```
                                710        720        730        740        750        760        770
CMW3173_ZAMBIA          ---------- GTGT------ ----ATGCA- ---------- ---------- ---CGGACAT TGAGGTGTAT
CMW3152_CAMEROON        ---------- ....------ ----.....- ---------- ---------- ---....... ..........
CMW3955_ZIMBABWE        ---------- ....------ ----.....- ---------- ---------- ---....... ..........
CMW5837_ETHIOPIA        TTGCAAGCTA ....TGCGCA TATT.....T GTCTTACTTG CATTTCGCTA GTTA.C.... ...CT..C.A
CMW5846_ETHIOPIA        TTGCAAGCTA ....TGCGCA TATT.....T GTCTTACTTG CATTTCGCTA GTTA.C.... ...CT..C.A
CMW8968_ETHIOPIA        TTGCAAGCTA ....TGCGCA TATT.....T GTCTTACTTG CATTTCGCTA GTTA.C.... ...CT..C.A
CMW5844_ETHIOPIA        TTGCAAGCTA ....TGCGCA TATT.....T GTCTTACTTG CATTTCGCTA GTTA.C.... ...CT..C.A
CMW8967_ETHIOPIA        TTGCAGGCTA ....TGCGCA TATT.....T GTCTTACTTG CATTTCGCTA GTTA.C.... ...CT..C.A
CMW8971_ETHIOPIA        TTGCAAGCTA ....TGCGCA TATT.....T GTCTTACTTG CATTTCGCTA GTTA.C.... ...CT..C.A
CMW2717_SOUTH_AFRICA    TTGCAAGCTA ....TGCGCA TATT.....T GTCTTACTTG CATTTCGCTA GTTA.C.... ...CT..C.A
CMW2740_SOUTH_AFRICA    TTGCAAGCTA ....TGCGCA TATT.....T GTCTTACTTG CATTTCGCTA GTTA.C.... ...CT..C.A
CMW3167_SOUTH_AFRICA    TTGCAAGCTA ....TGCGCA TATT.....T GTCTTACTTG CATTTCGCTA GTTA.C.... ...CT..C.A
CMW3164_LA_REUNION      TTGCAAGCTA ....TGCGCA TATT.....T GTCTTACTTG CATTTCGCTA GTTA.C.... ...CT..C.A

                                780        790        800        810        820        830        840
CMW3173_ZAMBIA          GCACGCACCT TACG------ -----GAC-- ---------- ---------- ---------- ----------
CMW3152_CAMEROON        .......... ....------ -----...-- ---------- ---------- ---------- ----------
CMW3955_ZIMBABWE        .......... ....------ -----...-- ---------- ---------- ---------- ----------
CMW5837_ETHIOPIA        ..---..AG. ....CTAGTT AGTTA...AA GCTTGGTTTG ACTTTGGCAA ATGCGTTCAC TTGCAAGCTT
CMW5846_ETHIOPIA        ..---..AG. ....CTAGTT AGTTA...AA GCTTGGTTTG ACTTTGGCAA ATGCGTTCAC TTGCAAGCTT
CMW8968_ETHIOPIA        ..---..AG. ....CTAGTT AGTTA...AA GCTTGGTTTG ACTTTGGCAA ATGCGTTCAC TTGCAAGCTT
CMW5844_ETHIOPIA        ..---..AG. ....CTAGTT AGTTA...AA GCTTGGTTTG ACTTTGGCAA ATGCGTTCAC TTGCAAGCTT
CMW8967_ETHIOPIA        ..---..AG. ....CTAGTT AGTTA...AA GCTTGGTTTG ACTTTGGCAA ATGCGTTCAC TTGCAAGCTT
CMW8971_ETHIOPIA        ..---..AG. ....CTAGTT AGTTA...AA GCTTGGTTTG ACTTTGGCAA ATGCGTTCAC TTGCAAGCTT
CMW2717_SOUTH_AFRICA    ..---..AG. ....CTAGTT AGTTA...AA CCTTGGTTTG ACTTTGGCAA ATGCGTTCAC TTGCAAGCTT
CMW2740_SOUTH_AFRICA    ..---..AG. ....CTAGTT AGTTA...AA CCTTGGTTTG ACTTTGGCAA ATGCGTTCAC TTGCAAGCTT
CMW3167_SOUTH_AFRICA    ..---..AG. ....CTAGTT AGTTA...AA CCTTGGTTTG ACTTTGGCAA ATGCGTTCAC TTGCAAGCTT
CMW3164_LA_REUNION      ..---..AG. ....CTAGTT AGTTA...AA CCTTGGTTTG ACTTTGGCAA ATGCGTTCAC TTGCAAGCTT
```

```
                          850        860        870        880        890        900        910
CMW3173_ZAMBIA        --TTGGACAT TGAGGGCTTG TA-------- ---------- ---------- -------CGC ACGCACCTTA
CMW3152_CAMEROON      --........ .......... ..-------- ---------- ---------- -------... ..........
CMW3955_ZIMBABWE      --........ .......... ..-------- ---------- ---------- -------... ..........
CMW5837_ETHIOPIA      AG......TA ...TTT.G.. C.TTGAAATA CAAGTCAACA TGCTAGCTAG CACTTCAT.A .T.G.A...G
CMW5846_ETHIOPIA      AG......TA ...TTT.G.. C.TTGAAATA CAAGTCAACA TGCTAGCTAG CACTTCA..A .T.G.A...G
CMW8968_ETHIOPIA      AG......TA ...TTT.G.. C.TTGAAATA CAAGTCAACA TGCTAGCTAG CACTTCA..A .T.G.A...G
CMW5844_ETHIOPIA      AG......TA ...TTT.G.. C.TTGAAATA CAAGTCAACA TGCTAGCTAG CACTTCA..A .T.G.A...G
CMW8967_ETHIOPIA      AG......TA ...TTT.G.. C.TTGAAATA CAAGTCAACA TGCTAGCTAG CACTTCA..A .T.G.A...G
CMW8971_ETHIOPIA      AG......TA ...TTT.G.. C.TTGAAATA CAAGTCAACA TGCTAGCTAG CACTTCA..A .T.G.A...G
CMW2717_SOUTH_AFRICA  AG......TA ...TTT.G.. C.TTGAAATA CAAGTCAACA TGCTAGCTAG CACTTCA..A .T.G.A...G
CMW2740_SOUTH_AFRICA  AG......TA ...TTT.G.. C.TTGAAATA CAAGTCAACA TGCTAGCTAG CACTTCA..A .T.G.A...G
CMW3167_SOUTH_AFRICA  AG......TA ...TTT.G.. C.TTGAAATA CAAGTCAACA TGCTAGCTAG CACTTCA..A .T.G.A...G
CMW3164_LA_REUNION    AG......TA ...TTT.G.. C.TTGAAATA CAAGTCAACA TGCTAGCTAG CACTTCA..A .T.G.A...G

                          920        930        940        950        960        970        980
CMW3173_ZAMBIA        CTTTGTTGGC GCAA------ ---------- --------AA AT-AAAGACT TGCAAGCTAA GCTTGATTGG
CMW3152_CAMEROON      .......... ....------ ---------- --------.. ..-....... .......... ..........
CMW3955_ZIMBABWE      .......... ....------ ---------- --------.. ..-....... .......... ..........
CMW5837_ETHIOPIA      G...A.A.-- -...GTATGC CACCTATAGC CAAGTACG.. .GC.TT.... .......... ....CG....
CMW5846_ETHIOPIA      G...A.A.-- -...GTATGC CACCTATAGC CAAGTACG.. .GC.TT.... .......... ....CG....
CMW8968_ETHIOPIA      G...A.A.-- -...GTATGC CACCTATAGC CAAGTANG.. .GC.TT.... .......... ....CG....
CMW5844_ETHIOPIA      G...A.A.-- -...GTATGC CACCTATAGC CAAGTACG.. .GC.TT.... .......... ....CG....
CMW8967_ETHIOPIA      G...A.A.-- -...GTATGC CACCTATAGC CAAGTACG.. .GC.TT.... .......... ....CG....
CMW8971_ETHIOPIA      G...A.A.-- -...GTATGC CACCTATAGC CAAGTACG.. .GC.TT.... .......... ....CG....
CMW2717_SOUTH_AFRICA  G...A.A.-- -...GTATGC CACCTATAGC CAAGTACG.. .GC.TT.... .......... ....CG....
CMW2740_SOUTH_AFRICA  G...A.A.-- -...GTATGC CACCTATAGC CAAGTACG.. .GC.TT.... .......... ....CG....
CMW3167_SOUTH_AFRICA  G...A.A.-- -...GTATGC CACCTATAGC CAAGTACG.. .GC.TT.... .......... ....CG....
CMW3164_LA_REUNION    G...A.A.-- -...GTATGC CACCTATAGC CAAGTACG.. .GC.TT.... .......... ....CG....
```

```
                          990        1010       1020       1030       1040       1050       1060
CMW3173_ZAMBIA        ACT------- ---------- ----GGAGT- ---------- ---------- ---------- --------CA
CMW3152_CAMEROON      ...------- ---------- ----.....- ---------- ---------- ---------- --------..
CMW3955_ZIMBABWE      ...------- ---------- ----.....- ---------- ---------- ---------- --------..
CMW5837_ETHIOPIA      .T.CTCTATT AGTTACATCT ACTT...C.A TGGCTGACAC GCAAAAAGCA AAGGGGG--A CTTGTTGG..
CMW5846_ETHIOPIA      .T.CTCTATT AGTTACATCT ACTT...C.A TGGCTGACAC GCAAAAAGCA AAGGGGG--A CTTGTTGG..
CMW8968_ETHIOPIA      .T.CTCTATT AGTTACATCT ACTT...C.A TGGCTGACAC GCAAAAAGCA AAGGGGGGGA CTTGTTGG..
CMW5844_ETHIOPIA      .T.CTCTATT AGTTACATCT ACTT...C.A TGGCTGACAC GCAAAAAGCA AAGGGGG--A CTTGTTGG..
CMW8967_ETHIOPIA      .T.CTCTATT AGTTACATCT ACTT...C.A TGGCTGACAC GCAAAAAGCA AAGGGGG--A CTTGTTGG..
CMW8971_ETHIOPIA      .T.CTCTATT AGTTACATCT ACTT...C.A TGGCTGACAC GCAAAAAGCA AAGGGGG--A CTTGTTGG..
CMW2717_SOUTH_AFRICA  .T.CTCTATT AGTTACATCT ACTT...C.A TGGCTGACAG GCAAAAAGCA AAGGGGG--A CTTGTTGG..
CMW2740_SOUTH_AFRICA  .T.CTCTATT AGTTACATCT ACTT...C.A TGGCTGACAG GCAAAAAGCA AAGGGGG--A CTTGTTGG..
CMW3167_SOUTH_AFRICA  .T.CTCTATT AGTTACATCT ACTT...C.A TGGCTGACAG GCAAAAAGCA AAGGGGG--A CTTGTTGG..
CMW3164_LA_REUNION    .T.CTCTATT AGTTACATCT ACTT...C.A TGGCTGACAG GCAAAAAGCA AAGGGGG--A CTTGTTGG..

                          1060       1070       1080       1090       1100       1110       1120
CMW3173_ZAMBIA        GACTTGA--- ---TATTCGT ---------- ---------- ---------- ---ACTTAAT GCTATCTTGC
CMW3152_CAMEROON      .......--- ---.......  ---------- ---------- ---------- ---.......  ..........
CMW3955_ZIMBABWE      .......--- ---.......  ---------- ---------- ---------- ---.......  ..........
CMW5837_ETHIOPIA      ..A....ACT TTT.C..... TTACAGCGTG CGCCGTGCGC CGTGCTGGGT CAG....... ..C..G..--
CMW5846_ETHIOPIA      ..A....ACT TTT.C..... TTACAGCGTG CGCCGTGCGC CGTGCTGGGT CAG....... ..C..G..--
CMW8968_ETHIOPIA      ..A....ACT TTT.C..... TTACAGCGTG CGCCGTGCGC CGTGCTGGGT CAG....... ..C..G..--
CMW5844_ETHIOPIA      ..A....ACT TTT.C..... TTACAGCGTG CGCCGTGCGC CGTGCTGGGT CAG....... ..C..G..--
CMW8967_ETHIOPIA      ..A....ACT TTT.C..... TTACAGCGTG CGCCGTGCGC CGTGCTGGGT CAG....... ..C..G..--
CMW8971_ETHIOPIA      ..A....ACT TTT.C..... TTACAGCGTG CGCCGTGCGC CGTGCTGGGT CAG....... ..C..G..--
CMW2717_SOUTH_AFRICA  ..A....ACT TTT.C..... TTACAGCGTG CACCGTGTGC CGTGCTGGGT CAG....... ..C..G..--
CMW2740_SOUTH_AFRICA  ..A....ACT TTT.C..... TTACAGCGTG CACCGTGTGC CGTGCTGGGT CAG....... ..C..G..--
CMW3167_SOUTH_AFRICA  ..A....ACT TTT.C..... TTACAGCGTG CACCGTGTGC CGTGCTGGGT CAG....... ..C..G..--
CMW3164_LA_REUNION    ..A....ACT TTT.C..... TTACAGCGTG CACCGTGTGC CGTGCTGGGT CAG....... ..C..G..--
```

```
                          11130       1140       1150       1160       1170       1180       1190
CMW3173_ZAMBIA          TATCTTACTA TCTT------ --ACTATCAA AAACCACAGC ACCCAGGATT GCCCACGTGG TCC-CCCACC
CMW3152_CAMEROON        .......... ....------ --........ .......... .......... C..G-..... ..GG......
CMW3955_ZIMBABWE        .......... ....ACTATC TT........ .......... .......... C..G-..... ...-......
CMW5837_ETHIOPIA        ---------- ---------- --........ .......... .......... C..GCG-... ...-......
CMW5846_ETHIOPIA        ---------- ---------- --T....... .......... .......... C..GCG-... ...-......
CMW8968_ETHIOPIA        ---------- ---------- --........ .......... .......... C..GCG-... ...-......
CMW5844_ETHIOPIA        ---------- ---------- --........ .......... .......... C..GCG-... ...-...C..
CMW8967_ETHIOPIA        ---------- ---------- --........ .......... .......... C..GCG-... ...-......
CMW8971_ETHIOPIA        ---------- ---------- --........ .......... .......... C..GCG-... ...-......
CMW2717_SOUTH_AFRICA    ---------- ---------- --........ .......... .......... C..GCA-... ...-......
CMW2740_SOUTH_AFRICA    ---------- ---------- --........ .......... .......... C..GCA-... ...-......
CMW3167_SOUTH_AFRICA    ---------- ---------- --........ .......... .......... C..GCA-... ...-......
CMW3164_LA_REUNION      ---------- ---------- --........ .......... .......... C..GCA-... ...-......

                          1200       1210       1220       1230       1240    1247
CMW3173_ZAMBIA          GTGGTACTAA CTAGGCGGCA CTTTGNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
CMW3152_CAMEROON        .......... ...A...... ..NNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
CMW3955_ZIMBABWE        .......... ..G....... .....G-TTA ACTGCGCAGA TNNNNNNNNN NNNNNNN
CMW5837_ETHIOPIA        .......... ..NNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNN
CMW5846_ETHIOPIA        .........G ..G....... .....-ATTA ACTGCGCAGA TCAGACGGGA TGCGGNN
CMW8968_ETHIOPIA        .......... ..G....... .....-ATTA ACTGCGCAGA TCAGACGGNN NNNNNNN
CMW5844_ETHIOPIA        .......... ..G....... .....-ATTA ACTGCGCAGA TCAGACGGGA TGCGGNN
CMW8967_ETHIOPIA        .......... ..G....... .....-ATTA ACTGCGCAGA TCAGACGGGA TGCGGNN
CMW8971_ETHIOPIA        .......... ..G....... .....-ATTA ACTGCGCAGA TCAGACGGNN NNNNNNN
CMW2717_SOUTH_AFRICA    .......... ..G....... .....-ATTA ACTGCGCAGA TCAGACGGGA TGCGGTG
CMW2740_SOUTH_AFRICA    .......... ..G....... .....GATTA ACTGCGCAGA TCAGACGGGA TGCGGTG
CMW3167_SOUTH_AFRICA    .......... ..G....... .....GATTA ACTGCGCAGA TCAGACGGGA TGCGGTG
CMW3164_LA_REUNION      .......... ..G....... .....-ATTA ACTGCGCAGA TCAGACGGGA TGCGGTG
```

Chapter Four

# Charaterisation of the Coniothyrium Stem Canker Pathogen on *Eucalyptus camaldulensis* in Ethiopia

## ABSTRACT

During a survey of *Eucalyptus* diseases in Ethiopia, a serious stem canker disease was discovered on *E. camaldulensis* trees, at several localities in the South and South Western parts of the country. The disease was characterised by the presence of discrete necrotic lesions, stem cankers, cracking of stems, production of kino pockets in the wood, as well as malformation of stems. These symptoms are similar to those caused by *Coniothyrium zuluense* in South Africa. The aim of this study was to identify the causal agent of the disease in Ethiopia. This was achieved by sequencing the ITS region of the rRNA operon and the β-tubulin gene for a representative set of isolates. Sequences for the Ethiopian isolates were compared with those from authenticated isolates collected in South Africa, Thailand and Mexico, as well as with *Coniothyrium*-like isolates collected from diseased *Eucalyptus* trees in Uganda. Based on these data, the *Coniothyrium* isolates from Ethiopia and Uganda grouped together in a separate clade, but closely related to *C. zuluense* from South Africa, Mexico and Thailand. This study represents the first definitive report of *C. zuluense* and the disease caused by it in Ethiopia and Uganda. In Ethiopia, Coniothyrium canker is causing considerable losses in yield and quality of timber and it impacts negatively on the lives of the subsistence farmers. Research will thus be required to minimize these losses.

## INTRODUCTION

*Eucalyptus* species, which originate from Australia and nearby islands, have been introduced and planted in many tropical and subtropical countries. Estimates indicate that plantations of *Eucalyptus* spp. cover approximately 10 million hectares of land, world-wide (Eldridge *et al.* 1997). These plantations provide furniture, timber, distillates, tannins, essential oils, nectar, pollen and fibre for the production of paper, rayon and viscose. They are also a valuable source of fuel wood and construction timber (Poynton 1979, Turnbull 1991).

In Ethiopia, the planting of exotic trees commenced with the introduction of *Eucalyptus* spp. in the 1890's. *Eucalyptus globulus* Labill, *E. camaldulensis* Dehn., *E. saligna* Sm., *E. grandis* Hill ex Maid and *E. citriodora* Hook are the most common species planted in Ethiopia. *E. camaldulensis* is widely planted, usually at lower elevation and warmer localities, while *E. globulus* is commonly planted in cooler areas. Plantations of *Eucalyptus* constitute the major proportion of exotic plantation species and cover about 100 000 ha of land. These *Eucalyptus* plantations provide wood for energy, construction material, transmission poles and fencing material (Pohjonen & Pukkala 1990, Persson 1995).

*Eucalyptus* spp. have showed great promise in most areas where they have been planted as exotics. However, diseases pose a serious threat to these economically important plantation species. A number of important diseases have been recorded on different *Eucalyptus* species and clones. These diseases infect stems, roots and leaves. Cryphonectria canker caused by *Cryphonectria cubensis* (Bruner) Hodges (Hodges, Alfenas & Ferreria 1986, Wingfield, Swart & Abear 1989, Conradie, Swart & Wingfield 1990), canker and die-back caused by *Botryosphaeria* spp. (Smith, Kemp & Wingfield 1994), vascular wilt of *Eucalyptus* caused by *Ceratocystis fimbriata* Ell. & Halst. (Roux *et al.* 2000), pink disease caused by *Erythricium salmonicolor* (Berk. & Broome) Burds. (Sharma, Mohanan & Florence 1984, Roux *et al.* 2001, Alemu, Roux & Wingfield 2002) and Leaf blotch caused by *Mycosphaerella* spp. (Park & Keane 1982, Crous 1998) are examples of diseases in commercial *Eucalyptus* plantations. Recently, a serious stem canker disease caused by *Coniothyrium zuluense* Wingfield, Crous & Coutinho has also been described causing losses to *Eucalyptus* trees in various countries (Wingfield, Crous & Coutinho 1996, Roux, Wingfield & Cibrián 2002, Van Zyl *et al.* 2002).

Stem canker caused by *C. zuluense* was reported for the first time in 1989 from an *E. grandis* clone in South Africa (Wingfield *et al.* 1996). Trees affected by Coniothyrium stem canker develop small, discrete, necrotic lesions on the young, green bark (Wingfield *et al.* 1996, Roux *et al.* 2002, Van Zyl *et al.* 2002). The canker disease has been found on several *E. grandis* clones, on hybrids of *E. grandis* with *E. urophylla* S. T. Blake and on *E. camaldulensis*, which is generally believed to be a relatively disease tolerant species (Wingfield *et al.* 1996). Initially, the pathogen was believed to be native to South Africa. It has, however, recently been described from *Eucalyptus* spp. in Thailand (Van Zyl *et al.* 2002) and Mexico (Roux *et al.* 2002).

During a disease survey of plantation forestry species in Western and South Western Ethiopia, several pathogens were identified (Alemu, Roux & Wingfield 2003). Symptoms of stem canker similar to those of Coniothyrium canker were observed on *E. camaldulensis* trees at a number of these localities (Alemu *et al.* 2003). *Coniothyrium* spp. are difficult to identify and morphological characteristics are generally considered insufficient to identify *C. zuluense* with certainty. This study was, therefore, conducted to confirm the identity of the causal agent of the canker disease of *E. camaldulensis.* An additional objective was to determine the phylogenetic relationship between the fungus occurring in Ethiopia and isolates from other parts of the world. To achieve this DNA sequences of the ITS regions of the rRNA operon and β-tubulin gene were used.

## MATERIALS AND METHODS

### *Sample collection and isolation*

Samples were collected from infected *E. camaldulensis* trees planted in Southern and South Western Ethiopia (Figure 1). Disease symptoms were used to select infected trees for sampling. Samples were collected from symptomatic plant parts including twigs, branches and stems of infected trees. Collections were made from plantations, community woodlots, and from *E. camaldulensis* trees planted around farmlands and homesteads. Segments of plant parts with disease symptoms were incubated in moist chambers at room temperature to induce sporulation. Masses of spores emerging from pycnidia were transferred to petri plates containing malt extract agar (MEA, 20 g Biolab Malt Extract; 15 g Biolab Agar), spread on

the agar surface with sterilised water and incubated at 25 °C for two weeks. Stock cultures of all isolates were maintained on 2% MEA slants at 5 °C. *Coniothyrium* cultures collected from Ethiopia are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

***DNA extraction***

Total genomic DNA was extracted from isolates (Table 1) grown in liquid MY medium (2% Malt Extract, 0.3% Yeast Extract Agar) for two weeks at 25 °C. Mycelium was harvested by centrifugation (8000 rpm, 30 min), freeze dried and ground to a fine powder in liquid nitrogen using a pestle and mortar. A modified version of the method of Raeder and Broda (1985) was used to extract DNA from the mycelium. Approximately 0.5 g of powdered mycelium was placed in 1.5 µl Eppindorf (Epps.) tubes and 1000 µl extraction buffer (100 mM Tris-HCl, pH 8; 50 mM EDTA; 500 mM NaCl; 5 g CTAB) was added to each tube. These suspensions were then incubated in a 60 °C water bath for 2 hours, and frequently mixed by inverting the tubes. Phenol (500 µl) was added and the solution was mixed using a vortex mixer. Thereafter, 300µl chloroform was added and mixed. The cell debris were removed by centrifugation at 12500 g, for 60 min at 4 °C. The upper aqueous layer of this mixture was transferred to new tubes, whereafter a further phenol:chlorophorm extraction was carried out by adding 200 µl phenol and 200 µl chloroform. This mixture was centrifuged at 12500 g for 5 min at 4 °C and the upper aqueous layer transferred to a fresh tube. To remove the excess phenol it was washed with 400 µl chloroform and centrifuged at 12500 g for 5 min at 4 °C. This step was repeated until a clear interface was obtained. Next, 0.1 volume of 3M NaAc (pH 5.5) and two volumes of absolute ethanol were added and the mixture was centrifuged for 30 min at 4 °C to precipitate the nucleic acid. The liquid phase was discarded and the precipitated nucleic acid was washed with 70% ethanol and centrifuged for 5 min at 4 °C to obtain a DNA pellet.

The DNA pellets were vacuum dried to remove excess ethanol and resuspended in 50 µl water. RNase A (1mg/ml) (Roche Diagnostics, South Africa) was added to the DNA solution to remove the contaminating RNA and incubated at 37 °C in a water bath over night. The presence of DNA in the samples was detected by using agarose gel electrophoresis in a 1% agarose gel, stained with ethidium bromide and visualised under UV light.

***PCR amplification***

The internal transcribed spacer (ITS) regions of the ribosomal RNA operon and the 5.8S gene were amplified using the polymerase chain reaction (PCR). PCR was conducted using primers ITS 1 (5' TCC GTA GGT GAA CCT GCG G '3) and ITS 4 (5' TCC TCC GCT TAT TGA TAT GC '3) to amplify the ITS 1, ITS 2 and 5.8S genes of the ribosomal RNA operon (White *et al.* 1990). The PCR reaction mixture contained DNA polymerase (*Taq*, 2.5 U/µl, Roche Diagnostics, South Africa), 2.5 mM dNTP's, PCR Buffer (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl), 2.5 mM $MgCl_2$, 0.15 mM of each primer, approximately 1 µl of DNA and 37 µl water to make up a final volume of 50 µl. Denaturation was performed at 96 °C for 1 min. This was followed by 35 cycles of primer annealing at 55 °C for 30 s, chain elongation was undertaken at 72 °C for 1 min and denaturation was conducted at 92 °C for 1 min. Final chain elongation was carried out at 72 °C for 5 min.

The β-tubulin gene was partially amplified using the forward primer Bt2a (5' GGT AAC CAA ATC GGT GCT GCT TTC 3') and the reverse primer Bt2b (5' ACC CTC AGT GTA G TG ACC CTT GGC 3') (Glass & Donaldson 1995). The PCR reaction mix included DNA polymerase (*Taq*, 2.5U/µl ), 0.2 mM dNTP's, PCR buffer (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl), 2.5 mM $MgCl_2$, 0.15 mM of each primer, 1 µl of DNA and 37 µl sterilised water to make up a final volume of 50 µl. Amplification was conducted using the following PCR reaction conditions: an initial denaturation at 94 °C for 1 min, which was followed by 40 cycles at 94 °C for 1 min, primer annealing at 51 °C for 30 s, chain elongation at 72 °C for 1 min and an additional chain elongation step at 72 °C for another 1 min. All PCR products were detected using agarose gel electrophoresis on 1% agarose gels stained with ethidium bromide under UV illumination.

***DNA sequencing***

The PCR products of both the ITS regions and the β-tubulin gene were purified using the High Pure PCR Product Purification Kit (QUIAGEN, GmbH, Hilden, Germany). The PCR products were sequenced in both directions using the Big Dye Cycle Sequencing kit with Amplitaq® DNA Polymerase FS (Perkin-Elmer, Warrington, UK), according to the manufacturer's protocol, on an ABI PRISM™ 3100 DNA Autosequencer (Perkin-Elmer). Primers ITS 1 and ITS 4 were used for sequencing the ITS regions and for the β-tubulin gene,

primers Bt2a and Bt2b were used. The sequences for the Ethiopian isolates were compared with ITS DNA sequences obtained from GenBank [National Centre for Biotechnology Information (NCBI), US National Institute of Health Bethesda (http:/www.ncbi.nlm.nih.gov/BLAST]. Once the possible identity of the fungus was determined using a BLAST search, additional sequence data of *Coniothyrium* spp. and *Mycosphaerella* spp. were included in the study and the ITS and β-tubulin data analyzed.

***Sequence analysis***

The ITS and β-tubulin gene sequences were aligned manually using PAUP 4.0 (Swofford, 1998). Gaps were inserted manually and treated as missing data. The sequences were analysed using parsimony with trees generated by heuristic searches, simple addition and Tree Bisection Reconstruction (TBR) branch swapping. Confidence intervals were determined using DNA BOOTSTRAP analysis (Bootstrap confidence intervals on DNA parsimony) (1000 replicates) (Felsenstein 1993). *Mycosphaerella molleriana* (Thumb.) Lindau. and *M. nubilosa* (Cooke) Hansf. were used as an outgroup for the combined data set. A Partition homogeneity test was used to check the combinability of the ITS and β-tubilin sequence data sets (Farris *et al.* 1995, Huelsenbeck, Bull & Cunningham 1996).

***Pathogenicity test***

An inoculation study was conducted on an 18-month-old *E. grandis* clone (ZG 14) (approximately 1 cm diameter) in the green house at a temperature of ~25 °C. Prior to inoculation, plants acclimatised to greenhouse conditions for one week. Seven of the *Coniothyrium* isolates from Ethiopia were randomly selected for the inoculation study (Table 1). Cultures were grown on MEA for two weeks before inoculation. A 9 mm cork borer was used to remove the bark and expose the cambium. Mycelial plugs, of equal size, overgrown with the test cultures, were placed into each wound with the mycelium facing the wood. All the wounds were covered with parafilm (Pechiney Plastic Packaging, Chicago) to prevent contamination and desiccation. Each isolate was inoculated on 10 trees and an additional ten trees were inoculated with sterile MEA to serve as controls.

After six weeks, development of symptoms was examined by measuring the lesion lengths on inoculated trees. A one-way ANOVA was conducted using Statistica for Windows (Statsoft. Inc. 1995) to statistically compare lesion development associated with the isolates and the control.

## RESULTS

### *Sample collection and Isolation*

Symptoms of Coniothyrium stem canker were observed in several *E. camaldulensis* growing localities in South and South Western Ethiopia. These areas were between Woliso and Jima and between Wolkite and Sodo (Figure 1). *E. camaldulensis* trees in Jiren plantation near Jima, and *E. camaldulensis* trees planted in woodlots as well as around farms and homesteads were seriously affected by the stem canker. About 50% of *E. camaldulensis* trees growing at these localities had symptoms of the disease. Stem malformation and extensive discoloration of the stems (Figure 2a-2d) were evident on most infected trees. Initially, small discrete lesions developed on young green bark (Figure 2a, 2d). When these lesions coalesced, large necrotic lesions developed on the stems, branches and twigs (Figure 2b). Kino pockets were observed in the wood associated with the bark lesions on infected trees (Figure 2c).

After one day in moisture chambers, pycnidial structures, producing slimy spore masses were found in the sunken necrotic lesions collected from infected trees. A *Coniothyrium* sp. was consistently isolated from these lesions and this fungus was morphologically similar to *C. zuluense* described from South Africa. In culture, isolates grew slowly and colonies were olive green in colour. The colonies of most isolates had similar growth and colour in culture.

### *PCR amplification and analysis of sequence data*

Amplification of the ITS regions and 5.8S gene for the *Coniothyrium* isolates used in this study yielded a fragment of about 500 base pairs (bp) in size. Amplification of the partial β-tubulin gene yielded fragments of approximately 400 bp.

The ITS regions and 5.8S gene were sequenced and after alignment yielded a total of 551 characters of which 495 characters were constant, 40 variable characters were parsimony uninformative and 16 characters were parsimony informative. A total of 485 characters were obtained when the β-tubulin gene was aligned. Of these, 397 characters were constant, 67 were parsimony uninformative and 21 characters were parsimony informative.

Comparison of the ITS and 5.8S gene sequences to sequences available in the NCBI data base revealed that the sequences of the samples from Ethiopia are most similar to that of *C. zuluense* (98%) followed by *Mycosphaerella vespa* (Carnegie & Keane) and *M. molleriana* (96% homology) and *M. nubilosa* (94% homology). Analysis of the ITS sequence data, using sequences obtained from Genbank and the data set from Van Zyl *et al.* (2002) produced 1 tree. The tree had a CI = 0.976 and RI = 0.944 (Figure 3), and showed that the *Coniothyrium* isolates from Ethiopia and Uganda grouped together in the larger *C. zuluense* clade (83% bootstrap). Two distinct sub-clades, were however, apparent. Isolates from South Africa, Thailand and Mexico grouped in one clade (97% bootstrap) and isolates from Ethiopia and Uganda grouped in another (80% bootstrap). *C. zuluense* isolates grouped more closely with *M. molleriana* and *M. nubilosa*, than with other species of *Coniothyrium*, including *C. ovatum* Swart and *C. fuckelii* Sacc.

A partition homogeneity test showed that the ITS and β-tubulin sequences could be combined (P value = 1). The combined sequences had a total of 956 characters of which 796 characters were constant, 116 variable characters were parsimony uninformative and 44 characters were parsimony informative. Analysis of the combined data sets generated 1 tree (Figure 4). The tree generated from the combined data set had a consistency index (CI) of 0. 969 and retention index (RI) of 0.942. Ethiopian and Uganda isolates grouped together with *C. zuluense* (100% bootstrap). Two sub-clades were, however, produced within *C. zuluense* (Figure 4). Isolates from South Africa, Thailand and Mexico grouped together in clade I with a 96% bootstrap support. This clade represents *C. zuluense*. Clade II contained the *Coniothyrium* isolates from Ethiopia and Uganda with a 100% confidence limit. The *Coniothyrium* isolates grouped separately from any of the *Mycosphaerella* isolates.

***Pathogenicity test***

Small lesions developed on *E. grandis* trees inoculated with Ethiopian *Coniothyrium* isolates (Figure 2f). Lesion lengths differed statistically from those of the control ($P<0.0001$) (R-square = 0.48). No variation was observed in lesion development between the *C. zuluense* isolates used in the inoculation study (Table 3, Figure 5).

## DISCUSSION

Coniothyrium stem canker, caused by *C. zuluense* is considered to be one of the most important new threats to plantation grown *Eucalyptus* species. Until recently, this disease was known only from South Africa (Wingfield *et al.* 1996), Thailand (Van Zyl *et al.* 2002) and Mexico (Roux *et al.* 2002). Although observations based on symptoms and morphology of the fungus have led to suggestions that the disease is present in Ethiopia (Alemu *et al.* 2003), this study provides the first clear evidence for its occurrence in the country and expands the geographic distribution of this important disease. This is particularly important, as it is virtually impossible to identify *C. zuluense* with certainty without DNA based comparisons.

Symptoms of Coniothyrium stem canker were first observed on *E. camaldulensis* in Ethiopia during a survey of plantation forestry diseases in 2000 and 2001 (Alemu *et al.* 2003). The disease is restricted to specific areas in Western Ethiopia, and is causing large-scale damage to trees in plantations, woodlots and around homesteads. It has not been found on other species of *Eucalyptus* in Ethiopia. This is probably due to the fact that they are planted in cooler areas, which would not be conducive to the development of *C. zuluense*. In South Africa Coniothyrium stem canker is only a problem in warmer sub-tropical areas (Wingfield *et al.* 1996) while the only other reports of this disease is from tropical areas such as Thailand (Van Zyl *et al.* 2002) and Mexico (Roux *et al.* 2002).

Comparison of ITS and the 5.8S gene sequences showed that Ethiopian isolates were most similar to those of *C. zuluense*. The next closest relatives were *Mycosphaerella* spp., including *M. vespa*, *M. molleriana* and *M. nubilosa*. This is particularly interesting as other *Coniothyrium* spp. such as *C. ovatum* and *C. fuckelii* were more distantly related to *C. zuluense* than the group of *Mycosphaerella* spp. noted above. Van Zyl *et al.* (2002) provided

the first DNA sequence data for *C. zuluense* and used *C. ovatum* and *C. fuckelii* as outgroup taxa. Our study, however, strongly suggests that *C. zuluense* is more closely related to *Mycosphaerella* spp., than to other *Coniothyrium* spp. for which sequence data are available. It was for this reason that we choose *Mycosphaerella* spp. as outgroup taxa. Our data provide preliminary evidence to suggest that *C. zuluense* is an anamorph of *Mycosphaerella*. This is particularly interesting, as many *Mycosphaerella* species are pathogens of *Eucalyptus* leaves and stems.

Results of our combined sequence data set separated the *C. zuluense* isolates into two distinct groups. One of these groups mainly constituted authentic *C. zuluense* isolates from South Africa, Thailand and Mexico. The Ethiopian isolates and one isolate from Uganda were identical and resided in a separate clade. These data might suggest that *C. zuluense* represents a species complex, and this deserves further scrutiny.

Pathogenicity tests showed that Ethiopian *Coniothyrium* isolates are pathogenic to *E. grandis*. Only very small lesions were produced, but they differed significantly from the controls. Wingfield *et al.* (1996) reported similar results for South African isolates in artificial inoculations. During an extensive survey of *Eucalyptus* diseases in Western and Southern Ethiopia (Alemu *et al.* 2003), Coniothyrium stem canker was not observed on *E. grandis*, or any other species than *E. camaldulensis*. The pathogenicity of *C. zuluense* under field conditions and on *E. camaldulensis*, however, needs to be investigated further.

*E. camaldulensis* is one of the most widely planted *Eucalyptus* spp. in Ethiopia. This species appears to be highly susceptible to Coniothyrium stem canker. The disease is wide spread in *E. camaldulensis* growing areas between Wolkite and Sodo as well as between Woliso and Jima. Near Jima, the disease was found on most *E. camaldulensis* trees in the Jiren plantation, east of Jima, whereas *E. camaldulensis* planted on the other side of the town showed no signs of infection. This might suggest that different seed sources of *E. camaldulensis* differ in their susceptibility and it raises the possibility of being able to select disease tolerant planting stock in the future. We recommend more intensive surveys for this disease and disease screening trials in the future.

## REFERENCES

Alemu Gezahgne, Roux, J. & Wingfield, M. J. (2002) First report of pink disease on *Eucalyptus camaldulensis* in Ethiopia. *Plant Pathology* **6**.

Alemu Gezahgne, Roux, J. & Wingfield, M. J. (2003) Diseases of exotic plantation *Eucalyptus* and *Pinus* species in Ethiopia. *South African Journal of Science* **99**: 29-33.

Conradie, E., Swart, W. J. & Wingfield, M. J. (1990) Cryphonectria canker of *Eucalyptus*, an important disease in plantation forestry of South Africa. *South African Forestry Journal* **152**: 43-49.

Crous, P. W. (1998) *Mycosphaerella* spp. and their anamorphs associated with leaf spot disease of *Eucalyptus*. *Mycologia Memoir* **21**: 1-170.

Eldridge, K., Davidson, J., Harwood, C. & Van Wyk, G. (1997) *Eucalyptus* domestication and breading. Oxford, Science Publication, UK.

Farris, J. S., Kallersjo, M., Kluge, A. G. & Bult, C. (1995) Testing significance of incongruence. *Cladistics* **10**: 315-319.

Felsentein, J. (1993) DNABOOT-Bootstrap Confidence Interval on DNA parsimony 3.1. Seattle, University of Washington DC.

Glass, N. L. & Donaldson, G. C. (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* **78**: 1323-1333.

Hodges, C. S., Alfenas, A. C. & Ferreria, F. A. (1986) The conspecificity of *Cryphonectria cubensis* and *Endothia eugeniae*. *Mycologia* **78**: 343-350.

Huelsenbeck, J. P., Bull, J. J. & Cunningham, C. W. (1996) Combining data in phylogenetic analysis. *Tree* **11**: 152-158.

Park, R. F. & Keane, P. J. (1982) Leaf diseases of *Eucalyptus* associated with *Mycosphaerella* spp. *Transactions of the British Mycological Society* **79**:101-115.

Persson, A. (1995) Exotics-prospects and risks from European and African viewpoint. *Buvisindi Agricultural Science* **9**: 47-62.

Pohjonen, V. & Pukkala, T. (1990) *Eucalyptus globulus* in Ethiopian forestry. *Forest Ecology and Management* **36**: 19-31.

Poynton, R. J. (1979) Tree planting in South Africa, Vol., 2. The *Eucalyptus*. Department of Forestry. Pretoria, South Africa.

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

Roux, J., Wingfield, M. J., Wingfield, B. D., Bouillett, J. P. & Alfenas, A. C. (2000) A serious new disease of *Eucalyptus* caused by *Ceratocystis fimbriata* in Central Africa. *European Journal of Forest Pathology* **30**: 175-184.

Roux, J., Heath, R. N., Van der Hoef, A. & Wingfield, M. J. (2001) First report of pink disease on *Eucalyptus* and *Podocarpus* in South Africa. *Phytopathology* **S78**. (Abstract).

Roux, J., Wingfield, M. J. & Cibrian, D. (2002) First report of Coniothyrium stem canker of *Eucalyptus* in Mexico. *Plant Pathology* **51**: 382.

Sharma, J. K., Mohanan, C. & Florence, E. J. M. (1984) Outbreak of pink disease caused by *Corticium salmonicolor* in *Eucalyptus grandis* in Kerala, India. *Tropical Pest Management* **30**: 253-255.

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

Swofford, D. L. (1998) PAUP*. *Phylogenetic Analysis Using Parsimony* (*and other methods). Version 4.0. Beta version. Sinauer Associates. Sunderland, Massachusetts.

Turnbull, J. W. (1991) Future use of *Eucalyptus*: opportunities and problems. In *Proceedings of the IUFRO symposium on intensive forestry. The role of Eucalyptus*: pp 2-27. 2-6 September, Durban, South Africa.

Van Zyl, L. M., Coutinho, T. A., Wingfield, M. J., Pongpanich, K. & Wingfield, B. D. (2002) Morphological and molecular relatedness of geographically diverse isolates of *Coniothyrium zuluense* from South Africa and Thailand. *Mycological Research* **106**: 51-59.

White, T. J., Bruns, T. Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: A guide to methods and applications* (A. M. Innis, D. H. Gelfand, J. J. Snisky & T. J. White eds): pp 315-322. Academic Press, San Diego.

Wingfield, M. J., Crous, P. W. & Coutinho, T. A. (1996) A serious canker disease of *Eucalyptus* in South Africa caused by a new species of *Coniothyrium*. *Mycopathologia* **136**: 139-145.

Wingfield, M. J., Swart, W. J. & Abear, B. J. (1989) First record of Cryphonectria canker of *Eucalyptus* in South Africa. *Phytophylactica* **21**: 311-313.

**Table 1.** List of fungal isolates used in this study

| Isolate No. | Origin | Species | Host | Collector | Accession No. | |
|---|---|---|---|---|---|---|
| | | | | | ITS | B-tubilin |
| CMW11220, CMW7399 | South Africa | *Coniothyrium zuluense* | *E. grandis* | L.M. van Zyl | AF376823 | AY244383 |
| CMW11221, CMW7459 | South Africa | ,, | ,, | ,, | AF376816 | AY244384 |
| CMW11225 | Ethiopia | ,, | ,, | Alemu Gezahgne & J. Roux | AY244415 | AY244390 |
| CMW11226 | Ethiopia | ,, | ,, | ,, | AY244413 | AY244391 |
| CMW11227 | Ethiopia | ,, | ,, | ,, | AY244414 | AY244392 |
| CMW11228 | Uganda | ,, | *Eucalyptus* spp. | J. Roux | AY244416 | AY244389 |
| CMW11230 | Mexico | ,, | ,, | M. J. Wingfield & J. Roux | AF385610 | AY244385 |
| CMW11231 | Mexico | ,, | ,, | ,, | AF385611 | AY244386 |
| CMW5232 | Thailand | ,, | ,, | M. J. Wingfield & van Zyl | AF376828 | AY244387 |
| CMW5234 | Thailand | ,, | ,, | ,, | AF376825 | AY244388 |
| CMW3032 | South Africa | *Mycosphaerella nubilosa* | *E. bicostata* | P.W. Crous | - | AY244393 |
| CMW8575 | Chile | *M. molleriana* | *E. globulus* | R. Ahumada | - | AY244394 |

[a] CMW numbers refer to the culture collection numbers of the Tree Pathology Co-operative Programme (TPCP), FABI, University of Pretoria, South Africa.

**Table 2.** Results of inoculation of an *E. grandis* clone with Ethiopian *Coniothyrium* isolates

| Isolates | Mean Lesion Length (mm) | 95% Confidence Limits |
|---|---|---|
| CMW11223 | 17.2 [a] | 15.65 –18.75 |
| CMW11234 | 17.9 [a] | 16.35-19.45 |
| CMW11233 | 16.6 [a] | 15.05-18.15 |
| CMW11238 | 16.7 [a] | 15.15-18.25 |
| CMW11238 | 17.9 [a] | 16.35-19.45 |
| CMW11225 | 16.8 [a] | 15.25-18.35 |
| CMW11235 | 18.8 [a] | 17.25-20.35 |
| Control | 11.0 [b] | 9.45-12.55 |

Each mean lesion length is the average of 10 measurements.

R-Square =0.48.

Mean values with the same letters did not differ significantly at P = 0.05.


**Figure 1**. Map of Ethiopia showing the plantation areas where surveys were conducted.


**Figure 2.** Symptoms of Coniothyrium stem canker on *E. camaldulensis*. (A) Discrete lesions on stem, (B) stem malformation and discoloration, (C) Kino pockets in *E. camaludensis* wood, (D) Development of necrotic lesions on branches, (E) lesions produced on ZG14 after artificial inoculation with *C. zuluense*. (1) control (2) *C. zuluense*.

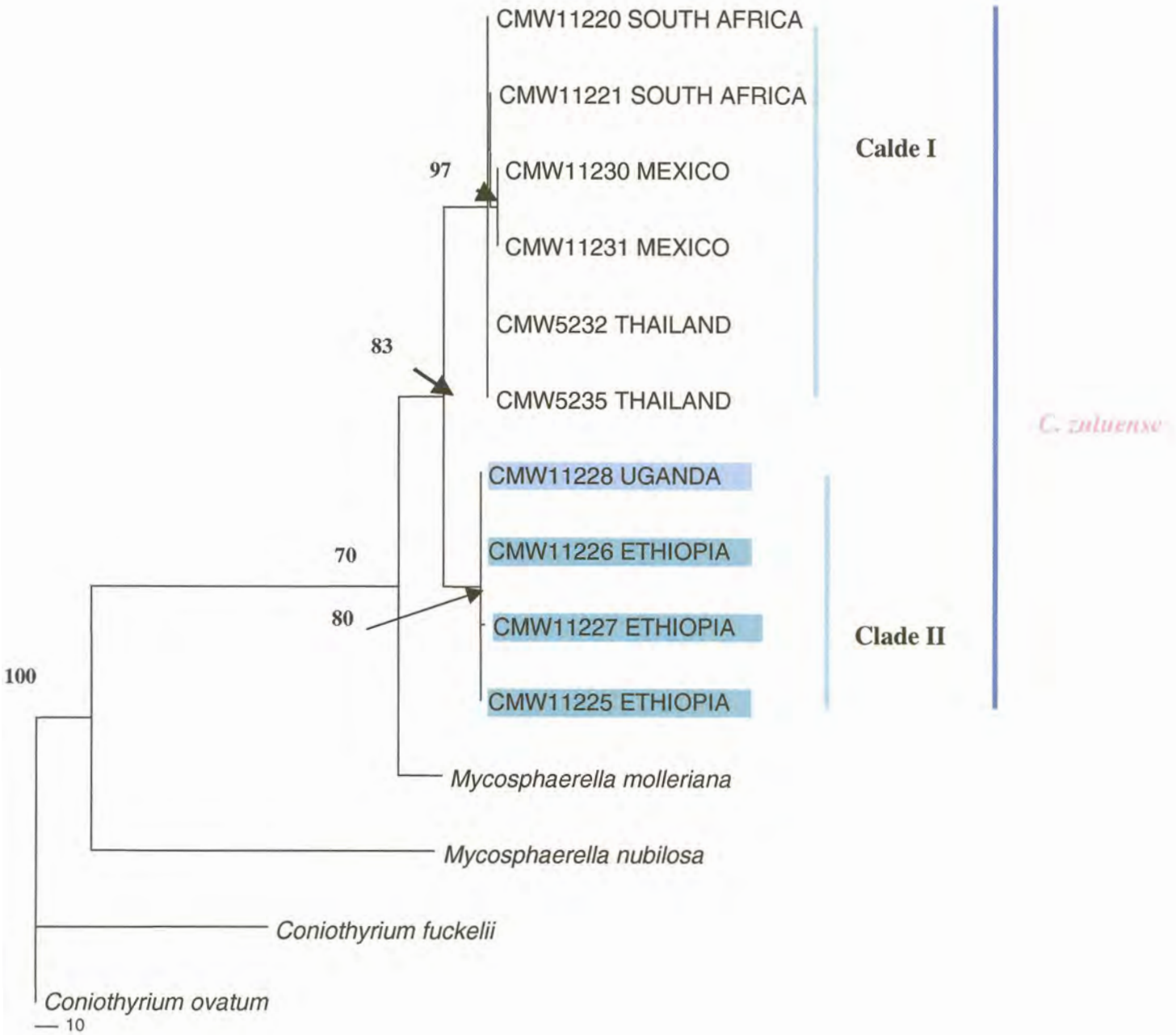



**Figure 3.** Phylogenetic tree of the ITS sequence data of *Coniothyrium* spp. and *Mycosphaerella* spp. CI=0.976 and RI=0.944. Bootstrap values are shown above the branches.

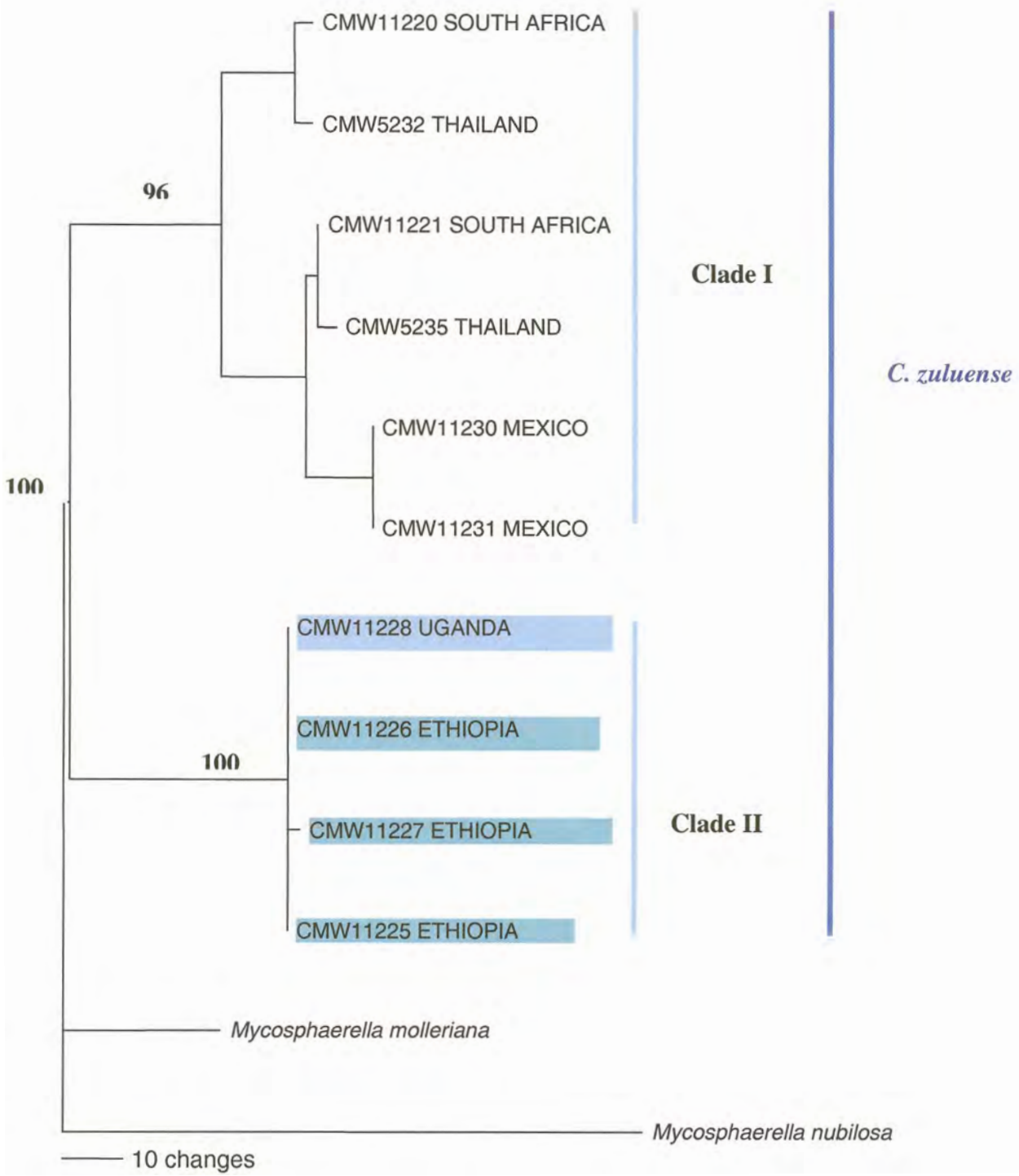



**Figure 4.** Phylogenetic tree of *Coniothyrium* spp. generated from the combined ITS and β-tubulin sequences. CI = 0.969 and RI = 0.942. Bootstrap values are shown at each branch.

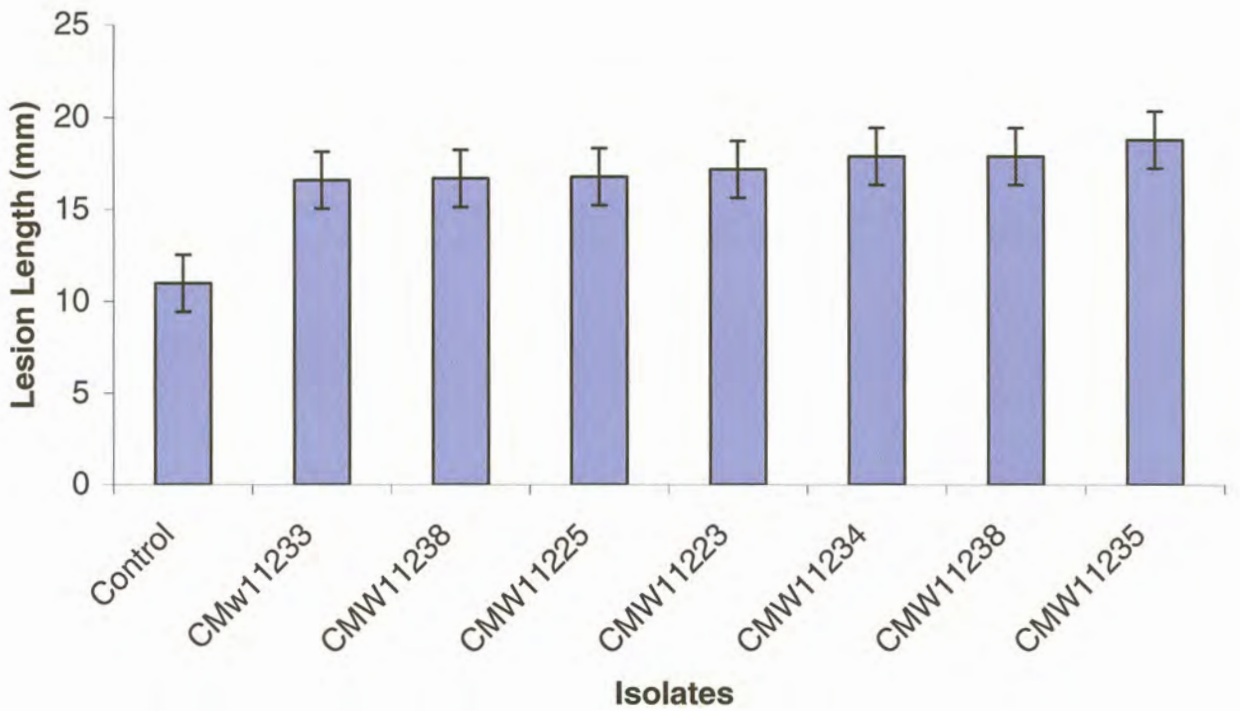



**Figure 5**. Means and confidence limits of lesion lengths of *Coniothyrium* isolates from the greenhouse inoculation trial.

**Figure 7**. Aligned sequences of the ITS and β-Tubulun genes of isolates used in this study. (-)= Gaps, (.)= homologous nucleotides (N)= Unknown bases.

```
                                10         20         30         40         50         60         70         80
CMW11220_South_Africa           TCCGTAGGTG GAACCTGCGG AGGGATCATT ACTGAGTGAG GGCGCAAGCC CGACCTCC-A ACCCCATGTT TTCCAACCAT
CMW11221_South_Africa           .......... .......... .......... .......... .......... ........-. .......... ...T......
CMW11230_Mexico                 .......... .......... .......... .......... .......... ........-. .......... ...T......
CMW11231_Mexico                 NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN N......... ...T......
CMW5232_Thailand                .......... .......... .......... .......... .......... ........-. .......... ..........
CMW5235_Thailand                .......... .......... .......... .......... .......... ........-. .......... ..........
CMW11228_Uganda                 .......... .......... .......... ......C... .....G.... ........-. .......... ...-......
CMW11226_Ethiopia               .......... .......... .......... ......C... .....G.... ........-. .......... ...-......
CMW11227_Ethiopia               NNNNNNNNNN NNN....... .......... ......C... .....G.... ........-. .......... ...-......
CMW11225_Ethiopia               .......... .......... .......... ......C... .....G.... ........-. .......... ...-......
Mycosphaerella_molleriana       .......... .......... .......... .......... .......... ........-. ....-..... .C.A.....C
Mycosphaerella_nubilosa         .......... -......... .......... ..C....... ....GC.... ........T. C...-..... .C...-...C

                                90         100        110        120        130        140        150        160
CMW11220_South_Africa           GTTGCCTCGG GGGCGACCCG GCCATCGCGC CGGGGCCCCC GGTGGACCCC TCCAACTCTG CATCTTTGCG TCTGAGTCAC
CMW11221_South_Africa           .......... .......... .......... .......... .......... .......... .......... ..........
CMW11230_Mexico                 .......... .......... .......... .......... .......... .......... .......... ..........
CMW11231_Mexico                 .......... .......... .......... .......... .......... .......... .......... ..........
CMW5232_Thailand                .......... .......... .......... .......... .......... .......... .......... ..........
CMW5235_Thailand                .......... .......... .......... .......... .......... .......... .......... ..........
CMW11228_Uganda                 .......... .......... ....C..... .......... ..C....... ..-....... .....C.... ..........
CMW11226_Ethiopia               .......... .......... ....C..... .......... ..C....... ..-....... .....C.... ..........
CMW11227_Ethiopia               .........TC .......... ....C..... .......... ..C....... ..-....... .....C.... ..........
CMW11225_Ethiopia               .......... .......... ....C..... .......... ..C....... ..-....... .....C.... ..........
Mycosphaerella_molleriana       .......... .......... ...GC..... .......... .........- ..-....... .....C.... ..........
Mycosphaerella_nubilosa         .......... .......... ...CC..... ........T. .CA.A..... ..-...GGCT GGATC.GTGC GTG....A.T
```

```
                                170        180        190        200        210        220        230        240
CMW11220_South_Africa           AAAATAAAAT CAATCAAAAC TTTCAACAAC GGATCTCTTG GTTCTGGCAT CGATGAAGAA CGCAGCGAAA TGCGATAAGT
CMW11221_South_Africa           .......... .......... .......... .......... .......... .......... .......... ..........
CMW11230_Mexico                 .......... .......... .......... .......... .......... .......... .......... ..........
CMW11231_Mexico                 .......... .......... .......... .......... .......... .......... .......... ..........
CMW5232_Thailand                .......... .......... .......... .......... .......... .......... .......... ..........
CMW5235_Thailand                .......... .......... .......... .......... .......... .......... .......... ..........
CMW11228_Uganda                 .......... .......... .......... .......... .......... .......... .......... ..........
CMW11226_Ethiopia               .......... .......... .......... .......... .......... .......... .......... ..........
CMW11227_Ethiopia               .......... .......... .......... .......... .......... .......... .......... ..........
CMW11225_Ethiopia               .......... .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_molleriana       .....C..-. .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_nubilosa         .C..CC..-. ....T..... .......... .......... .......... .......... .......... ..........

                                250        260        270        280        290        300        310        320
CMW11220_South_Africa           AATGTGAATT GCAGAATTCA GTGAATCATC GAATCTTTGA ACGCACATTG CGCCCTCTGG TATTCCGGAG GGCATGCCTG
CMW11221_South_Africa           .......... .......... .......... .......... .......... .......... .......... ..........
CMW11230_Mexico                 .......... .......... .......... ...C...... .......... .......... .......... ..........
CMW11231_Mexico                 .......... .......... .......... ...C...... .......... .......... .......... ..........
CMW5232_Thailand                .......... .......... .......... .......... .......... .......... .......... ..........
CMW5235_Thailand                .......... .......... .......... .......... .......... .......... .......... ..........
CMW11228_Uganda                 .......... .......... .......... .......... .......... .......... .......... ..........
CMW11226_Ethiopia               .......... .......... .......... .......... .......... .......... .......... ..........
CMW11227_Ethiopia               .......... .......... .......... .......... .......... .......... .......... ..........
CMW11225_Ethiopia               .......... .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_molleriana       .......... .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_nubilosa         .......... .......... .......... .......... .......... .......... .......... ..........
```

```
                                330        340        350        360        370        380        390        400
CMW11220_South_Africa           TTCGAGCGTC ATTACACCAC TCCAGCCTCG CTGGGTATTG GGCGCCGCGG CCTCCGCGCG CCTT-AATGT CTCCGGCCGA
CMW11221_South_Africa           .......... .......... .......... .......... .......... .......... ....-..... ..........
CMW11230_Mexico                 .......... .......... .......... .......... .......... .......... ....-..... ..........
CMW11231_Mexico                 .......... .......... .......... .......... .......... .......... ....-..... ..........
CMW5232_Thailand                .......... .......... .........? .......... .......... .......... ....-..... ..........
CMW5235_Thailand                .......... .......... .......... .......... .......... .......... ....-..... ..........
CMW11228_Uganda                 .......... .......... .......... .......... .......... .......... ....-..... ..........
CMW11226_Ethiopia               .......... .......... .......... .......... .......... .......... ....-..... ..........
CMW11227_Ethiopia               .......... .......... .......... .......... .......... .......... ....-..... ..........
CMW11225_Ethiopia               .......... .......... .......... .......... .......... .......... ....-..... ..........
Mycosphaerella_molleriana       .......... .......... ...G...... .......... .......... .......... ...CG..-.. ..........
Mycosphaerella_nubilosa         .......... ...T...... .......C.. ..T....... .......... .......... ...C-..... ..........

                                410        420        330        440        450        460        470        480
CMW11220_South_Africa           GCCGACCGTC TCCAAGCGTT GTGGCACAAC TGTTTCGCTT TCGGG-ACCG GTCCGGCGAC GCGCCGTTAA ACCCTTTCAC
CMW11221_South_Africa           .......... .......... .......... .......... .....-.... .......... .......... ..........
CMW11230_Mexico                 .......... .......... .......... .......... .....-.... .......... .......... ..........
CMW11231_Mexico                 .......... .......... .......... .......... .....-.... .......... .......... ..........
CMW5232_Thailand                .......... .......... .......... .......... .....-.... .......... .......... ..........
CMW5235_Thailand                .......... .......... .......... .......... .....-.... .......... .......... ..........
CMW11228_Uganda                 .......... ..T....... .......... .......... .....-.... ...T...... .......... ..........
CMW11226_Ethiopia               .......... ..T....... .......... .......... .....-.... ...T...... .......... ..........
CMW11227_Ethiopia               .......... ..T....... .......... .......... .....-.... ...T...... .......... ..........
CMW11225_Ethiopia               .......... ..T....... .......... .......... .....-.... ...T...... .......... ..........
Mycosphaerella_molleriana       .......... .......... .......... .......... .....-.... ...T....G. .......... ..........
Mycosphaerella_nubilosa         .......... ..TC...... .......T.. .........G A....G.... ...T....-. .......... ..........
```

```
                                490        500        510        520        530        540        550        560
CMW11220_South_Africa           CAAAGGTTGA CCTCGGATCA GGTAGGGATA CCCGCTGAAC TTAAGCATAT CAATTAAAGC GGAGGATGGT AACCAAA---
CMW11221_South_Africa           .......... .......... .......... .......... .......... ..-....-.. .......... .......---
CMW11230_Mexico                 .......... .......... .......... .......... .......... .????????? ??????.... .......---
CMW11231_Mexico                 .......... .......... .......... .......... .......... ..T.A.-GCG .AG.A..... .......---
CMW5232_Thailand                .......... .......... .......... .......... .......... ....-..-.. .......... .......---
CMW5235_Thailand                .......... .......... .......... .......... .......... ....-..-.. .......... .......---
CMW11228_Uganda                 .......... .......... .......... .......... .......... ..-....-.. .......... .......---
CMW11226_Ethiopia               .......... .......... .......... .......... .......... ..-....... .......... .......A--
CMW11227_Ethiopia               .......... .......... .......... .......... .......... ..-....... .......... .......---
CMW11225_Ethiopia               .......... .......... .......... .......... .......... ....-..-.. .......... .......---
Mycosphaerella_molleriana       -......... .......... .......... ?????????? ?????????? ?????????? ??????.... .......A--
Mycosphaerella_nubilosa         .......... .......... .......... ?????????? ?????????? ?????????? ?????????. .......AA-

                                570        580        590        600        610        620        630        640
CMW11220_South_Africa           TCGGTGCTGC TTTCTGGCAG AACATCTCCG GCGAGCACGG CCTCGACGGC TCCGGCGTGT AGGTCTAGCA GGAGTGGGAT
CMW11221_South_Africa           .......... .......... .......... .......... .........T .......... .......... ..........
CMW11230_Mexico                 .......... .......... .......... .......?.. .......... .......... .........G ..........
CMW11231_Mexico                 .......... .......... .......... .......... .......... .......... .........G ..........
CMW5232_Thailand                .......... .......... .......... .......... .......... .......... .......... ..........
CMW5235_Thailand                .......... .......... .......... .......... .........T .......... .......... ..........
CMW11228_Uganda                 .......... .......... .......... .......... ......T... .......... ......GA.. ...A..A..G
CMW11226_Ethiopia               .......... .......... .......... .......... ......T... .......... ......GA.. ...A..A..G
CMW11227_Ethiopia               .......... .......... .......... .......... ......T... .......... ......GA.. ...A..A..G
CMW11225_Ethiopia               .......... .......... .......... .......... ......T... .......... ......GA.. ...A..A..G
Mycosphaerella_molleriana       .......... .......... .......... .......T.. .......... ..T....... .T..G..... ATGC...C.G
Mycosphaerella_nubilosa         .......... .......... .C........ ....A..T.. .........T G......... GA..G..A.. ACGCGAAAGA
```

```
                                650        660        670        680        690        700        710        720
CMW11220_South_Africa           CGAAGGAGAA GAGGATACTG ACGCGAGGCA GGTACAATGG CACGTCTGAC CTCCAGCTCG AGCGCATGAA CGTGTACTTC
CMW11221_South_Africa           ....T..... .......... .......... .......... .......... .......... .......... ..........
CMW11230_Mexico                 ...G...... .......... .......... .......... .......... .......... .......... ..........
CMW11231_Mexico                 ...G...... .......... .......... .......... .......... .......... .......... ..........
CMW5232_Thailand                .......... .......... .......... .......... .......... .......... .......... ..........
CMW5235_Thailand                ....T..... .......... .......... .......... .......... .......... .......... ..........
CMW11228_Uganda                 ..G.A...G. .......... .......... .......... .......... .......... .......... ..........
CMW11226_Ethiopia               ..G.A...G. .......... .......... .......... .......... .......... .......... ..........
CMW11227_Ethiopia               ..G.A...G. .......... .......... .......... .......... .......... .......... ..........
CMW11225_Ethiopia               ..G.A...G. .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_molleriana       T.G....... .C...C.... .......A.. .......... .........T .......... .......... ..........
Mycosphaerella_nubilosa         GCCT.AG... CGC..C.... .TAT.GT... .......... .......... .......... .......... ...C......

                                730        740        750        760        770        780        790        800
CMW11220_South_Africa           AACGAGGTAT GGCCTGAGGC AGCAACTATC -TCCAATCCA CACAC----- --TAACGCGA TACGCAGGCA TCCGGCAACA
CMW11221_South_Africa           .......... .....T.... .......... C.T...-... .....----- --......... .......... ..........
CMW11230_Mexico                 .......... .....T.... .......... C.T...-... .....----- --......... .......... ..........
CMW11231_Mexico                 .......... .....T.... .......... C.T...-... .....----- --......... .......... ..........
CMW5232_Thailand                .......... .......... .......... -......... .....----- --......... .......... ..........
CMW5235_Thailand                .......... .....T.... .......... G.T..C-... .....----- --......... .......... ..........
CMW11228_Uganda                 .......... .....T.... .......... C.T...-... .....----- --...T.... C......... ..........
CMW11226_Ethiopia               .......... .....T.... .......... C.T...-... .....----- --...T.... C......... ..........
CMW11227_Ethiopia               .......... .....T.... .......... C.T...-... .....----- --...T.... C......... ..........
CMW11225_Ethiopia               .......... .....T.... .......... C.T...-... .....----- --...T.... C......... ..........
Mycosphaerella_molleriana       .......... .....C.... ...C.T.... CCT.T.-.A. ..ACA---CC AC.G..CGC. A..AT..... ..T.......
Mycosphaerella_nubilosa         ........GC .A.ACCGCT. TTTCCA.... AGG.T.-TGG ..GTGAGGAT AC.G..T..C A..A.....G ..........
```

```
                                 810        820        830        840        850        860        870        880
CMW11220_South_Africa        AGTATGTCCC GCGTGCCGTC CTCGTCGACT TGGAGCCGGG CACCATGGAC GCTGTCCGCG CTGGTCCGTT CGGTCAGCTC
CMW11221_South_Africa        .......... .......... ..G....... .......... .......... .......... .C........ ..........
CMW11230_Mexico              .......... .......... ..G....... .......... .......... .......... .C........ ..........
CMW11231_Mexico              .......... .......... ..G....... .......... .......... .......... .C........ ..........
CMW5232_Thailand             .......... .......... .......... .......... .......... .......... .......... ..........
CMW5235_Thailand             .......... .......... ..G....... .......... ...T...... ......-... .C........ ..........
CMW11228_Uganda              .......... .......... .......... .......... .......... .......... .C.....C.. ..........
CMW11226_Ethiopia            .......... .......... .......... .......... .......... .......... .C.....C.. ..........
CMW11227_Ethiopia            .......... .......... .......... .......... .......... .......... .C.....C.. ..........
CMW11225_Ethiopia            .......... .......... .......... .......... .......... .......... .C.....C.. ..........
Mycosphaerella_molleriana    .......... .......... .......... .......... T......... ..C....... .......T.. ..........
Mycosphaerella_nubilosa      .......... A......... ........T. .......A.. T......... ..C.....T. .-..C..A.. ...A......

                                 890        900        910        920        930        940        950      958
CMW11220_South_Africa        TTCCGCCCGG ACAACTTCGT CTTCGGTCAG TCGGGTGCTG GCAACAACTG GGCCAAGGGT CACTAC-ACT GAGGGTA
CMW11221_South_Africa        .......... .......... .......... .......... .......... .......... ......-... .......
CMW11230_Mexico              .......... .......... .......... .......... .......... .......... ......-... .......
CMW11231_Mexico              .......... .......... .......... .......... .......... .......... ..-...-... .......
CMW5232_Thailand             .......... .......... .......... .......... .......... .......... ......C... .......
CMW5235_Thailand             ...-...... .......... .......... .......... .......... .......... ......-... .......
CMW11228_Uganda              .......... .......... ......C... .......... .......... .......... ......-... .......
CMW11226_Ethiopia            .......... .......... ......C... .......... .......... .......... .-....C... .......
CMW11227_Ethiopia            .......... .......... ......C... .......... .......... .......... ......-... .......
CMW11225_Ethiopia            .......... .......... ......C... .......... .......... .......... ......-... .......
Mycosphaerella_molleriana    .......... .......... ......C... ..C....... .......... .......... .....NNNNN NNNNNNN
Mycosphaerella_nubilosa      ........A. .T........ ......C... .......... .A........ .......... .-...AC... .......
```

# Chapter Five
# Identification of the Causal Agent of Botryosphaeria Stem Canker in Ethiopian *Eucalyptus* plantations

## ABSTRACT

Plantations of exotic *Eucalyptus* spp. constitute more than 30% of Ethiopia's plantation forests, providing fuel and construction timber to the country. Species such as *E. camaldulensis, E. saligna, E. grandis, E. citriodora* and *E. globulus* are the most commonly planted. During a disease survey of *Eucalyptus* spp. in 2000 and 2001, Botryosphaeria stem canker was observed in most plantations. Characteristic symptoms included tip die-back, coppice failure and stem cankers characterised by kino exudation. The aim of this study was to identify the species responsible for Botryosphaeria stem canker in Ethiopia. Culture and conidial morphology, as well as DNA-based identification involving RFLP's and sequencing of the ITS regions of the ribosomal DNA gene and the elongation factor 1-alpha (EF1-α) gene, were used to identify isolates. Pathogenicity studies were conducted in the greenhouse and under field conditions. Results showed that *B. parva* is responsible for Botryosphaeria stem canker of *Eucalyptus* spp. in Ethiopia. This is the first report of the fungus from the country. The results of greenhouse and field inoculation studies showed that the Ethiopian isolates are highly virulent. Careful site species selection and breeding trials are needed to reduce the impact of this disease in Ethiopia.

## INTRODUCTION

In Ethiopia, the planting of *Eucalyptus* species commenced with the introduction of *E. globulus* Labil. in the late 1890's (Pohjonen & Pukkala 1990, Persson 1995). *Eucalyptus* spp., including *E. camaldulensis* Dehnh., *E. saligna* Sm., *E. grandis* Hill ex Maid and *E. citriodora* Hook are also commonly planted today. Together, they constitute the major proportion of the plantation resource, which covered an estimated 100 000 ha in 1990 (Pohjonen & Pukkala 1990, Persson 1995). The wood from these plantations is used as a source of fuel, construction timber and for the production of poles and posts.

Fungi in the genus *Botryosphaeria* are associated with diseases on a wide range of hosts. On *Eucalyptus* spp., these fungi are known as saprophytes, opportunistic pathogens and endophytes (Davison & Tay 1983, Barnard *et al.* 1987, Shearer, Tippett & Bartle 1987, Smith, Kemp & Wingfield 1994, Smith, Wingfield & Petrini 1996). Damage due to *Botryosphaeria* spp. is more pronounced when plants are under stress caused by drought, frost, water logging and insect damage (Wene & Schoenewesis 1980, Swart, Wingfield & Knox-Davies 1987, Pusey 1989, Old *et al.* 1990). Recently, it has been recognised that *Botryosphaeria* spp. also exist as symptomless endophytes in *Eucalyptus* spp. For example *B. dothidea* (Moug.) Ces. & De. Not. (anamorph = *F. aesculi* Corda) has been reported as an endophyte in *E. nitens* (Deane Et Maid.) Maid. in England (Fisher, Petrini & Sutton 1993) and in *E. grandis, E. camaldulensis, E. nitens* and *E. smithii* R. T. Baker in South Africa (Smith *et al.* 1996). When trees or tree parts are affected by stress, these fungi become active and can cause die-back.

The taxonomy of *Botryosphaeria* spp. is complicated and has been the subject of considerable debate (Sivanesan 1984, Butin 1993, Jacobs & Rehner 1998). This is because many of the species are almost impossible to distinguish from each other in culture. Furthermore, sexual structures of different *Botryosphaeria* spp. on infected tissues are morphologically similar and often indistinguishable. In the past, there has been a tendency to describe new species for collections from different hosts and this

has caused substantial confusion. Names such as *B. dothidea*, *B. ribis* Gressenb. & Dugg., (anamorph = *Fuscicoccum ribis* Gressenb. & Dugg.) and *B. berengeriana* De Not. have been used interchangeably (Slippers *et al.* 2003). In recent years, molecular techniques, particularly DNA sequencing have been used to clarify questions pertaining to *Botryosphaeria* taxonomy (Jacobs & Rehner 1998, Zhou & Stanosz 2001, Slippers *et al.* 2003). These data are showing that names used in past descriptions of diseases, must be treated with some circumspection.

*Botryosphaeria ribis* has been found associated with *Eucalyptus* spp. in different countries. In Florida, *B. ribis* has been associated with seed capsule abortion and twig die-back of *E. camaldulensis*, where it subsequently resulted in the abandonment of commercial seed production (Webb 1983). Infection by *B. ribis* has also been found associated with basal cankers and coppice failure of *E. grandis* in Florida (Barnard *et al.* 1987). In Australia, *B. ribis* is associated with twig, branch and stem cankers of *E. marginata* Donn. ex Sm. (Davison & Tay 1983). This fungus was also responsible for the death of *E. radiata* Sieb. ex DC. in species selection trials in Western Australia (Shearer *et al.* 1987).

In Africa, Botryosphaeria die-back and canker, caused by *B. dothidea, B. rhodina* (Cooke) Von Arx (anamorph = *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.) and *B. eucalyptorum* Crous, H. Smith et M. J. Wingf. (anamorph = *Fusicoccum eucalyptorum* Crous, H. Smith et M. J. Wingf.) has been reported from several countries including South Africa, Republic of Congo and Uganda (Smith *et al.* 1994, 2001, Roux *et al.* 2000, 2001). In South Africa, wide-spread twig die-back and stem cankers were observed on *E. grandis, E. nitens* and *E. smithii*, clones of *E. grandis*, hybrids of *E. grandis* with *E. camaldulensis* as well as with *E. urophylla* S. T. Blake. *B. dothidea* and *B. eucalyptorum* were identified from symptomatic trees (Smith *et al.* 1994, 2001) and are considered to be the most common cause of *Eucalyptus* disease in South Africa. In the Republic of Congo, *B. rhodina* was found associated with root disease on *E. grandis* (Roux *et al.* 2000). Similarly, *B. rhodina* was associated with stem cankers on *Eucalyptus* spp. in the Republic of Congo and Uganda (Roux *et al.* 2000, 2001).

A recent disease survey conducted in *Eucalyptus* plantations of Ethiopia has shown that symptoms, typical of Botryosphaeria canker and die-back are present on several *Eucalyptus* spp. (Alemu, Roux & Wingfield 2003). The aim of this study is to identify the *Botryosphaeria* spp. associated with stem canker of *Eucalyptus* species in Ethiopia. To achieve this, morphological characterization, PCR-RFLP analysis and DNA sequencing were used.

## MATERIALS AND METHODS

### *Symptoms, sample collection and fungal isolation*

In 2000 and 2001, disease surveys were conducted in plantations of *Eucalyptus* spp. in Southern and South Western Ethiopia, from Munessa Shashemen, Wondo Genet, Menagesha and Jima (Figure 1). Different symptoms were noted on several *Eucalyptus* spp. Segments of symptomatic plant parts were incubated in moist chambers for 2-3 days to enhance development of fruiting structures. These were then transferred to MEA (2% Biolab Malt Extract and 1.5% Biolab Agar) and incubated at 25°C. Isolation from symptomatic tissue was also made directly onto MEA. Isolations were also made onto MEA from fruiting structures occurring on *Eucalyptus* twigs collected from the forest floor. All isolates have been deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

*Botryosphaeria* isolates were inoculated on sterilised pine needles placed on the surface of water agar (2% Biolab Agar) in Petri dishes for 2-3 weeks at 25 °C to induce sporulation. Conidial masses from fruiting structures were spread on the surface of MEA in a sterile drop of water. Germinating single conidia were isolated after 12-16 hr.

***Morphological characterisation***

Isolates were initially characterized based on culture morphology on the MEA. Conidia from each of these cultures were mounted in lactophenol and examined using a Zeiss Axioskop light microscope. Widths and lengths of ten conidia were measured for each isolate and their length:width ratios were calculated based on the mean length and width of each isolate (Table 1).

***DNA extraction***

Total genomic DNA was extracted from selected Ethiopian *Botryosphaeria* isolates (Table 2). These isolates were selected to represent different morphological groups. Mycelium used for DNA extraction was scraped directly from MEA plates covered with mycelium using a sterile scalpel and placed into 1.5 µl Eppendorf tubes. The DNA extraction method of Raeder and Broda (1985), with slight modifications, was used to extract DNA. Extraction buffer, 200 µl (100 mM Tris-HCl, pH 8; 50 mM EDTA; 500 mM NaCl; 5 g CTAB) was added to each Eppendorf tube and the mycelium ground into small pieces with a sterile toothpick. When the mycelium was thoroughly broken, a further 800 µl of extraction buffer was added and incubated in a 60 °C water bath for one hour. Thereafter, 500 µl phenol and 300 µl chloroform was added and mixed. The cell debris was precipitated by centrifugation (12000 g, for 60 min) at 4 °C. The upper aqueous layer was transferred into new tubes and a further phenol:chlorophorm extraction was carried out by adding 200 µl phenol and 200 µl chloroform. This mixture was centrifuged at 12000 g for 5 min at 4 °C. The upper aqueous layer was again transferred into clean tubes. Following this, 400 µl chloroform was added to remove the excess phenol. When a clear interface was obtained, 0.1 volume of 3M NaAc (pH 5.5) and two volumes of absolute ethanol were added and the mixture centrifuged for 30 min at 4 °C to precipitate the nucleic acids. The ethanol was discarded and the precipitated nucleic acids were washed by the addition of 500 µl ethanol (70%) and centrifuged for 5 min at 4 °C. The ethanol was removed and the DNA pellets vacuum dried to remove excess ethanol. The DNA pellet was resuspended in 50 µl sterilised water. RNase A (1mg/ml) was added to the

DNA solution to remove the contaminating RNA and incubated over night at 37 °C in a water bath. A 1% agarose gel, stained with ethidium bromide was run and the DNA visualised under UV light.

***PCR amplification***

The internal transcribed spacer regions and 5.8S gene of the ribosomal RNA operon and the elongation factor gene (EF1-α) of the *Botryosphaeria* isolates used in this study were amplified using the Polymerase Chain Reaction (PCR). To amplify the ITS rDNA regions, primers ITS 1 (5' TCC GTA GGT GAA CCT GCG G '3) and ITS 4 (5' TCC TCC GCT TAT TGA TAT GC '3) (White *et al.* 1990) were used. For the EF1-α gene, forward primer EF1-728F (5'-CAT CGA GAA GTT CGA GAA GG-3') and reverse primer EFI-986R (5'-TAC TTG AAG GAA CCC TTA CC-3') was used (Carbone, Anderson & Kohn 1999).

The PCR reaction mixture contained DNA polymerase (*Taq*, 2.5U/µl, Roche), 0.2 mM DNTP's, 10x Buffer, with 1 mM $MgCl_2$ supplied by the manufacturer, 25 mM $MgCl_2$ and 0.75 mM of each primer and approximately 1µl of DNA. Sterile water (37 µl) was added to give a total reaction volume of 50 µl. Denaturation was performed at 96 °C for 1 min. This was followed by 35 cycles of primer annealing at 55 °C for 30 s, chain elongation at 72 °C for 1 min and denaturation at 92 °C for 1 min. Final chain elongation was carried out at 72 °C for 5 min.

***Restriction Fragment Length Polymorphisms (RFLP)***

The ITS amplicons of the Ethiopian *Botryosphaeria* isolates used in this study were digested with *Cfo* I restriction endonuclease to determine the RFLP profile of the isolates (Jacobs 2002, Slippers *et al.* 2002). The RFLP reaction mix contained 20 µl DNA template, 0.5 µl enzyme and 2.5 µl enzyme buffer. The mixture was digested at 37 °C in a water bath for 6 hr. The RFLP fragments were separated on a 3% agarose gel stained with ethidium bromide and visualised under UV light. A standard 100 bp molecular marker was used to estimate the fragment sizes. These banding patterns were compared with those published by Jacobs (2002) and Slippers *et al.* (2002).

***DNA sequencing***

PCR products were purified using the High Pure PCR Product Purification Kit (QUIAGEN, GmbH, Hilden, Germany) and sequenced in both directions. The Big Dye Cycle Sequencing kit with Amplitaq® DNA Polymerase, FS (Perkin-Elmer, Warrington, UK), was used following the manufacturer's protocols, on an ABI PRISM$^{TM}$ 377 DNA Autosequencer (Perkin-Elmer). Primers ITS 1 and ITS 4 were used for sequencing the ITS rDNA regions, and primers EF1-728F and EF1-986R were used to sequence the EF1-α gene.

***Sequence analysis***

The possible identity of the Ethiopian isolates was considered by comparing their ITS sequences with those in the GenBank database [National Centre for Biotechnology Information (NCBI) US National Institute of Health Bethesda (http:/www.ncbi.nlm.nih.gov/BLAST)]. The Ethiopian *Botryosphaeria* sequences were aligned against *Botryosphaeria* sequences obtained from GenBank and from Slippers *et al.* (2003). Alignment of both ITS and EF 1-α gene sequences was done manually using PAUP version 4.0b (Swofford 1998). Gaps were treated as missing data. The sequences were analysed using parsimony, with trees generated by heuristic searches with simple addition and Tree Bisection Reconstruction (TBR) branch swapping. In the phylogenetic analysis *Guignardia philoprina* (Ellis) Viala & Ravaz was used as outgroup. Confidence intervals were determined using DNA BOOTSTRAP analysis (Bootstrap confidence intervals on DNA parsimony) (1000 replicates) (Felsenstein 1993). A partition homogeneity test (Farris *et al.* 1995, Huelsenbeck, Bull & Cunningham 1996) was used to consider whether the data sets for the ITS and EF1-α sequences could be combined.

***Pathogenicity tests***

Greenhouse inoculation studies were conducted on an 18-month-old *E. grandis* clone (ZG14). The trees were maintained in a greenhouse for one week to allow them to

acclimatise to the greenhouse environment. Thirteen *Botryosphaeria* isolates from Ethiopia were used in the greenhouse inoculation trial (Table 3).

Isolates used for inoculation tests were grown on MEA for ten days. A cork borer with a diameter of 9 mm was used to remove the bark and expose the wood for inoculation. Mycelial plugs of equal size were placed into each wound with the mycelial surface facing the xylem. After inoculation, the wounds were covered with Parafilm (Pechiney Plastic Packaging, Chicago) to prevent contamination and desiccation of the inoculum. Each isolate was inoculated on ten trees. Ten trees were also inoculated with sterile MEA to serve as a control.

After six weeks, disease development was evaluated by measuring the lesion lengths on inoculated trees. These measurements were subjected to statistical analysis (One-way ANOVA) using Statistica for Windows (StatSoft. Inc. 1995) to determine whether the lesions associated with the various *Botryosphaeria* isolates differed statistically from each other.

For the field inoculation trials, three representative isolates (CMW11059, CMW11065 and CMW11073) were selected based on the results of the greenhouse inoculation trial and these are representative of the range of lesion sizes (Table 3). Isolates were inoculated onto two-year-old coppice stems of *E. citriodora* trees in a plantation at Wondo Genet. Each isolate was inoculated into 20 trees and 20 trees were inoculated with sterile MEA as a control. A 9 mm cork borer was used to remove the bark and the same protocol used in the greenhouse trial was used. All inoculation wounds were covered with masking tape to prevent desiccation. Lesion development was evaluated after 8 weeks. A one-way analysis of variance ($P<0.0001$) was carried out to determine statistically, the differences in lesion development. Comparison of means was made using Dunnett's t Test method available in Statistica for Windows (StatSoft. Inc. 1995).

## RESULTS

### *Symptoms, sample collection and fungal isolation*

Symptoms of Botryosphaeria canker were commonly found in *Eucalyptus* plantations at Munessa Shashemene, Wondo Genet, Jima and Menagesha. Disease symptoms were found on different *Eucalyptus* species including *E. globulus, E. saligna, E. grandis* and *E. citriodora*. Symptoms of Botryosphaeria stem canker were observed on both coppice stems and first generation stands and on trees of all ages (Figure 2a-d). Bark cracking, production of copious amounts of kino (Figure 2a, 2d), stem discoloration and malformation, tip die-back and death (Figure 2b), as well as the occurrence of kino pockets in the xylem were the most common symptoms observed. When the bark was removed from symptomatic trees, well-developed kino pockets (Figure 2d) were visible in the cambium and xylem. Of all *Eucalyptus* spp., *E. citriodora* plantations at Wondo Genet and Jima/Belete were the most severely damaged by this stem canker disease. Large basal cankers (Figure 2a), as well as two to three layers of black kino rings (Figure 2c) were commonly found on *E. citriodora* trees, indicating different seasons of infection. Isolates of *Botryosphaeria* associated with these stem cankers were easily collected from all samples.

### *Morphological characterisation*

The Ethiopian *Botryosphaeria* isolates grown on MEA showed some variation in colony growth and morphology. Some of the isolates had fluffy light brown aerial mycelium, whereas others had flat colony growth with little aerial mycelium (Figure 3d, 3e). Considerable variation was observed between the conidial lengths of *Botryosphaeria* isolates obtained from Ethiopia (Table 1). The lengths of the individual conidia for all isolates ranged from 12.5 µm to 27.5 µm and the average conidial length for different isolates ranged from 15.25 µm to 24.25 µm. The widths of the conidia showed limited variation and ranged from 5 µm to 7.5 µm. The conidia were grouped into three categories, namely (a) Those with long, narrow conidia, (b) those with long, wide conidia and (c) those with short conidia (Figure 3a-c). No teleomorph structures were observed for isolates examined in this study.

***PCR amplification***

Amplification of the ITS regions and 5.8S gene yielded a PCR product with a fragment length of approximately 500 base pairs (bp). For the EF1-α gene, fragments of approximately 300 base pairs were obtained.

***Restriction Fragment Length Polymorphisms (RFLP)***

All of the *Botryosphaeria* isolates from Ethiopia produced the same banding pattern (Figure 4) when the ITS PCR products were cut with *Cfo* I. This suggested that these isolates might represent the same species, even though they displayed substantial morphological variation. Comparison of the RFLP pattern for the Ethiopian isolates with banding patterns described for *Botryosphaeria* spp. (Jacobs 2002, Slippers *et al.* 2002) showed that the Ethiopian isolates had a banding pattern similar to that of *B. parva* (Figure 4).

***DNA sequencing and analysis***

When compared with sequences in GenBank, the ITS sequences of the Ethiopian *Botryosphaeria* isolates, most closely matched those of *B. ribis*. Alignment of these sequences with sequences of *B. ribis* and with representative sequences of other *Botryosphaeria* spp. (Slippers *et al.* 2003) yielded a total of 518 characters. Analysis of the data set for the EF 1-α sequences produced a total of 343 characters.

The partition homogeneity test revealed that the ITS and EF1-α data sets could be combined. The phylogenetic tree generated for the combined sequences of the ITS and EF 1-α produced five clades (Figure 5). Based on this tree, the Ethiopian *Botryosphaeria* isolates resided within the *B. parva* group. Other clades were similar to those defined by Slippers *et al.* (2003) and included *B. ribis* (Clade II), *B. eucalyptorum* (Clade III), *B. lutea* (clade IV) and *B. dothidea* (clade V). All clades were supported with bootstrap values of 100%. This phylogenetic tree was generated

based on a total of 855 characters, where 254 variable characters were parsimony uninformative and 194 characters were parsimony informative. The phylogenetic tree generated from the combined sequences had a CI value of 0.928 and RI value 0.905.

***Pathogenicity tests***

All Ethiopian *Botryosphaeria* isolates used in the greenhouse inoculation trial produced lesions on the *E. grandis* clone (Figure 6a, b). The mean lesion lengths produced ranged from 24.9 mm and 91.8 mm (Table 3). Isolate CMW11073, produced the largest lesions while the smallest average lesions were associated with isolate CMW11065. No lesions developed on seedlings inoculated with the sterile MEA. Statistical analysis showed that the lesions produced by the majority of isolates were significantly different from the control ($P<0.0001$) (Table 3). An R-square value of 0.47 was recorded for the data obtained in the greenhouse trial. Isolates CMW11073, CMW10095, CMW11066, CMW11064, CMW11063, CMW11069, CMW11059, CMW10094, CMW11067 and CMW11068 produced lesions that were significantly different from the control. The average lesion lengths associated with isolates CMW11071, CMW11070 and CMW11065 (Table 3, Figure 7) were not statistically different from the controls.

The three isolates used in the field inoculation trial produced lesions ranging in average length between 63 mm and 255.1 mm. The largest lesion was recorded for isolate CMW11073 (average = 255.1mm) and the smallest lesion (average = 63.35) was that associated with CMW11065 (Table 4). Some trees inoculated as controls also developed lesions. However, the controls were statistically different ($P=0.0001$) to those where *Botryosphaeria* isolates were used for inoculation (Table 4, Figure 6c-e). An R-square value of 0.71 was recorded for the data obtained from the field study. The results of the field inoculation trial also showed that the lesions associated with isolates CMW11073 and CMW11059 were statistically different to those of the control. CMW11065 produced lesions that did not vary significantly from the control (Table 4, Figure 8). The field and greenhouse trials, therefore, gave similar results.

## DISCUSSION

Results of this study and a prior survey in 2000/2001 have shown that Botryosphaeria canker is the most common disease of *Eucalyptus* in Ethiopia. This disease affects all the major *Eucalyptus* spp. including *E. globulus, E. grandis, E. saligna* and *E. citriodora* (Alemu *et al.* 2003). The results of the current study have, furthermore, shown that *B. parva* is the major cause of Botryosphaeria canker in Ethiopian *Eucalyptus* plantations. This is the first report of this fungus from Ethiopia.

Ethiopian *Botryosphaeria* isolates used in this study showed some variation in colony growth, as well as in conidial length and shape. Based on culture morphology two groups could be distinguished. When the morphology of the conidia was considered, three morphological groups emerged. The morphological variation detected in this study, was however, not consistent with the results of the DNA-based comparison, which showed that the Ethiopian *Botryosphaeria* isolates represent a single species. Results of this study support the view (Jacobs & Rehner 1998, Smith & Stanosz 2001, Slippers *et al.* 2003) that morphological characteristics are insufficient to identify many *Botryosphaeria* spp. with confidence. They also provide additional evidence to suggest that names used for *Botryosphaeria* spp. in the past, are questionable.

Analysis of the banding patterns of the RFLP of ITS rDNA PCR product has been successfully used to distinguish between *Botryosphaeria* spp. obtained from different hosts (Jacobs 2002, Slippers *et al.* 2002). In this study the RFLP analysis showed that all Ethiopian isolates might represent a single species. It was, however, not useful in determining a species name for the fungus because *B. ribis* and *B. parva* have the same banding pattern (Slippers *et al.* 2002).

Ethiopian *Botryosphaeria* isolates had identical ITS sequences, which were sufficient only to determine that the isolates represented either *B. ribis* or *B. parva*. Inability to distinguish between these two species based on ITS sequences has been reported previously by Smith & Stanosz (2001) and Zhou & Stanosz (2001). However, the combination of the ITS rDNA and EF1-α sequence data was useful to separate *B.*

*ribis* and *B. parva* and showed that Ethiopian isolates belong to *B. parva*. These combined sequences were also used by Slippers *et al.* (2003) who showed that *B. ribis* and *B. parva* represent two distinct species. It is interesting that only one species of *Botryosphaeria* is associated with die-back in Ethiopia, while three species, *B. parva, B. dothidea* and *B. eucalyptorum* are associated with die-back on this host in South Africa (Smith *et al.* 1994, Smith *et al.* 2001, Slippers *et al.* 2003).

*Botryosphaeria parva* was first recorded in 1985 as a new species from Kiwifruit in New Zealand (Pennycook & Samuels 1985). There has, however, been considerable controversy surrounding its taxonomic status. It has, for example, been suggested that *B. parva* represents a synonym of *B. ribis* (Smith & Stanosz 2001, Zhou & Stanosz 2001). More recent studies have, however, shown that *B. ribis* and *B. parva* are distinct (Zhou, Smith & Stanosz 2001, Slippers *et al.* 2003). *Botryosphaeria parva* was previously most frequently found associated with fruit trees (Pennycook & Samuels 1985) and little information is available on the importance of this species in *Eucalyptus* plantations. Recently, Slippers *et al.* (2003) showed that *B. parva* is dominant in plantations of *Eucalyptus* spp. in South Africa. The results of the current study also showed that this fungus is important in *Eucalyptus* plantations of Ethiopia.

Greenhouse and field inoculation trials revealed that most *Botryosphaeria* isolates obtained from *Eucalyptus* in Ethiopia are pathogenic to *E. grandis* (clone ZG 14) and to *E. citriodora*. The *B. parva* isolates used in this study showed variation in pathogenicity both in the greenhouse and field study. Development of lesions on some trees inoculated as controls might have been due to contamination at the time of inoculation, wound stress or entophytic infections. The variations in virulence of the three isolates were concordant between greenhouse and field inoculation studies. These findings are similar to those of Jacobs (2000) who showed that *B. parva* is pathogenic to Mango, but isolates varied substantially in pathogenicity.

*Botryosphaeria* spp. have long been recognised as stress related opportunistic pathogens (Schoeneweiss 1981, Pusey 1989). A contemporary view is that they are latent pathogens that commonly occur in leaf and branch tissues of healthy woody

plants, and cause disease when trees are stressed (Fisher *et al.* 1993, Smith *et al.* 1996). In this respect, they are very similar to fungi such as *Sphaeropsis sapinea* (Fr.:Fr.) Dyko & Sutton (Syn=Diplodia pinea (Desm.) Kickx), which biologically and phylogenetically is a typical species of *Botryosphaeria* (Smith *et al.* 1996). The latter fungus is commonly found in healthy pine tissue but causes serious damage under conditions of stress such as after hail damage (Zwolinski, Swart & Wingfield 1990). Hence, *B. parva* must be considered an important pathogen in Ethiopia, where it almost certainly resides in healthy trees, but causes die-back and death of trees under stress conditions.

Plantations in Ethiopia are commonly developed on marginal sites where moisture stress is a limiting factor for tree growth. This could favour disease development associated with *B. parva*. Moreover, the association of Botryosphaeria canker with *Eucalyptus* coppice stands is of great concern, because regenerating *Eucalyptus* species by coppicing is widely practiced in Ethiopia, particularly by small scale tree growers. This practice evidently stresses trees, facilitating infection by *B. parva*. In the future, efforts will need to be made to match species and genotypes to sites and thus to minimise the impact of this opportunistic pathogen.

## REFERENCES

Alemu Gezahgne, Roux, J. & Wingfield, M. J. (2003) Diseases of exotic plantation *Eucalyptus* and *Pinus* species in Ethiopia. *South African Journal of Science* **99:** 29-33.

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

Butin, H. (1993) Morphological adaptation and spore pleomorphism in the form-complex *Dichomera-Camarosporium* and *Fusicoccum-Dothiorella*. *Sydowia* **45**: 161-166.

Carbone, I., Anderson, J. B. & Kohn, L. M. (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* **91**: 553-556.

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

Farris, J. S., Kallersjo, M., Kluge, A. G. & Bult, C. (1995) Testing significance of incongruence. *Cladistics* **10**: 315-319.

Felsentein, J. (1993) DNABOOT-Bootstrap Confidence Interval on DNA parsimony 3.1. Seattle, University of Washington DC.

Fisher, P. J., Petrini, O. & Sutton, B. C. (1993) A comparative study of fungal endophytes in leaves, xylem and bark of *Eucalyptus nitens* in Australia and England. *Sydowia* **45**: 1-14.

Huelsenbeck, J. P., Bull, J. J. & Cunningham, C. W. (1996) Combining data in phylogenetic analysis. *Tree* **11**: 152-158.

Jacobs, K. A. & Rehner, S. A. (1998) Comparison of cultural and morphological characters and ITS sequences in anamorphs of *Botrysphaeria* and related taxa. *Mycologia* **90**: 601-610.

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

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

Pennycook, S. R. & Samuels, G. J. (1985) *Botryosphaeria* and *Fusicoccum* species associated with ripe fruit rot of *Actinidia deliciosa* (Kiwifruit) in New Zealand. *Mycotaxon* **24**: 445-448.

Persson, A. (1995). Exotics-prospects and risks from European and African view point. *Agricultural Science* **9**: 47-62.

Pohjonen, V. & Pukkala, T. (1990) *Eucalyptus globulus* in Ethiopian forestry. *Forest Ecology and Management* **36**:19-31.

Pusey, P. L. (1989) Influence of water stress on susceptibility of nonwooded peach bark to *Botryosphaeria dothidea*. *Plant Disease* **73**: 1000-1003.

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

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

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

Schoeneweiss, D. F. (1981) The role of environmental stress in diseases of woody plants. *Plant Disease* **65**: 308-314.

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

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

Slippers, B., Coutinho, T. A., Wingfield, B. D., Crous, P. W. & Wingfield, M. J. (2002) Identifying species in the genus *Botryosphaeria*. In Proceedings of the 7th International Mycological Congress: pp 231 (765). Oslo. Norway. (Abstract)

Slippers, B., Crous, P. W., Denman, S., Coutinho, T. A., Wingfield, B. D. & Wingfield M. J. (2003) Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. *Mycologia* (In press).

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

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

Smith, H., Crous, P. W., Wingfield, M. J., Coutinho, T. A. & Wingfield, B. D. (2001) *Botryosphaeria eucalyptorum* sp. nov., a new species in the *B. dothidea*-complex on *Eucalyptus* in South Africa. *Mycologia* **93**: 277-285.

Smith, H., Wingfield, M. J., Crous, P. W., & Coutinho T. A. (1996) *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. *South African Journal of Botany* **62**: 86-88.

Smith, D. R. & Stanosz, G. R. (2001) Molecular and morphological differentiation of *Botryosphaeria dothidea* (anamorph *Fusicoccum aesculi*) from some other fungi with *Fusicoccum* anamorphs. *Mycologia* **93**: 505-515.

Swart, W. J., Wingfield, M. J. & Knox-Davies, P. S. (1987) Factors associated with *Sphaeropsis sapinea* infection of pine trees in South Africa. *Phytophylactica* **19**: 505-510.

Swofford, D. L. (1998) PAUP*. *Phylogenetic Analysis using Parsimony* (*and other methods). Version 4.0. Beta version. Sinauer Associates. Sunderland, Massachusetts.

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

Wene, E. G. & Schoeneweiss D. F. (1980) Localized freezing predisposition to *Botryosphaeria dothidea* canker in differentially frozen woody stems. *Canadian Journal of Botany* **58**: 1455-1458.

White, T. J., 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*. (M. A. Innis, D. H. Gelfand, J. J. Snisky & J. W. White eds.): pp 315-322. San Diego, Academic Press.

Zhou, S. & Stanosz, G. R. (2001) Relationships among *Botryosphaeria* species and associated anamorphic fungi inferred from the analysis of ITS and 5.8S rDNA sequences. *Mycologia* **93**: 516-527.

Zhou, S., Smith, D. R. & Stanosz, G. R. (2001) Differentiation of *Botryosphaeria* species and related anamorphic fungi using Inter Simple or Short Sequence Repeat (ISSR) fingerprinting. *Mycological Research* **105**: 919-926.

Zwolinski, J. B., Swart, W. J. & Wingfield, M. J. (1990) Intensity of dieback induced by *Sphaeropsis sapinea* in relation to site conditions. *European Journal of Forest Pathology* **20**: 167-174.

**Table 1.** Conidial sizes of *Botryosphaeria* isolates from *Eucalyptus* in Ethiopia

| Isolate | Origin | Host | Range and Average Length (µm) | Range and Average Width (µm) | Length: Width (ratio) |
|---|---|---|---|---|---|
| CMW10088 | Wondo Genet | *Eucalyptus* sp. | (15) 18.3 (22.5) | (5.25) 5 (5.5) | 3.65 |
| CMW10089 | Wondo Genet | *Eucalyptus* sp. | (20) 24.3 (27.5) | (7) 7.5 (7.75) | 3.23 |
| CMW10092 | Menagesha | *E. globulus* | (15) 17.3 (20) | (5) 5.8 (7.5) | 3.17 |
| CMW10093 | Wondo Genet | *E. saligna* | (12.5) 15 (17.5) | (4.75) 5 (5.5) | 3 |
| CMW10094 | Wondo Genet | *E. saligna* | (17.5) 19 (20) | (5) 5 (5.25) | 3.8 |
| CMW10095 | Wondo Genet | *E. grandis* | (15) 15.3 (17.5) | (5) 5 (5.5) | 3.05 |
| CMW10096 | Wondo Genet | *Eucalyptus* sp. | (15) 16.3 (17.25) | (5) 5 (5.25) | 3.25 |
| CMW11059 | Jima/Belete | *E. citriodora* | (15) 17.5 (20) | (5) 5 (5.5) | 3.5 |
| CMW11060 | Jima/Belete | *E. citriodora* | (17.5) 18.3 (20) | (4.75) 5 (5.5) | 3.17 |
| CMW11061 | Jima/Belete | *E. citriodora* | (15) 17.8 (22.5) | (5) 5.3 (7.5) | 3.38 |
| CMW11062 | Jima/ Belete | *E. citriodora* | (17.5) 19.5 (22.5) | (7) 7.5 (7.5) | 2.6 |
| CMW11063 | Jima/Belete | *E. citriodora* | (15) 16.5 (17.5) | (4.75 5 (5.5) | 3.3 |
| CMW11064 | Jima/Belete | *E. citriodora* | (17.5) 21.8 (25) | (5) 5 (5.5) | 4.35 |
| CMW11066 | Jima/Belete | *E. citriodora* | (17.5) 20.8 (25) | (5) 5.3 (7.5) | 3.95 |
| CMW11068 | Munessa | *E. globulus* | (15) 18.5 (22.5) | (5) 5 (5.5) | 3.7 |
| CMW11069 | Menagesha | *E. globulus* | (15) 16.8 (20) | (5) 5.8 (7.5) | 2.91 |
| CMW11070 | Menagesha | *E. globulus* | (17.5) 17.5 (20) | (5.25) 5 (5.5) | 3.55 |
| CMW11071 | Menagesha | *E. globulus* | (17.5) 18.5 (20) | (5) 6.5 (7.5) | 2.85 |
| CMW11072 | Menagesha | *E. globulus* | (17.5) 19.8 (22.5) | (5) 5 (5.5) | 3.95 |

Each mean values and ranges are based on measurements from 10 conidia.

CMW numbers are culture collection numbers of the Forestry and Agricultural Biotechnology Institute, University of Pretoria.

**Table 2.** Isolates used in the DNA sequence analysis.

| Culture No. | Identity | Host | Origin | Collector | Accession No. | |
|---|---|---|---|---|---|---|
| | | | | | ITS | EF 1-α |
| CMW7780 | *B. dothidea* | *Fraxinus excelsior* | Switzerland | B. Slippers | AY236947 | AY236896 |
| CMW8000 | *B. dothidea* | *Prunus* sp. | Switzerland | B. Slippers | AY236949 | AY236898 |
| CMW10125 | *B. eucalyptorum* | *E. grandis* | S. Africa | H. Smith | AF283686 | AY236891 |
| CMW10126 | *B. eucalyptorum* | *E. grandis* | S. Africa | H. Smith | AF283687 | AY236892 |
| CMW992/3 | *F. luteum* | *Actinidia deliciosa* | New Zealand | G. J. Smuels | AF027745 | AY236894 |
| CMW9076 | *B. lutea* | *Malus X domestica* | New Zealand | S. R .Pennycook | AY236946 | AY236893 |
| CMW7772 | *B. ribis* | *Ribis* sp. | New York | B. Slippers/ G. Hudler | AY236935 | AY236877 |
| CMW7773 | *B. ribis* | *Ribis* sp. | New York | B. Slippers/ G. Hudler | AY236936 | AY236878 |
| CMW9071 | *B. parva* | *Ribis* sp. | Australia | M. J. Wingfield | AY236938 | AY236880 |
| CMW994 | *B. parva* | *Malus sylvestris* | New Zealand | G. J Samuels | AY243395 | AY236883 |
| CMW9077 | *B. parva* | *Actinidia deliciosa* | New Zealand | S. R. Pennycook | AY236939 | AY236884 |
| CMW10122 | *B. parva* | *E. grandis* | S. Africa | H. Smith | AF283681 | AY236882 |
| CMW11060 | *Botryosphaeria* sp. | *E. citriodora* | Ethiopia | Alemu Gezahgne & J. Roux | AY210474 | AY210480 |
| CMW11062 | ,, | ,, | ,, | ,, | AY210475 | AY210481 |
| CMW11064 | ,, | ,, | ,, | ,, | AY210476 | AY210482 |
| CMW10089 | ,, | *E. globulus* | ,, | ,, | AY210477 | AY210483 |
| CMW10095 | ,, | *E. grandis* | ,, | ,, | AYS20478 | AY210485 |
| CMW10094 | ,, | *E. saligna* | ,, | ,, | AY210479 | AY210484 |
| CMW7063 | *Guignardia philoprina* | *Taxus baccata* | Netherlands | H. A. van der Aa | AY236979 | AY236905 |

Isolates from Ethiopia were sequenced in this study. All other sequences are from the study of Slippers *et al.* (2003).
CMW numbers are culture collection numbers of the Forestry and Agricultural Biotechnology Institute, University of Pretoria.

**Table 3.** Mean lesion lengths and confidence limits for greenhouse inoculations using *Botryosphaeria* isolates from Ethiopia.

| Isolate | Mean Lesion length (mm) | 95% Confidence limits |
|---|---|---|
| CMW11059 | $54.2^{bcd}$ | 38.35-70.05 |
| CMW11063 | $66.0^{abc}$ | 49.29-72.71 |
| CMW11064 | $71.7^{ab}$ | 55.85-87.55 |
| CMW11065 | $24.9^{de}$ | 9.05-40.75 |
| CMW11066 | $72.8^{ab}$ | 56.95-88.65 |
| CMW11067 | $48.1^{bcd}$ | 32.25-63.95 |
| CMW11068 | $43.5^{bcd}$ | 27.65-59-35 |
| CMW11069 | $60.8^{abc}$ | 44.95-76.65 |
| CMW11070 | $34.9^{cde}$ | 19.05-50.75 |
| CMW11071 | $39.6^{cde}$ | 23.75-55.45 |
| CMW11073 | $91.8^{a}$ | 75.95-107.65 |
| CMW10095 | $83.2^{ab}$ | 67.35-99.05 |
| CMW10094 | $49.5^{bcd}$ | 33.65-65.35 |
| Control | $10.9^{e}$ | 4.95-26.75 |

CMW numbers are culture collection numbers of the Forestry and Agricultural Biotechnology Institute, University of Pretoria.

Means are averages of 10 measurements.

Means with the same letter are not significantly different from each other at $P<0.05$ significance level.

**Table 4.** Mean lesion lengths and confidence limits for trees inoculated with *Botryosphaeria* sp. on *E. citriodora* in the field.

| Isolates | Mean Lesion length (mm) | 95% Confidence limits |
|---|---|---|
| CMW11059 | 226.8[a] | 197.95-255.65 |
| CMW11065 | 63.35[b] | 34.50-92.20 |
| CMW11073 | 255.1[a] | 226.25-283.95 |
| Control | 29.35[b] | 0.50-58.20 |

CMW numbers are culture collection numbers of the Forestry and Agricultural Biotechnology Institute, University of Pretoria.

Means are averages of 20 measurements.

Means with the same letter are not significantly different from each other at $P<0.05$ significance level.


**Figure 1**. Map of Ethiopia showing the plantation areas where samples were collected.


**Figure 2**. Symptoms of Botryosphareia stem canker in Ethiopia. (a) Basal canker, (b) Wilting and die-back, (c) Black kino rings, (d) Xylem discoloration.

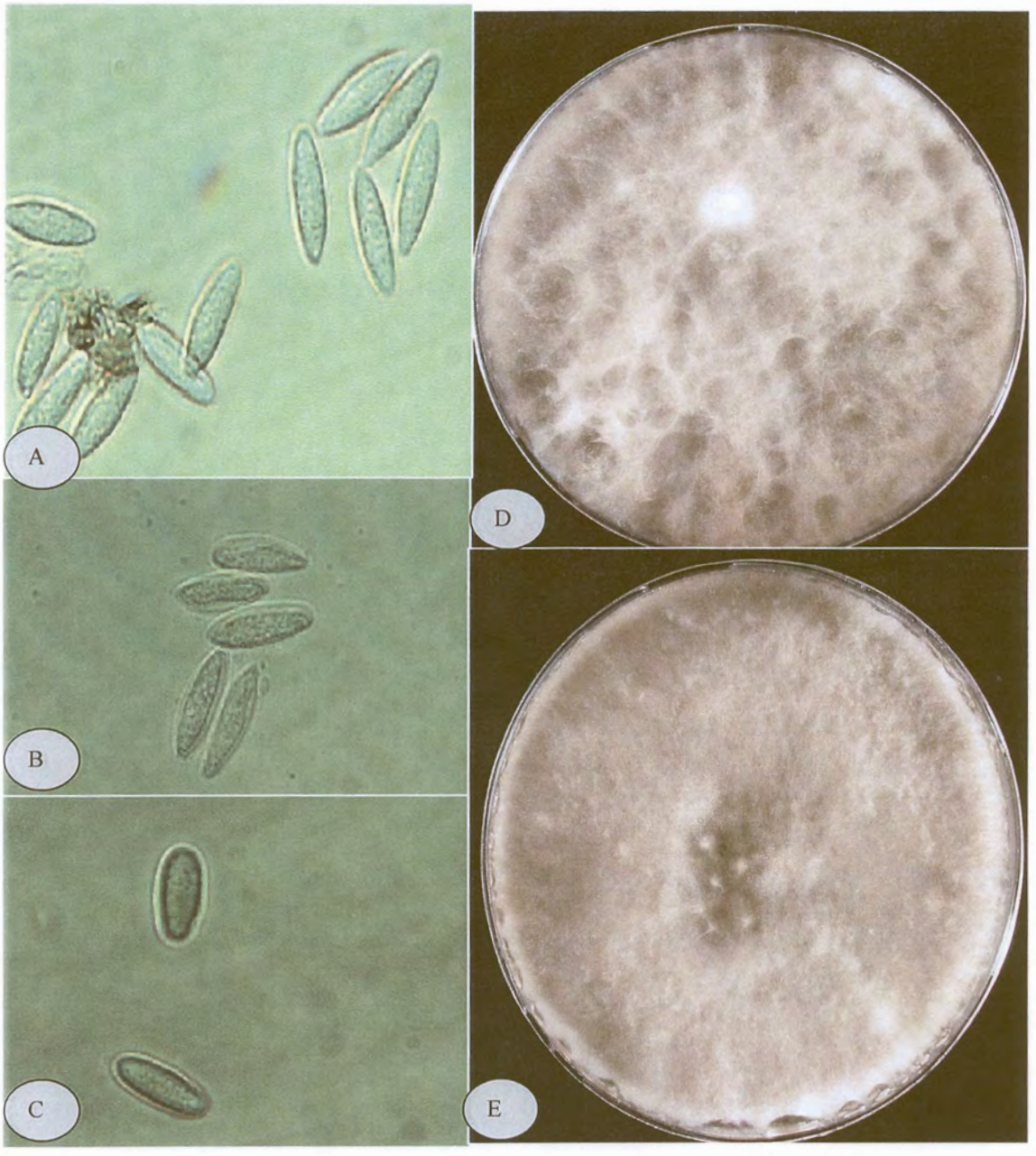



**Figure 3.** Culture and conidial morphology for *B. parva*, (A) Long, narrow and cylindrical conidia, (B) Long, wide and cylindrical conidia, (C) short, and wide conidia, (D) fluffy light brown aerial mycelial growth and (E) flat light brown mycelial growth.

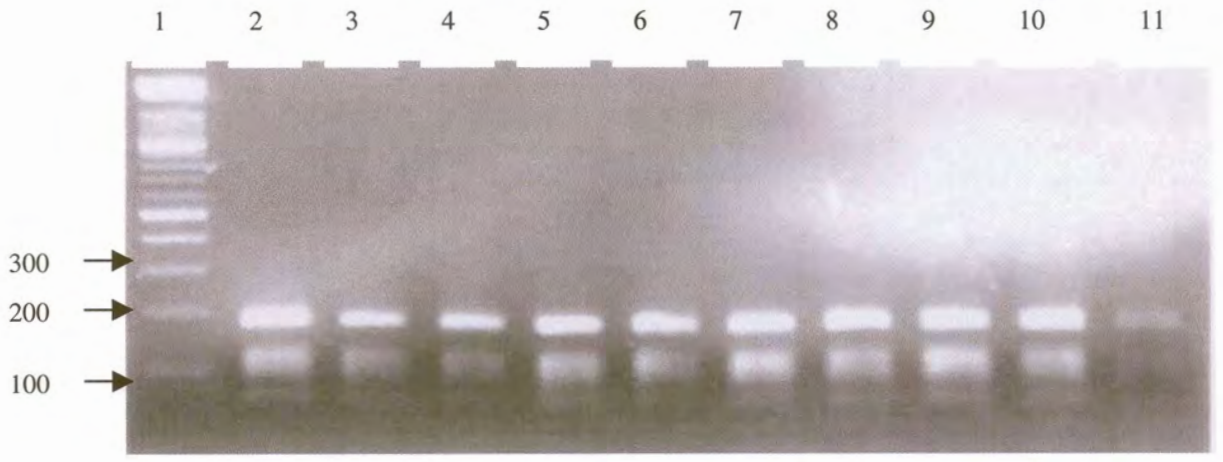



**Figure 4.** Restriction fragment patterns of *Botryosphaeria* isolates from Ethiopia after electrophoresis on a 3% agarose gel stained with ethidium bromide. Lane 1=100 base pair Molecular Weight Marker, Lane 2-11 representative isolates CMW11060, CMW11062, CMW11063, CMW11064, CMW10089, CMW10090, CMW10091, CMW10094 and CMW10095 respectively.

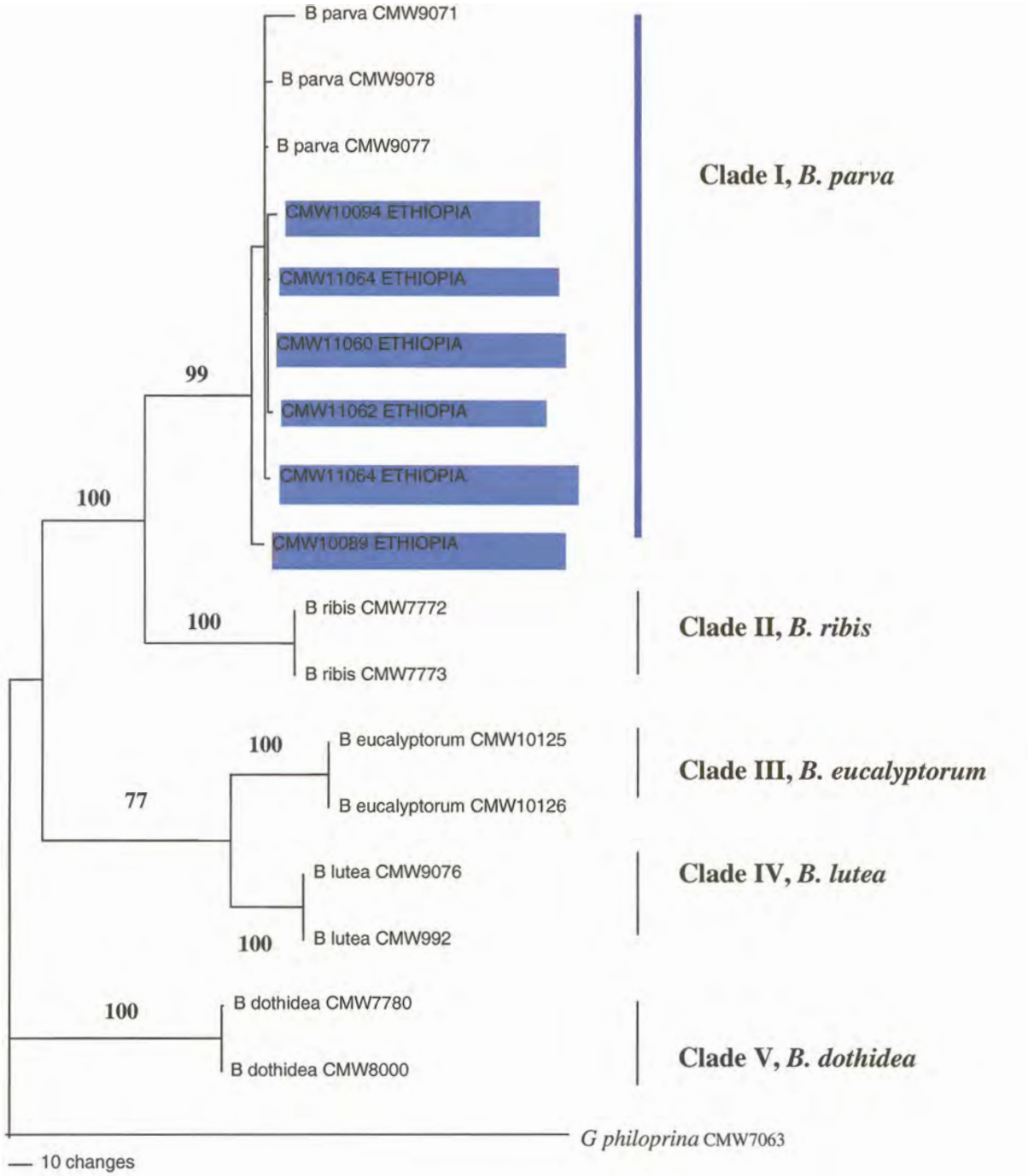



**Figure 5**. Phylogenetic tree for combined ITS rDNA and EF1-α sequences. (CI=0.928, RI=0.905). Bootstrap values are shown on the branches.

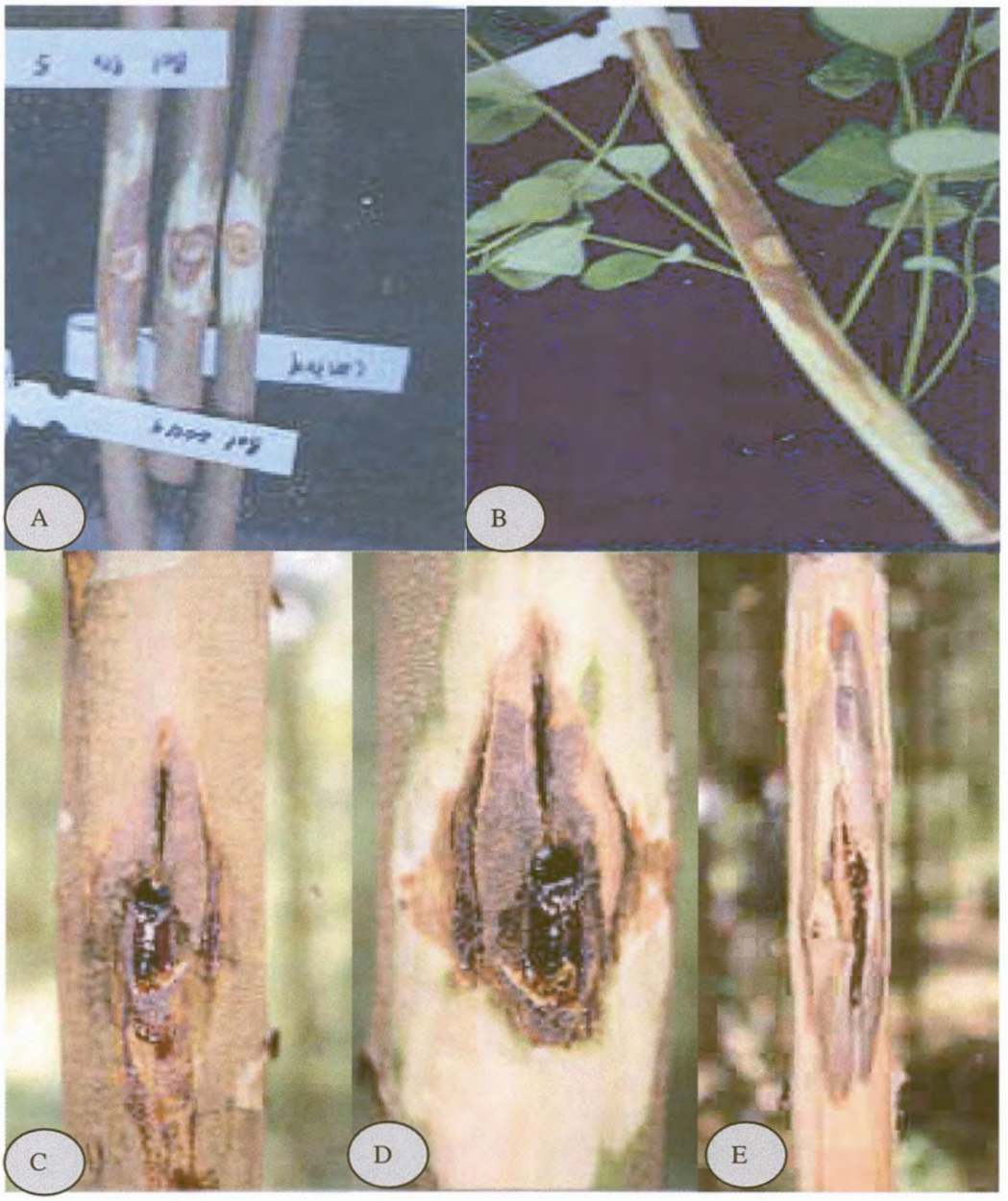



**Figure 6.** Results of pathogenecity tests. (A) Lesions from the greenhouse inoculation trial. (B) Development of epicormic shoots on *E. grandis* clone. (C, D, E) Lesion development in the field inoculation trial.

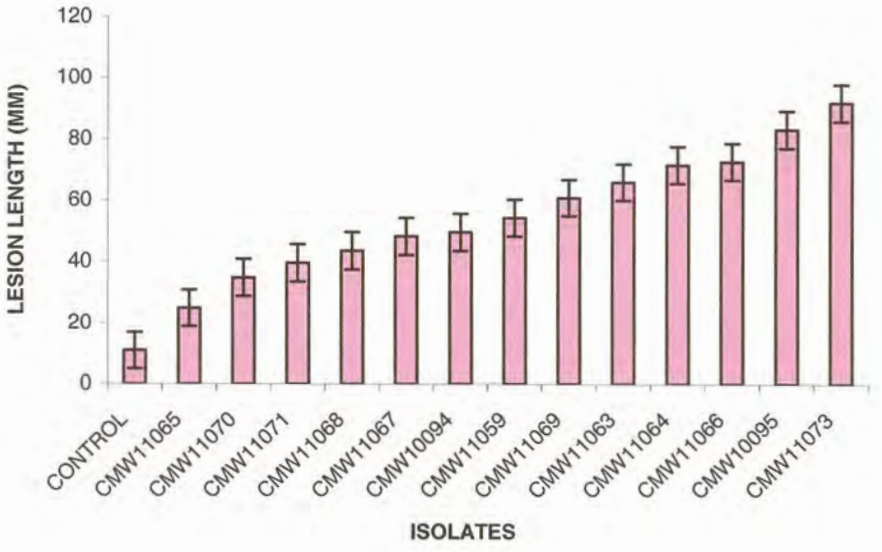



**Figure 7.** Lesion lengths and confidence limits for *Botryosphaeria* isolates and controls used in the greenhouse inoculation study (P=0.05).

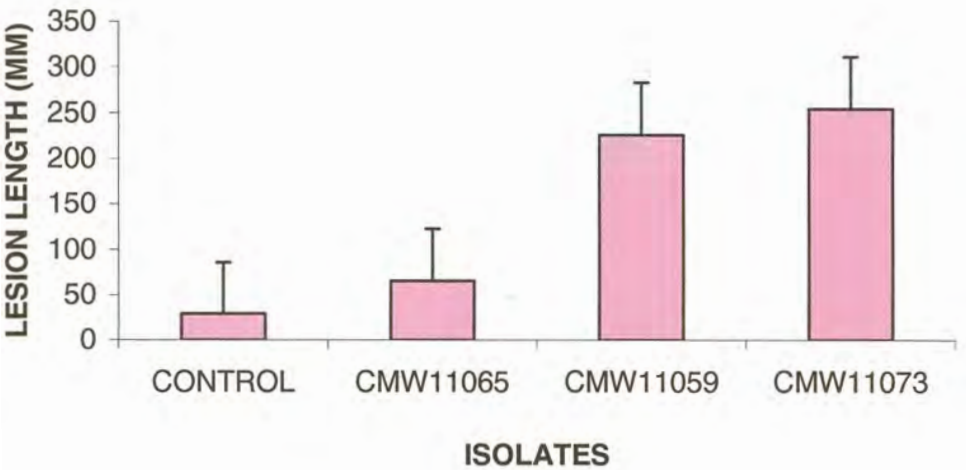



**Figure 8.** Lesion lengths and confidence limits for *Botryosphaeria* isolates and controls used in the field inoculation trial (P=0.05).

**Figure 9**. Aligned sequences of the ITS and Elongation Factor (1-alpha) genes of *Botryosphaeria* isolates. (-) = Gaps, (.) = homologous nucleotides (N) = Unknown bases

```
                                10        20        30        40        50        60        70
B_parva_CMW9071                 GAAGTTCGAG AAGGTAAGAA -AG-TTTTTC C-TTCCGCTG CACGCGC--T GGGTGCCAGG TGCTGGGT--
B_PARVA_CMW9078                 ....G.---- -----..... -..-..... .-........ .......--. .......... ........--
B_PARVA_CMW9077                 ....G.---- -----..... -..T....-. .-........ .......--. .......... ........--
B_DOTHIDEA_CMW7780              ....G.---- -----...C. C.CA....-. TG.G..--.. ....-----. ---------. ........--
B_DOTHIDEA_CMW8000              ....G.---- -----...C. C.CA....-. TG.G..--.. ....-----. ---------. ........--
CMW10094ETHIOPIA                ....G.---- -----..... -..T....-. .-........ .......--. .......... ........--
CMW10089ETHIOPIA                ....G.---- -----..... -..T....-. .-........ .......--. .......... ........--
CMW11064ETHIOPIA                ....G.---- -----..... -..T....-. .-........ .......--. .......... ........--
CMW11060ETHIOPIA                ....G.---- -----..... -..T....-. .-........ .......--. .......... ........--
CMW11062ETHIOPIA                ....G----- -----..... -..T....-. .-........ .......--. .......... ........--
CMW10095ETHIOPIA                ....G----- -----..... -..T....-. .-........ .......--. .......... ........--
B_eucalyptorum_CMW10125         .......... .......... -.AT....-. .-........ .....TTCC. ......---- ---.....--
B_eucalyptorum_CMW10126         .......... .......... -.AT....-. .-........ .....TTCC. ......---- ---.....--
B_lutea_CMW9076                 .......... .......... G.-T....-. -G........ .C.....GA. ..-...---- ---....A--
B_lutea_CMW992                  .......... .......... G.-T....-. -G........ .C.....GA. ..-...---- ---....A--
B_RIBIS_CMW7772                 ....G.---- -----..... -..T....-. .-........ .......--. ......TG.. ........GC
B_RIBIS_CMW7773                 ....G.---- -----..... -..T....-. .-........ .......--. ......TG.. ........GC
G_PHILOPRINA_CMW7063            ....G.---- -----..... C..------. .--------- ---------- ---------- ----------
```

```
                                  80         90         100        110        120        130        140
B_parva_CMW9071          ---------- --TCCCGCAC TCAATTTGCC TTATC--GCT TCGGTGAGGG GCA-TTT--T GGTGGTGGGG
B_PARVA_CMW9078          ---------- --......... .......... .....--... .......... ...-...--. ..........
B_PARVA_CMW9077          ---------- --......... .......... .....--... .......... ...-...--. ..........
B_DOTHIDEA_CMW7780       ---------- --...T..G. CGG....... .....-A-.. CT........ ...A...C-. --.........
B_DOTHIDEA_CMW8000       ---------- --...T..G. CG........ .....-A-.. CT........ ...A...C-. --.........
CMW10094ETHIOPIA         ---------- --.........  ........T. .....--... ....A..... ...-...--. ..........
CMW10089ETHIOPIA         ---------- --......... .......... .....--... .......... ...-...--. ..........
CMW11064ETHIOPIA         ---------- --......... .......... .....--... .......... ...-...--. ..........
CMW11060ETHIOPIA         ---------- --......... .......... .....--... .......... ...-...--. ..........
CMW11062ETHIOPIA         ---------- --......... .......... .....--... .......... ...-...--. ..........
CMW10095ETHIOPIA         ---------- --......... .......... .....--... .......... ...-...--. ..........
B_eucalyptorum_CMW10125  ---------- --......... .......... .....--... ..A....... ...-...--. .....---..
B_eucalyptorum_CMW10126  ---------- --......... .......... .....--... ..A....... ...-...--. .....---..
B_lutea_CMW9076          ---------- --.G....... .........T. .....--... .......... ...-...--. ..........
B_lutea_CMW992           ---------- --.G....... .........T. .....--... .......... ...-...--. ..........
B_RIBIS_CMW7772          TGGGTGCTGG GT........ .......... .....--... .......... ...-...--. ..........
B_RIBIS_CMW7773          TGGGTGCTGG GT........ .......... .....--... .......... ...-...--. ..........
G_PHILOPRINA_CMW7063     ---------- --------.. ..-C....-- A..C.CA.-A ..-...C..C .---.C.CGC A-.CTCACA-
```

```
                                150         160         170         180         190         200         210
B_parva_CMW9071                 T-TGGCCCGC GCTAAGCCTC GTTTGGGCT- CGGCAAAATG TCCGCATC-- TGGTTTTTTT GCGACCGGCG
B_PARVA_CMW9078                 .-........ .......... .........- .......... ........-- .......... ..........
B_PARVA_CMW9077                 .-........ .......... .........- .......... ........-- .......... ..........
B_DOTHIDEA_CMW7780              -C........ .......... ......T..T .........C ........-- ...A...... .T........
B_DOTHIDEA_CMW8000              -C........ .......... ......T..T .........C ........-- ...A...... .T........
CMW10094ETHIOPIA                .-........ .......... ?........- .......... ........-- .......... ..........
CMW10089ETHIOPIA                .-........ .......... .........- .......... ........-- .......... ..........
CMW11064ETHIOPIA                .-........ ......T... .........- .......... ........-- .......... ..........
CMW11060ETHIOPIA                .-........ .......... .........- .......... ........-- .......... ..........
CMW11062ETHIOPIA                .-........ .......... .........- .......... ........-- .......... ..........
CMW10095ETHIOPIA                .-........ .......... .........- .......... ........-- .......... ..........
B_eucalyptorum_CMW10125         .C-....... .....T...G .....T...T .........C ....T...-- .........- ..........
B_eucalyptorum_CMW10126         .C-....... .....T...G .....T...T .........C ....T...-- .........- ..........
B_lutea_CMW9076                 .C-....... .......... .....T...T .........C .........- .........- ..........
B_lutea_CMW992                  .C-....... .......... .....T...T .........C .........- .........- ..........
B_RIBIS_CMW7772                 .-........ .......... ...C.....- .......... ........-- .......... ..........
B_RIBIS_CMW7773                 .-........ .......... ...C.....- .......... ........-- .......... ..........
G_PHILOPRINA_CMW7063            CC....ATT. TG.GCC...? -..ACCC..C .TCA.....- --------.AA --T....... .T.G..CTTT
```

```
                                220        230        240        250        260        270        280
B_parva_CMW9071                 TGCGAC-CGA AGCG--CGCC CCTCGCCAGA ------CACG CCAC------ --GCATGTGC G-----ACCA
B_PARVA_CMW9078                 ......-... ....--.... .......... ------.... ....------ --......... .-----....
B_PARVA_CMW9077                 ......-... ....--.... .......... ------.... ....------ --......... .-----....
B_DOTHIDEA_CMW7780              ......-... C...AA.A.. ....A...-. CGCTTC..-. ....TCACGT TC.TC.A...  .-----....
B_DOTHIDEA_CMW8000              ......-... C...AA.A.. ....A...-. CGCTTC..-. ....TCACGT TC.TC.A...  .-----....
CMW10094ETHIOPIA                ......-... ....--.... .......... ------.... ....------ --......... .-----....
CMW10089ETHIOPIA                ......-... ....--.... .......... ------.... ....------ --......... .-----....
CMW11064ETHIOPIA                ......-... ....--.... .......... ------.... ....------ --......... .-----....
CMW11060ETHIOPIA                ......-... ....--.... .......... ------.... ....------ --......... .-----....
CMW11062ETHIOPIA                ......-... ....--.... .......... ------.... ....------ --......... .-----....
CMW10095ETHIOPIA                ......-... ....--.... .......... ------.... ....------ --......... .-----....
B_eucalyptorum_CMW10125         ......-... .AT.--.... ........-- ---T-G..-- ---------- -------... .--CGG-...
B_eucalyptorum_CMW10126         ......-... .AT.--.... ........-- ---T-G..-- ---------- -------... .--CGG-...
B_lutea_CMW9076                 ......-... C...--.A.. .......... --CTCG..-. ....AC---- ------.... .ATCGG----
B_lutea_CMW992                  ......-... C...--.A.. .......... --CTCG..-. ....AC---- ------.... .ATCGG----
B_RIBIS_CMW7772                 ......-... ....--.A.. .......... ------.... ....------ --......... .-----....
B_RIBIS_CMW7773                 ......-... ....--.A.. .......... ------.... ....------ --......... .-----....
G_PHILOPRINA_CMW7063            .TA.TGGG.C C----A.AA. ..C-...... GTTCTCG.TA ---------- --....C.CA A---GG.---
```

```
                                290        300        310        320        330        340        350
B_parva_CMW9071          -GACGCTAAC -----AGCCA TCCC---AGG AAGCCACCGA GTTGATTCGA GCTCCGGC-T CGACTCTCCC
B_PARVA_CMW9078          -......... -----..... ....---... .......... .......... ........-. ..........
B_PARVA_CMW9077          -......... -----..... ....---... .......... .......... ........-. ..........
B_DOTHIDEA_CMW7780       T-.T...... CA-----..G C.A.AAC... .......... .........G ........C- ...TC-CT..
B_DOTHIDEA_CMW8000       T-.T...... CA-----..G C.A.AAC... .......... .........G ........C- ...TC-CT..
CMW10094ETHIOPIA         -......... -----..... ....---... .....NNNNN NNNNNNNNNN NNNNNNNNNN NNNN......
CMW10089ETHIOPIA         -......... -----G...C ....---... .......... .......... ........-. ..........
CMW11064ETHIOPIA         -......... -----..... ....---... .......... .......... ........-. ..........
CMW11060ETHIOPIA         -......... -----..... ....---... .......... .......... ........-. ..........
CMW11062ETHIOPIA         -......... -----..... ....---... .......... .......... ........-. ..........
CMW10095ETHIOPIA         -......... -----..... ....---... .......... .......... ........-. ..........
B_eucalyptorum_CMW10125  -........T -----GC... ....---... .......... .....C.... ........-. ..........
B_eucalyptorum_CMW10126  -........T -----GC... ....---... .......... .....C.... ........-. ..........
B_lutea_CMW9076          --........ -----GA..G ..T.---... .......... .......... ........-. ..........
B_lutea_CMW992           --........ -----GA..G ..T.---... .......... .......... ........-. ..........
B_RIBIS_CMW7772          -......... -----G.... ....---... .......... .......... ........-. ..........
B_RIBIS_CMW7773          -......... -----G.... ....---... .......... .......... ........-. ..........
G_PHILOPRINA_CMW7063     --.G..--.. GCGCTGA.AG ....---.AA .TAGG.AT.. .A.C..G.C- -..T...GG. A...-.....
```

```
                                360        370        380        390        400        410        420
B_parva_CMW9071          ACCCAATGTG TACC-TACCT CTGTTGCTTT GGCGGGCCGC GGTCCTCCGC -ACCGG-CGC CC-TTC-GGG
B_PARVA_CMW9078          ....T..... ....-..... .......... .......... .......... -.....-... ..-...-...
B_PARVA_CMW9077          .......... ....-..... .......... .......... .......... -.....-... ..-...-...
B_DOTHIDEA_CMW7780       CA..CT.TGT GTA.C..... .......... .......... .......... GG....C.C. ..TCC.C...
B_DOTHIDEA_CMW8000       CA..CT.TGT GTA.C..... .......... .......... .......... GG....C.C. ..TCC.C...
CMW10094ETHIOPIA         ....T..... ....-..... .......... .......... .......... -.....-... ..-...-...
CMW10089ETHIOPIA         .......... ....-..... .......... .......... .......... -.....-... ..-...-...
CMW11064ETHIOPIA         ....T..... ....-..... .......... .......... .......... -.....-... ..-...-...
CMW11060ETHIOPIA         ....T..... ....-..... .......... .......... .......... -.....-... ..-...-...
CMW11062ETHIOPIA         ....T..... ....-..... .......... .......... .......... -.....-... ..-...-...
CMW10095ETHIOPIA         .......... ....-..... .....T.... .......... .......... -.....-... ..-...-...
B_eucalyptorum_CMW10125  ....T..... ....-..... .......... .......... .......... -.....-.T. ..T..--...
B_eucalyptorum_CMW10126  ....T..... ....-..... .......... .......... .......... -.....-.T. ..T..--...
B_lutea_CMW9076          ....C..... ....-..... .......... .......... .......... -....AC.C. .G-...-...
B_lutea_CMW992           ....C..... ....-..... .......... .......... .......... -....AC.C. .G-...-...
B_RIBIS_CMW7772          .......... ....-..... .......... .......... .......... -.....-... ..-...G...
B_RIBIS_CMW7773          .......... ....-..... .......... .......... .......... -.....-... ..-...G...
G_PHILOPRINA_CMW7063     ....TT...T ...AA..... T......... .....C..-. .-..--G.AA G..-AAC..G .--.C.-..-
```

```
                                430        440        450        460        470        480        490
B_parva_CMW9071          GGGCTGGCCA GCGCCCGCCA GAGGACCAT- AAAACTCCAG TCAGTGAAC- TTCGCAGTCT GAAAAACAAG
B_PARVA_CMW9078          .......... .......... .........- .......... .........- .......... ..........
B_PARVA_CMW9077          .......... .......... .........- .......... .........- .......... ..........
B_DOTHIDEA_CMW7780       ...-...... .......... .........C ...-...... .....A...G A.-....... .........-T
B_DOTHIDEA_CMW8000       ...-...... .......... .........C ...-...... .....A...G A.-....... .........-T
CMW10094ETHIOPIA         .......... .......... .........- .......... .........- .......... ..........
CMW10089ETHIOPIA         .......... .......... .........- .......... .........- .......... ..........
CMW11064ETHIOPIA         .......... .......... .........- .......... .........- .......... ..........
CMW11060ETHIOPIA         .......... .......... .........- .......... .........- .......... ..........
CMW11062ETHIOPIA         ..-....... .......... .........- .......... .........- .......... ..........
CMW10095ETHIOPIA         .......... .......... .........- .......... .........- .......... ..........
B_eucalyptorum_CMW10125  ..-....... ...T...... .........C .......... .....A...G ..-....... ..........
B_eucalyptorum_CMW10126  ..-....... ...T...... .........C .......... .....A...G ..-....... ..........
B_lutea_CMW9076          ....C..... .......... .........C .......... .....A...G -......... ..G.......
B_lutea_CMW992           ....C..... .......... .........C .......... .....A...G -......... ..G.......
B_RIBIS_CMW7772          .......... .......... .........- .......... .........- .......... ..........
B_RIBIS_CMW7773          .......... .......... .........- .......... .........- .......... ..........
G_PHILOPRINA_CMW7063     ---....T.. ...G...... .......--.C .......ATA ----.T.TTA ..-.TC.... ..GT.-.T.T
```

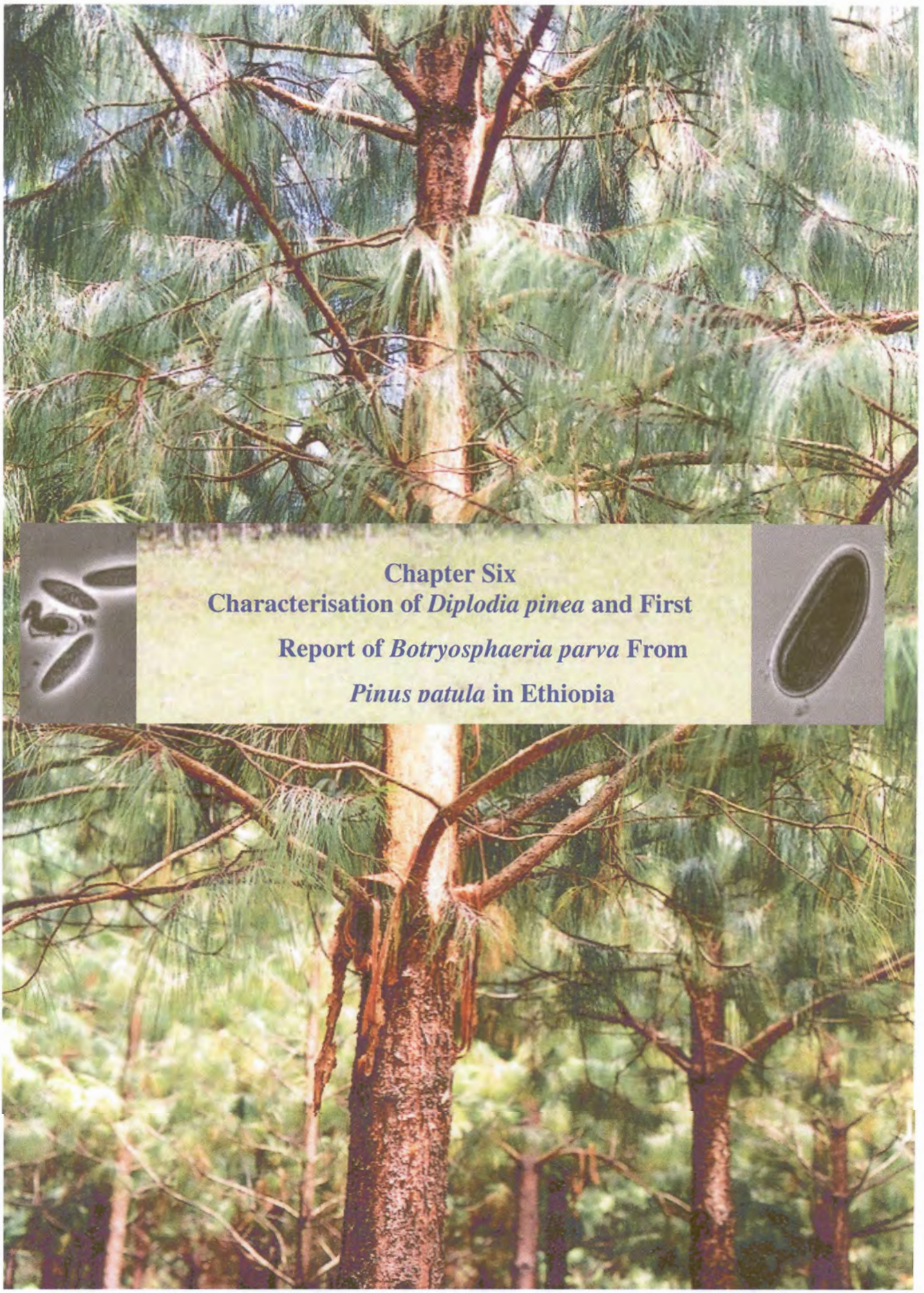
```
                                500        510        520        530        540        550        560
B_parva_CMW9071          TTAATAAACT AAAACTTTCA ACAACGGATC TCTTGGTTCT GGCATCGATG AAGAACGCAG CGAAATGCGA
B_PARVA_CMW9078          .......... .......... .......... .......... .......... .......... ..........
B_PARVA_CMW9077          .......... .......... .......... .......... .......... .......... ..........
B_DOTHIDEA_CMW7780       .......... .......... .......... .......... .......... .......... ..........
B_DOTHIDEA_CMW8000       .......... .......... .......... .......... .......... .......... ..........
CMW10094ETHIOPIA         .......... .......... .......... .......... .......... .......... ..........
CMW10089ETHIOPIA         .......... .......... .......... .......... .......... .......... ..........
CMW11064ETHIOPIA         .......... .......... .......... .......... .......... .......... ..........
CMW11060ETHIOPIA         .......... .......... .......... .......... .......... .......... ..........
CMW11062ETHIOPIA         .......... .......... .......... .......... .......... .......... ..........
CMW10095ETHIOPIA         .......... .......... .......... .......... .......... .......... ..........
B_eucalyptorum_CMW10125  .......... .......... .......... .......... .......... .......... ..........
B_eucalyptorum_CMW10126  .......... .......... .......... .......... .......... .......... ..........
B_lutea_CMW9076          .......... .......... .......... .......... .......... .......... ..........
B_lutea_CMW992           .......... .......... .......... .......... .......... .......... ..........
B_RIBIS_CMW7772          .......... .......... .......... .......... .......... .......... ..........
B_RIBIS_CMW7773          .......... .......... .......... .......... .......... .......... ..........
G_PHILOPRINA_CMW7063     A.....G-T. .......... .......... .......... .......... .......... ..........
```

```
                          570        580        590        600        610        620        630
B_parva_CMW9071      TAAGTAATGT GAATTGCAGA ATTCAGTGAA TCATCGAATC TTTGAACGCA CATTGCGCCC CTTGGTATTC
B_PARVA_CMW9078      .......... .......... .......... .......... .......... .......... ..........
B_PARVA_CMW9077      .......... .......... .......... .......... .......... .......... ..........
B_DOTHIDEA_CMW7780   .......... .......... .......... .......... .......... .......... T.........
B_DOTHIDEA_CMW8000   .......... .......... .......... .......... .......... .......... T.........
CMW10094ETHIOPIA     .......... .......... .......... .......... .......... .......... ..........
CMW10089ETHIOPIA     .......... .......... .......... .......... .......... .......... ..........
CMW11064ETHIOPIA     .......... .......... .......... .......... .......... .......... ..........
CMW11060ETHIOPIA     .......... .......... .......... .......... .......... .......... ..........
CMW11062ETHIOPIA     .......... .......... .......... .......... .......... .......... ..........
CMW10095ETHIOPIA     .......... .......... .......... .......... .......... .......... ..........
B_eucalyptorum_CMW10125 .......... .......... .......... .......... .......... .......... ..........
B_eucalyptorum_CMW10126 .......... .......... .......... .......... .......... .......... ..........
B_lutea_CMW9076      .......... .......... .......... .......... .......... .......... ..........
B_lutea_CMW992       .......... .......... .......... .......... .......... .......... ..........
B_RIBIS_CMW7772      .......... .......... .......... .......... .......... .......... ..........
B_RIBIS_CMW7773      .......... .......... .......... .......... .......... .......... ..........
G_PHILOPRINA_CMW7063 .......... .......... .......... .......... .......... .......... .C........
```

```
                                640        650        660        670        680        690        700
B_parva_CMW9071          CGAGGGGCAT GCCTGTTCGA GCGTCATTTC AACCCTCAAG CTCTGCTTGG TATTGGGCCC CGTCCTCCAC
B_PARVA_CMW9078          .......... .......... .......... .......... .......... .......... ..........
B_PARVA_CMW9077          .......... .......... .......... .......... .......... .......... ..........
B_DOTHIDEA_CMW7780       ...A...... .......... ........A. .......... .......... ........A. ......T--T
B_DOTHIDEA_CMW8000       ...A...... .......... ........A. .......... .......... ........A. ......T--T
CMW10094ETHIOPIA         .......... .......... .......... .......... .......... .......... ..........
CMW10089ETHIOPIA         .......... .......... .......... .......... .......... .......... ..........
CMW11064ETHIOPIA         .......... .......... .......... .......... .......... .......... ..........
CMW11060ETHIOPIA         .......... .......... .......... .......... .......... .......... ..........
CMW11062ETHIOPIA         .......... .......... .......... .......... .......... .......... ..........
CMW10095ETHIOPIA         .......... .......... .......... .......... .......... .......... ..........
B_eucalyptorum_CMW10125  .......... .......... .......... .......... ..T....... .......... ......-.T-
B_eucalyptorum_CMW10126  .......... .......... .......... .......... ..T....... .......... ......-.T-
B_lutea_CMW9076          .......... .......... .......... .......... .......... ........T. ......-.T-
B_lutea_CMW992           .......... .......... .......... .......... .......... ........T. ......-.T-
B_RIBIS_CMW7772          .......... .......... .......... .......... .......... ........T. ..........
B_RIBIS_CMW7773          .......... .......... .......... .......... .......... ........T. ..........
G_PHILOPRINA_CMW7063     ..G....... .......... ........A. .......... .......... ........-- -...AC..C.
```

```
                          710        720        730        740        750        760        770
B_parva_CMW9071       GGACGC---G CCTCAAAGAC CTCGGCGGTG GCGTCTTGCC TCAAGCGTAG TAGAA--AAC ACCTCGCTTT
B_PARVA_CMW9078       .......---. ...T...... .......... .......... .......... .....--... ..........
B_PARVA_CMW9077       .......---. .......... .......... .......... .......... .....--... ..........
B_DOTHIDEA_CMW7780    .CGG..-GC. .......... .......... .......... .......... .....CAT.. .T.......C
B_DOTHIDEA_CMW8000    .CGG..-GC. .......... .......... .......... .......... .....CAT.. .T.......C
CMW10094ETHIOPIA      .......---. ...T...... .......... .......... .......... .....--... ..........
CMW10089ETHIOPIA      .......---. ...T...... .......... .......... .......... .....--... ..........
CMW11064ETHIOPIA      .......---. ...T...... .......... .......... .......... .....--... ..........
CMW11060ETHIOPIA      .......---. ...T...... .......... .......... .......... .....--... ..........
CMW11062ETHIOPIA      .......---. ...T...... .......... .......... .......... .....--... ..........
CMW10095ETHIOPIA      .......---. ...T...... .......... .......... .......... .....--... ..........
B_eucalyptorum_CMW10125 -.TG.ACGC. .......... .......... .......... .......... .....--.T. ..........
B_eucalyptorum_CMW10126 -.TG.ACGC. .......... .......... .......... .......... .....--.T. ..........
B_lutea_CMW9076       -.TG.ACGC. ....G..... .......... .......... .......... .....-A.-. ..........
B_lutea_CMW992        -.TG.ACGC. ....G..... .......... .......... .......... .....-A.-. ..........
B_RIBIS_CMW7772       .......---. ...T...... .......... .......... .......... .....--... ..........
B_RIBIS_CMW7773       .......---. ...T...... .......... .......... .......... .....--... ..........
G_PHILOPRINA_CMW7063  ..GT..---. ...T...AT. AGT....... C.....G..T .......... .--..--T.. TT........
```

```
                          780        790        800        810        820        830 833
B_parva_CMW9071      GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTTT-GAATT ATTTCTCAAG GTTGACCTCG GAT
B_PARVA_CMW9078      .......... .......... .......... ....-..... .......... .......... ...
B_PARVA_CMW9077      .......... .......... .......... ....-..... .......... .......... ...
B_DOTHIDEA_CMW7780   ........G. .......... .......... ...CT...C. -......... .......... ...
B_DOTHIDEA_CMW8000   ........G. .......... .......... ...CT...C. -......... .......... ...
CMW10094ETHIOPIA     .......... .......... .......... ....-..... .......... .......... ...
CMW10089ETHIOPIA     .......... .......... .......... ....-..... .......... .......... ...
CMW11064ETHIOPIA     .......... .......... .......... ....-..... .......... .......... ...
CMW11060ETHIOPIA     .......... .......... .......... ....-..... .......... .......... ...
CMW11062ETHIOPIA     .......... .......... .......... ....-..... .......... .......... ...
CMW10095ETHIOPIA     .......... .......... .......... ....-..... .......... .......... ...
B_eucalyptorum_CMW10125 ........T. .......... .......... ....-..... -......... .......... ...
B_eucalyptorum_CMW10126 ........T. .......... .......... ....-..... -......... .......... ...
B_lutea_CMW9076      .......... .......... .......... ....-..... -......... .......... ...
B_lutea_CMW992       .......... .......... .......... ....-..... -......... .......... ...
B_RIBIS_CMW7772      .......... .......... .......... ....-..... .......... .......... ...
B_RIBIS_CMW7773      .......... .......... .......... ....-..... .......... .......... ...
G_PHILOPRINA_CMW7063 ....TC.GG. CGAG..T..T G.CA.--... .CCC--.--. ....T.T... .......... ...
```



# Chapter Six
# Characterisation of *Diplodia pinea* and First Report of *Botryosphaeria parva* From *Pinus patula* in Ethiopia

## ABSTRACT

*Pinus patula* is extensively used in Ethiopian reafforestation. In recent disease surveys a dark grey fungus resembling *Diplodia* sp. was isolated from cones of *P. patula* in Ethiopia. *Diplodia pinea* has a cosmopolitan distribution on *Pinus* spp. and is an important pathogen on exotic *Pinus* spp. in the tropics and Southern Hemisphere. *D. pinea* is a stress related opportunistic pathogen that can result in branch and shoot die-back, root disease and blue stain of the sapwood. *D. pinea,* including the two morphotypes (A and C), and *D. scrobiculata* are commonly associated with *Pinus* spp. The aim of this study was to characterise dark grey fungal isolates obtained from the cones of *P. patula* in Ethiopia. These were compared based on morphology and DNA based techniques. Morphological comparisons showed that the fungi isolated from pine cones represent a species of *Fusicoccum* as well as *D. pinea*. DNA sequences for the ITS, 5.8S and β-tubulin gene regions confirmed the identification of *D. pinea* and showed that the fungus with the *Fusicoccum* anamorph is closet to *Botryosphaeria parva* or *B. ribis*. Analysis of SSR sequences showed that the *D. pinea* isolates from Ethiopia represent the A morphotype of the fungus, which is widely distributed in countries where pines are grown as exotics. Pathogenicity tests showed that isolates of both *Fusicoccum* sp. and *D. pinea* were able to cause lesions but the *D. pinea* isolates were most pathogenic.

## INTRODUCTION

In Ethiopia, *P. patula* Schiede & Deppe and *P. radiata* D. Don. have been used extensively for afforestation, having been introduced into the country more than 100 years ago. *P. patula* is still planted in Ethiopia whereas planting of *P. radiata* has been abandoned probably due to Dothistroma needle blight. Plantations of *Pinus* spp. cover approximately 30 000 ha, of which *P. patula* constitutes the major proportion (Anonymous 1994). Information available on the disease situation in these *P. patula* plantations is very limited. In a recent disease survey conducted in Ethiopia, a fungus resembling *Diplodia pinea* (Desm.) Kick (= *Sphaeropsis sapinea* (Fr.:Fr.) Dykco & Sutton) was isolated from cones of *P. patula* (Alemu, Roux & Wingfield 2003).

*Diplodia pinea* has a world-wide distribution and an extensive host range mainly among the conifers (Birch 1937, Eldridge 1961, Gibson 1979). This fungus is known from *Pinus* spp., wherever they are planted (Currie & Toes 1978, Gibson 1979) and it is known as an opportunistic pathogen (Marks & Minko 1969, Swart, Knox-Davies & Wingfield 1985). Stress, associated with environmental conditions and mechanical damage, predispose trees to disease caused by *D. pinea.* The fungus exists in pine cones and stems as an endophyte (Smith *et al.* 1996), where it lives in the plant without necessarily showing disease symptoms. Expression of symptoms commences when the trees are under stress (Swart *et al.* 1985, Smith *et al.* 1996, Stanosz *et al.* 1997), such as that caused by hail damage, drought or frost (Marks & Minko 1969, Bega *et al.* 1978, Swart *et al.* 1985, Swart & Wingfield 1991).

*Diplodia pinea* is associated with various disease symptoms (Puntinhalingam & Waterson 1970, Swart *et al.* 1985). These include shoot blight, shoot die-back, stem canker (Gilmour 1964, Marks & Minko 1969, Swart *et al.* 1985, Stanosz *et al.* 1997), root diseases (Wingfield & Knox-Davies 1980) and staining of wood (Da Costa 1955, Eldridge 1961). In South Africa, for example, *D. pinea* was found associated with seedling root rot (Wingfield & Knox-Davies 1980) and associated with hail damage (Swart *et al.* 1985). For this reason, *P. radiata* is not planted in summer rainfall areas in South Africa (Swart, Wingfield & Knox-Davies 1987, Swart & Wingfield 1991).

In the past, four morphotypes (A, B, C and I) have been described for *D. pinea* (Wang *et al.* 1985, Palmer, Swart & Wingfield 1987, De Wet *et al.* 2002, Hausner *et al.* 1999). Burgess, Wingfield & Wingfield (2001) showed that the I morphotype represents the anamorph of *Botryosphaeria obtusa* (Schw.) Shoemaker. In a recent study, De Wet *et al.* (2003) showed that the A and C morphotypes, once encompassed in *S. sapinea*, are closely related to each other and treated them in *D. pinea.* They also showed that the B morphotype of *S. sapinea* represent a new *Diplodia* sp. recently described as *D. scrobiculata* J. de Wet, B. Slippers & M. J. Wingfield (De Wet *et al.* 2003).

All of the abovementioned fungi, broadly treated as *D. pinea* are known to be pathogenic to a wide range of *Pinus* spp. (Wang *et al.* 1985, Palmer *et al.* 1987, De Wet *et al.* 2002). The C morphotype of *D. pinea* is more pathogenic than the A morphotype or *D. scrobiculata* (De Wet *et al.* 2002). The A morphotype is known to occur on a wide range of conifers world-wide (Morelet & Chandelier 1993, Smith & Stanosz 1995, Hausner *et al.* 1999, De Wet *et al.* 2000). *D. scrobiculata* is best known from the North Central United States and is only mildly pathogenic (Wang *et al.* 1985, Smith & Stanosz 1995, Stanosz, Swart & Smith 1999, De Wet *et al.* 2000, Burgess *et al.* 2001). The C morphotype has been reported only from Indonesia (De Wet *et al.* 2002).

Very little information is available regarding diseases affecting *P. patula* in Ethiopia. In a recent survey, Alemu *et al.* (2002), identified a fungus similar to *Armillaria fuscipes* Petch as a cause of mortality in certain plantation of *Pinus patula* in Western and South Western Ethiopia. Alemu *et al.* (2003), also reported the presence of *D. pinea* in cones of *P. patula* in Ethiopia, but these authors did not identify the morphotype of the *Diplodia* isolates. The aim of this study was to identify and characterise these isolates. This was based both on morphological characteristics and DNA based techniques. Pathogenicity of the isolates was also considered in greenhouse trials.

## MATERIALS AND METHODS

### *Fungal isolations and morphological characterisation*

During a disease survey conducted in 2000, cones were randomly collected from the forest floor in a *P. patula* plantation at Munessa Shashemene and isolations were made from them. The cones were opened and ~3 mm sections were taken from the pith of the cones. These were surface-sterilised with ethanol (100%), washed with sterile water, plated onto 2% MEA (20 g Biolab Malt Extract, 15 g Biolab Agar) and incubated at 25 °C. Isolates with dark grey mycelium, typical of *D. pinea* were selected and transferred to MEA in Petri dishes. Pure cultures were deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute, University of Pretoria.

Isolates were transferred to water agar (WA) (15 g Biolab Agar, 1L $H_2O$) with sterilised pine needles laid on the surface and incubated for three weeks to promote production of fruiting structures. Single conidial isolates were made from the resultant fruiting structures. This was achieved by spreading spore masses on MEA in a drop of sterile water and incubating plates for 24 hr at 25 °C. After 24 hr, single germinating spores were picked and transferred to MEA and incubated at 25 °C.

Conidia were mounted in lactophenol and examined using a Zeiss Axioskop light microscope. Ten spores were selected randomly from each isolate and the lengths and widths of the conidia were measured (Table 1).

### *DNA extraction*

Total genomic DNA was extracted from isolates selected to represent the different morphological groups (Table 2). Mycelium for DNA extraction was obtained by scraping the surface of the agar plates with a sterile scalpel and placing mycelial mats into 1.5 µl Eppendorf tubes. The mycelium was freeze dried and ground to a fine powder in liquid nitrogen using a pestle and mortar. The method of Raeder and Broda (1985) was used to extract DNA from the mycelium.

The DNA pellets were vacuum dried to remove excess ethanol and re-suspended in 50 µl sterilised water. RNase A (1mg/ml) (Roche Diagnostics, South Africa) was added to the DNA solution to remove the contaminating RNA and incubated at 37 °C in a water bath over night. The concentration of the DNA in the samples was detected by comparison with a standard on a 1% agarose gel, stained with ethidium bromide and visualised under UV light.

***PCR amplification***

The internal transcribed spacer (ITS) regions of the ribosomal RNA operon and the 5.8S gene were amplified using the polymerase chain reaction (PCR). PCR was conducted using primers ITS 1 (5' TCC GTA GGT GAA CCT GCG G '3) and ITS 4 (5' TCC TCC GCT TAT TGA TAT GC '3) (White *et al.* 1990). The PCR reaction mixture contained DNA polymerase (*Taq*, 2.5U/µl, Roche), 2.5 mM dNTP's, 10x PCR Buffer (10 mM Tris-HCl, HCl, 1.5 mM $MgCl_2$, 50 mM KCl), 0.15 mM of each primer, 1 µl of DNA and 37 µl sterilised water to make up a final volume of 50 µl. Denaturation was performed at 96 °C for 1 min. This was followed by 35 cycles of primer annealing at 55 °C for 30 s, chain elongation at 72 °C for 1 min and denaturation at 92 °C for 1 min. Final chain elongation was carried out at 72 °C for 5 min.

The β-tubulin gene was partially amplified using the forward primer Bt2a (5' GGT AAC CAA ATC GGT GCT GCT TTC 3') and the reverse primer Bt2b (5' ACC CTC AGT GTA G TG ACC CTT GGC 3') (Glass & Donaldson 1995). The PCR reaction mix included DNA polymerase (*Taq*, 2.5U/µl), 2.5 mM dNTP's, 10x Buffer, 25 mM $MgCl_2$ supplied by the manufacturer, 0.15 mM of each primer, 1 µl of DNA and 37 µl sterilized water. PCR reaction conditions involved an initial denaturation at 94 °C for 1 min, which was followed by 40 cycles at 94 °C for 1 min, primer annealing at 51 °C for 30 s, chain elongation at 72 °C for 1 min and an additional chain elongation step at 72 °C for an additional minute.

***Morphotype determination***

Three sets of unlabelled Simple Sequence Repeat (SSR) markers (Burgers *et al.* 2001) were used in PCR reactions to determine the morphotype of the *D. pinea* isolates. The sets of the SSR primers used included SS7 (forward primer TB23 5' GAC AGA CAT CTA GGC CCT GC 3' and reverse primer TB24 5' GAT CAG TCG GTC GAG ACG AG 3'), SS9 (forward primer TB37 5' CAG CGG TTT CAT TGA AAT GCC 3') and reverse primer TB38 5' GAC TTG TCT CCT ACC GAT TCC 3') as well as SS10 (forward primer TB41 5' GCC AAC CCT AAT GCT TCC ATG 3' and reverse primer TB42 5' CAG CGG CGA TTG CGG TAT GG 3') (Burgess *et al.* 2001). The PCR reactions and conditions used were similar to those described by Burgess *et al.* (2001). All PCR products were detected on 1% agarose gels stained with ethidium bromide and visualised under UV illumination.

***DNA sequencing***

All PCR products were purified using the High Pure PCR Product Purification Kit (QUIAGEN, GmbH, Hilden, Germany). The PCR products were sequenced in both directions using the Big Dye Cycle Sequencing kit with Amplitaq® DNA Polymerase, FS (Perkin-Elmer, Warrington, UK), according to the manufacturer's protocol, on an ABI PRISM$^{TM}$ 3100 DNA Autosequencer (Perkin-Elmer). Primers ITS 1 and ITS 4 were used for sequencing the ITS regions whereas the β-tubulin genes were sequenced using primers Bt2a and Bt2b. For the morphotype determination the SSR primers mentioned above were used.

***Sequence analysis***

The identity of the isolated fungi was determined by comparing the ITS rDNA sequences of the Ethiopian isolates against sequences obtained from GenBank [National Centre for Biotechnology Information (NCBI), US National Institute of Health Bethesda (http:/www.ncbi.nlm.gov/BLAST)]. Thereafter, the ITS rDNA and β-tubulin gene sequences of the isolates were combined and aligned manually using

PAUP 4.0b (Swofford 1998) against the sequence data set of *D. pinea* and *Botryoshpaeria* spp. obtained from De Wet *et al.* (2000) and Slippers *et al.* (2003) (Table 2). Gaps were inserted manually and were treated as missing data. The sequences were analysed using parsimony, with trees generated by heuristic searches with simple addition and Tree Bisection Reconstruction (TBR) branch swapping. Confidence intervals were determined using DNA BOOTSTRAP analysis (Bootstrap confidence intervals on DNA parsimony) (1000 replicates) (Felsenstein 1993). *Guignardia philoprina* (Ellis) Viala & Ravaz as well as *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl. were used as outgroup taxa in the phylogenetic analysis.

SSR sequences of the Ethiopian isolates were aligned against each other and with representative sequences of the three morphotypes of *D. pinea* obtained from De Wet *et al.* (2003). The sequences were analysed using parsimony, with trees generated by heuristic searches with simple addition and Tree Bisection Reconstruction (TBR) branch swapping. A phylogenetic tree showing the relationships of the isolates was obtained using the mid point rooting option.

***Pathogenicity trials***

Greenhouse inoculation trials using isolates CMW10717, 11240, 11246, 11250, 11252 and 11253 were conducted to evaluate the pathogenicity of the fungi in question. The greenhouse pathogenicity tests were conducted on 2-year-old *P. taeda* seedlings. Prior to inoculation, the trees were kept in the greenhouse for ten days to allow them to acclimatise to the environment. Six isolates were selected to represent the two conidial forms emerging from morphological and DNA sequence results.

Isolates used in the inoculation trials were grown on MEA for ten days. A 9 mm cork borer was used to wound the trees and expose the cambium. Mycelial plugs of equal size were placed in the wounds with mycelium facing the exposed cambium. Each isolate was inoculated onto 20 trees. Plugs of sterile MEA, were also inoculated onto 20 trees, to serve as controls. The inoculated wounds were covered with Parafilm (Pechiney Plastic Packageing, Chicago, USA) to prevent desiccation.

After six weeks, lesion lengths were measured to evaluate disease development on inoculated plants. One-way analysis of variance (ANOVA) using Statistica for Windows (StatSoft. Inc. 1995) was carried out to evaluate statistical differences between treatments. Mean variation was compared using Dunnett's T -test available in Statsitica for Windows (StatSoft. Inc. 1995).

## RESULTS

### *Fungal isolations and morphological characterisation*

Several dark coloured isolates were obtained from the pine cones collected from *P. patula* plantations at Munessa Shashemene. Cultures showed grey to black mycelial growth on MEA. These cultures also had a fluffy mycelial growth covering the whole surface of the Petri dish. No distinct variation was detected in mycelial growth of the isolated fungi. In total, twenty isolates, each from a different cone were retained for further study.

Of the 20 isolates inoculated onto water agar, only 15 produced fruiting structures on the pine needles and conidial morphology was determined for these. Evaluation of conidial morphology showed that two different fungi were present. One group had conidia similar to those of *Fusicoccum* spp. (Figure 1a) and the other group had conidia similar to those of *D. pinea* (Figure 1b). The walls of the conidia in the latter group were smooth and the conidia were aseptate. The lengths of the conidia that resembled *D. pinea* varied between 34 µm and 35 µm (Table 1). Their widths ranged from 16 µm to 17 µm. The length of the conidia that resembled *Fusicoccum* spp. ranged from 17 µm to 19 µm and their average widths ranged from 5.2 µm to 5.7 µm (Table 1). No sexual structures were found. Four of the 15 isolates examined had conidia similar to *D. pinea* whereas 11 of the isolates had conidia similar to *Fusicoccum* sp.

### *PCR amplification* and *DNA sequence comparisons*

A fragment size of approximately 500 base pairs (bp) was obtained when the ITS rDNA of *D. pinea* and the *Fusicoccum* isolates were amplified with primers ITS1 and

ITS4. Partial amplification of the β-tubulin gene of the *D. pinea* and *Fusicoccum* isolates with primers Bt2a and Bt2b produced a fragment size of approximately 400 bp.

The ITS sequence data of the *D. pinea* isolates were compared with sequences in Genbank. Sequences of *Diplodia* isolates showed that they were closely related to *D. pinea*. Similarly, when sequences of the *Fusicoccum* isolates were compared, they showed a high degree of homology to sequences of *Botryosphaeria ribis* Grossenb. & Dugg. (anamorph =*Fusicoccum ribis* Grossenb. & Dugg.) and *B. parva* (Pennycook & Samuels).

The ITS rDNA and β-tubulin sequences of the isolates used in this study were combined and aligned against each other and against sequence data obtained from De Wet *et al.* (2002) and Slippers *et al.* (2003). After alignment a total of 1029 characters was obtained (Figure 6). Of these 486 characters were constant whereas 278 characters were variable and parsimony uninformative, while 265 characters were parsimony informative. Phylogenetic analysis using parsimony produced 9 trees. The topologies of these trees were the same with only minor variation in arrangements within the groups. The phylogenetic tree (CI=0.886, RI=0.886) (Figure 2) showed that sequences of the Ethiopian isolates grouped together with *D. pinea* isolates with a 100% bootstrap value and the Ethiopian *Fusicoccum* isolates resided in a clade containing *B. parva* and *B. ribis* with 97% bootstrap value. The Ethiopian *Fusicoccum* isolates, however, formed their own subgroup of which the exact identity is unclear (Figure 2). This clade was supported by a bootstrap value of 100%.

***Morphotype determination***

To determine the morphotype of the *D. pinea* isolates a further sequence analysis was conducted using three SSR markers. Alignment of the combined SSR sequences of the *D. pinea* isolates with sequences obtained from De Wet *et al.* (2003) produced a total of 1051 base pairs (Figure 7). Sequence analysis using mid point rooting produced a single tree. This phylogenetic tree showed that the *D. pinea* isolates from Ethiopia group together with the 'A' morphotype of *D. pinea* (Figure 3).

***Pathogenicity trials***

All isolates tested in the two inoculation studies produced lesions on the two-year-old *P. taeda* seedlings (Figure 3). The mean lesion lengths produced by these isolates in the first inoculation trial was in a range of 30 mm to 56.8mm (Table 3) and in the second inoculation trial the lesion lengths produced were between 29.75 mm and 95.93 mm (Table 4). The *Diplodia* isolates produced the largest lesions compared to the *Fusicoccum* isolates. Both these fungi developed lesions that were significantly different (P>0.0001) compared to the lesions of the controls (Table 3, Figure 5). Analysis of variance showed statistically significant differences, in pathogenicity for the *D. pinea* and *Fusicoccum* sp., *D. pinea isolates* were more pathogenic than the *Fusicoccum* sp.

## DISCUSSION

*Pinus patula* is the only *Pinus* sp. currently planted in Ethiopia and is of great importance to the country. During a survey conducted in *P. patula* plantations in Ethiopia, *D. pinea* was isolated from pine cones (Alemu *et al.* 2003). The results of the present study showed that two fungal species are associated with *P. patula* cones in Ethiopia. Based on morphological characteristics and sequence analysis, they were identified as a *Fusicoccum* sp. and *D. pinea*. This is the first report defining the morphotype of *D. pinea* and the presence of a *Botryosphaeria* sp. from *Pinus* spp. in Ethiopia. Our results, although preliminary, suggests that in contrast to other countries, the *Botryosphaeria* sp. is more commonly associated with *P. patula* cones than *D. pinea*.

Results of DNA based comparisons confirmed identifications based on morphology. Use of sequence analysis of the ITS and β-tubulin sequence, however, could not assist in determining the morphotype of *D. pinea* in Ethiopia. Further sequence analysis using SSR markers, however, showed that the *D. pinea* obtained from cones of *P. patula* from plantations at Munessa Shashemene in Ethiopia, belong to the A morphotype. De Wet *et al.* (2003) used SSR sequences to determine the relationships

of the morphotypes of *Diplodia*. The A morphotype has been frequently found associated with seed and seed chaff (Anderson, Belcher & Miller 1984), indicating the endophytic nature of the fungus. The A morphotype is also the most widely distributed morphotype of *D. pinea* (Wang *et al.*, 1985, De wet *et al.* 2000). Its presence in Ethiopia is, therefore, not surprising.

Identification of the *Fusicoccum* isolates to species level, was not possible using only conidial morphology. Evaluation of the ITS and β-tubulin sequences, however, showed that the *Fusicoccum* sp. associated with *P. patula* in Ethiopia is closely related to *B. ribis* and *B. parva*. Several *Fusicoccum* spp. are known anamorphs of *Botryosphaeria* spp. (Sutton 1980, Pennycook & Samuels 1985). *Botryosphaeria* spp. are also known as opportunistic wound and stress related pathogens and as symptomless endophytes on several hosts (Smith *et al.* 1996). It has been shown that *Botryosphaeria* spp. cause die-back and cankers on a wide range of woody plants including *Eucalyptus* spp. (Smith, Kemp & Wingfield 1994). In Hawaii, *B. dothidea* has been found associated with wilting and dying *P. taeda* and *P. elliottii* Engelm. (Hodges 1983). The importance of the *Fusicoccum* sp. in pine plantations of Ethiopia, however, needs further investigation.

The results of the greenhouse inoculation studies showed that both *D. pinea* and the *Fusicoccum* sp. are pathogenic to *P. taeda*. The *D. pinea* isolates, however, produced larger lesions than those of the *Fusicoccum* sp. The A morphotype of *D. pinea* has been shown to be highly pathogenic to several *Pinus* spp. (Wang *et al.* 1985, Palmer *et al.* 1987). In Swaziland symptoms similar to those of *D. pinea* were observed on *P. elliottii* Englem and *P. taeda* (Wingfield & Knox-Davies 1980) suggesting that *P. taeda* is susceptible to *D. pinea* infection. This was confirmed in our study where *P. taeda* was used as a substitute for *P. patula*, due to the lack of available trees.

The occurrence of *D. pinea* in Ethiopian *P. patula* plantations could have a serious impact on the management, utilisation and future development of *P. patula*. It has been shown that *D. pinea* was introduced into several countries, apparently with seeds (Burgess *et al.* 2001, Smith *et al.* 1996). It is therefore, essential to manage future introductions of pine seed into Ethiopia to minimize the risks of introduction of the

other morphotypes of *D. pinea*. For example, the C morphotype of this fungus is considerably more pathogenic than isolates of the A morphotype (De Wet *et al.* 2002). Multiple introductions increase the clonal diversity and risk of disease from this pathogen. Species site matching and selection for disease resistance have to be considered to minimise severe damage from *D. pinea*. The importance of the *Fusicoccum* sp. in *P. patula* plantation also needs further investigation.

## REFERENCES

Alemu Gezahgne, Coetzee, M. P. A., Roux, J., & Wingfield, M. J. (2002) Armillaria root rot in Ethiopia. *South African Journal of Science* **98**: IV. (Abstract).

Alemu Gezahgne, Roux, J. & Wingfield, M. J. (2003) Diseases of Exotic plantation *Eucalyptus* and *Pinus* species in Ethiopia. *South African Journal of Science* **99**: 29-33.

Anderson, R. L., Belcher, E. & Miller, T. (1984) Occurrence of seed fungi inside slash pine seeds produced in seed orchards in the United States. *Seed Science and Technology* **12**: 795-799.

Anonymous (1994) Ethiopian Forestry Action Plan Final report, vol. I, pp 14 and Vol. II, pp 34. Addis Ababa, Ethiopia.

Bega, R. V., Smith, R. S., Martinez, A. P. & Davis, C. I. (1978) Severe damage to *Pinus radiata* and *P. pinaster* by *Diplodia pinea* and *Lophodermium* spp. on Molokai and Lanai in Hawaii. *Plant Disease Reporter* **62**: 329-331.

Birch, T. T. C. (1937) *Diplodia pinea* in New Zealand. *Review of Applied Mycology* **16**: 148.

Burgess, T., Wingfield, B. D. & Wingfield, M. J. (2001) Comparison of genotypic diversity in native and introduced populations of *Sphaeropsis sapinea* isolated from *Pinus radiata*. *Mycological Research* **105**: 1331-1339.

Currie, D. & Toes, E. (1978) Stem volume loss due to severe *Diplodia* infection in a young *Pinus radiata* stand. *New Zealand Journal of Forestry* **23**: 143-148.

Da Costa (1955) Effects of blue stain on the strength of *Pinus radiata*. *CSIRO Forest Products Newsletter* **209**, Australia.

De Wet, J., Wingfield, M. J., Coutinho, T. A. & Wingfield B. D. (2000) Characterisation of *Sphaeropsis sapinea* isolates from South Africa, Mexico, and Indonesia. *Plant Disease* **84**: 151-156.

De Wet, J., Wingfield, M. J., Coutinho, T. A. & Wingfield B. D. (2002) Characterisation of the 'C' morphotype of the pine pathogen *Sphaeropsis sapinea*. *Forest Ecology and Management* **161**: 181-188.

De Wet, J., Burgess, T., Slippers, B., Preisig, O., Wingfield, B. D. & Wingfield, M. J. (2003) Multiple gene genealogies and microsatelite markers reflect relationships between morphotypes of *Sphaeropsis sapinea* and distinguish a new species of *Diplodia*. *Mycological Research* (Submitted).

Eldridge, K. J. (1961) Significance of *Diplodia pinea* in plantations. *Review of Applied Mycology* **41**: 339

Felsentein, J. (1993) DNABOOT-Bootstrap Confidence Interval on DNA parsimony. 3.1. Seattle, University of Washington DC.

Gibson, I. A. S. (1979) *Diseases of trees widely planted as exotics in the tropics and southern hemisphere.* Part II. The genus *Pinus*. Commonwealth Forestry Institute, University of Oxford, Oxford UK.

Gilmour, J. W. (1964) Survey of Diplodia whorl canker in *Pinus radiata*. *New Zealand Forestry Service Research Leaflet* **5**, pp 4.

Glass, N. L. & Donaldson, G. C. (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* **78**: 343-350.

Hausner, G., Hopkin, A. A., Davis, C. N. & Reid, J. (1999) Variation in culture and rDNA among isolates of *Sphaeropsis sapinea* from Ontario and Manitoba. *Canadian Journal of Plant Pathology* **21**: 256-264.

Hodges (1983) Pine mortality in Hawaii, USA associated with *Botryosphaeria dothidea*. *Plant Disease* **67**: 555-556.

Marks, G. C. & Minko, G. (1969) The pathogenicity of *Diplodia pinea* to *Pinus raditata* D. Don. *Australian Journal of Botany* **17**: 1-12.

Morelet, P. M. & Chandelier, P. (1993) A cause of Variability in *Sphaeropsis sapinea*. *European Journal of Forest Pathology* **23**: 317-320.

Palmer, M. A., Swart, E. L. & Wingfield, M. J. (1987) Variation among isolates of *Sphaeropsis sapinea* in the north central United States. *Phytopathology* **77**: 944-948.

Pennycook, S. R. & Samuels, G. J. (1985) *Botryosphaeria* and *Fusicoccum* species associated with ripe fruit rot of *Actinidia deliciosa* (Kiwifruit) in New Zealand. *Mycotaxon* **24**: 445-448.

Punithalingam, E. & Waterson, J. M. (1970) *Diplodia pinea*. CMI description of plant pathogenic fungi and Bacteria 273. Commonwealth Mycological Institute and Association of Applied Biologist, Kew, Surry, England.

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

Slippers, B., Crous, P. W., Denman, S., Coutinho, T. A., Wingfield, B. D. & Wingfield M. J. (2003) Combined multiple gene genealogies and phenotypic characters differentiating several species previously identified as *Botryosphaeria dothidea*. *Mycologia* (In press).

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

Smith, D. R. & Stanosz, G. R. (1995) Confirmation of two distinct populations of *Sphaeropsis sapinea* in the north central United states using RAPDs. *Phytopathology* **85**: 699-704.

Smith, H., Wingfield, M. J., Crous, P. W. & Coutinho, T. A. (1996) *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. *South African Journal of Botany* **62**: 86-88.

Stanosz, G. R., Smith, D. R., Guthmiller, M. R. & Stanosz, J. C. (1997) Persistence of *Sphaeropsis sapinea* on or in asymptomatic stems of red pine nursery seedlings. *Mycologia* **89**: 525-530.

Stanosz, G. R., Swart, W. J. & Smith, D. R. (1999) RAPD marker and isozyme characterization of *Sphaeropsis sapinea* from diverse coniferous hosts and locations. *Mycological Research* **103**: 1193-1202.

Sutton, B. C. (1980) The Coelomycetes. Commonwealth Mycological Institute. Kew.

Swart, W. J., Knox-Davies, P. J. & Wingfield, M. J. (1985) *Sphaeropsis sapinea*, with special reference to its occurrence on *Pinus* spp. in South Africa. *South African Forestry Journal* **35**: 1-8.

Swart, W. J. & Wingfield, M. J. (1991) Biology and control of *Sphaeropsis sapinea* in *Pinus* species in South Africa. *Plant Disease* **75**: 761-766.

Swart, W. J., Wingfield, M. J. & Knox-Davies, P. J. (1987) Factors associated with *Sphaeropsis sapinea* infection of pine trees in South Africa. *Phytophylactica* **19**: 505-510.

Swofford, D. L. (1998) PAUP*. *Phylogenetic Analysis Using Parsimony*. (* and other methods). Version 4.0. Beta version. Sinauer Associates. Sunderland, Massachusetts.

Wang, C-G., Blanchette, R. A., Jackson, W. A. & Palmer, M. A. (1985) Difference in conidial morphology among isolates of *Sphaeropsis sapinea*. *Plant Disease* **69**: 838-841.

White, T. J., 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*. ( M. A. Innis, D. H. Gelfand, J. J. Snisky & J. W. White eds.). San Diego: Academic Press.

Wingfield, M. J. & Knox-Davies, P. S. (1980) Association of *Diplodia pinea* with a root disease of pines in South Africa. *Plant Disease* **64**: 221-223.

**Table 1.** Average conidial lengths of fungal isolates obtained from *Pinus patula* cones.

| Isolate No. | Species | Average Conidial Length (µm) | Average Conidial Width (µm) |
|---|---|---|---|
| CMW11249 | *Diplodia pinea* | 34.6 | 15.6 |
| CMW11250 | „ | 33.6 | 16.5 |
| CMW11252 | „ | 33.2 | 16.1 |
| CMW10717 | „ | 34.3 | 15.8 |
| CMW11240 | *Fusicoccum sp.* | 17.4 | 5.5 |
| CMW11241 | „ | 18.8 | 5.7 |
| CMW11242 | „ | 17.1 | 5.5 |
| CMW11243 | „ | 17.5 | 5.3 |
| CMW11244 | „ | 18.2 | 5.7 |
| CMW11245 | „ | 17.2 | 5.2 |
| CMW11246 | „ | 16.8 | 5.7 |
| CMW11247 | „ | 17.2 | 5.5 |
| CMW11248 | „ | 18.5 | 5.2 |
| CMW11251 | „ | 18.9 | 5.5 |
| CMW11253 | „ | 18.3 | 5.7 |

CMW numbers are those of the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria.

Values of conidial length and width are averages of 10 measurements.

**Tables 2.** Isolates used in the DNA sequence analyses

| Culture Nr. | Identity | Morhotype | Host | Origin | Collector |
|---|---|---|---|---|---|
| CMW189 | *Diplodia pinea* | B | *Pinus resinosa* | United States | M. A. Palmer |
| CMW190 | ,, | A | *P. banksiana* | United States | M. A. Palmer |
| CMW4876 | ,, | C | *P .patula* | Indonesia | M. J. Wingfield |
| CMW11250[a] | ,, | A | *P. patula* | Ethiopia | Alemu Gezahgne & Jolanda Roux |
| CMW11246[a] | ,, | A | *P. patula* | Ethiopia | Alemu Gezahgne & Jolanda Roux |
| CMW10717[a] | ,, | A | *P. patula* | Ethiopia | Alemu Gezahgne & Jolanda Roux |
| CMW4891 | *Lasiodiplodia theobromae* | - | | South Africa | W. A. Smith |
| CMW7780 | *B. dothidea* | - | *Fraxinus excelsior* | Switzerland | B. Slippers |
| CMW8000 | *B. dothidea* | - | *Prunus* sp. | Switzerland | B. Slippers |
| CMW10125 | *B. eucalyptorum* | - | *E. grandis* | S. Africa | H. Smith |
| CMW10126 | *B. eucalyptorum* | - | *E. grandis* | S. Africa | H. Smith |
| CMW992/3 | *F. luteum* | - | *Actinidia deliciosa* | New Zealand | G.J. Smuels |
| CMW9076 | *B. lutea* | - | *Malus X domestica* | New Zealand | S.R .Pennycook |
| CMW7772 | *B. ribis* | - | *Ribis* sp. | New York | B. Slippers/ G. Hudler |
| CMW7773 | *B. ribis* | - | *Ribis* sp. | New York | B. Slippers/ G. Hudler |
| CMW9071 | *B. parva* | - | *Ribis* sp. | Australia | M.J. Wingfield |
| CMW994 | *B. parva* | - | *Malus sylvestris* | New Zealand | G.J Samuels |
| CMW9077 | *B. parva* | - | *Actinidia deliciosa* | New Zealand | S.R. Pennycook |
| CMW9071 | *B. parva* | - | *Ribis* sp. | Australia | M.J. Wingfield |
| CMW10122 | *B. parva* | - | *E. grandis* | S. Africa | H. Smith |
| CMW11246[a] | *B. parva* | - | *Pinus patula* | Ethiopia | Alemu Gezahgne & Jolanda Roux |
| CMW10717[a] | *B. parva* | - | *Pinus patula* | Ethiopia | Alemu Gezahgne & Jolanda Roux |
| CMW7060 | *B. stevensii* | - | *Fraxinus ecelsior* | Netherlands | H. A. van der Aa |
| CMW7774 | *B. obtusa* | - | *Ripes* spp. | New York, USA | B. Slippers/G. Hudler |
| CMW7775 | *B. obtusa* | - | *Ripes* spp. | New York, USA | B. Slippers/G. Hudler |
| CMW10130 | *B. rhodina* | - | *Vitex donniana* | Uganda | J. Roux |
| CMW9074 | *B. rhodina* | - | *Pinus* sp. | Mexico | T. Burgess |
| CMW7063 | *Guignardia phyiloprina* | - | *Taxus baccata* | Netherlands | H.A. van der Aa |

[a]/ Sequences of the isolates from Ethiopia were obtained in this study. All other sequences are those from the studies of Slippers *et al.* (2003) and De Wet *et al.* 2000, 2003.

**Table 3**. Lesion lengths and confidence limits for trees inoculated with *D. pinea* and *Fusicoccum* sp. obtained from *Pinus patula* cones in Ethiopia.

| Isolates | Species | Trial 1 | | Trial 2 | |
|---|---|---|---|---|---|
| | | Mean Lesion Length (mm)[1] | 95% Confidence limits[1] | Mean Lesion Length (mm)[2] | 95% Confidence limits[2] |
| CMW11250 | *Diplodia pinea* | 56.80[a] | 48.819-64.781 | 38.45[bc] | 30.873-46.026 |
| CMW10717 | ,, | 54.30[a] | 46.319-62.281 | 51.3[b] | 43.732-58.876 |
| CMW11252 | ,, | 48.65[ab] | 40.669-56.631 | 95.93[a] | 87.185-104-681 |
| CMW11246 | *Fusicoccum* sp. | 38.85[b] | 30.869-46.831 | 52.30[b] | 44.723-59.876 |
| CMW11240 | ,, | 37.90[b] | 29.918-45.881 | 37.40[bc] | 29.823-44.976 |
| CMW11253 | ,, | 30.00[bc] | 22.019-37.981 | 29.75[c] | 22.173-37.326 |
| CONTROL | — | 14.35[c] | 6.369-22.331 | 11.95[d] | 4.373-19.526 |

Each value is the average of 20 measurements.

Means followed by the same letters are not significantly different from each other at P=<0.05 significance level.

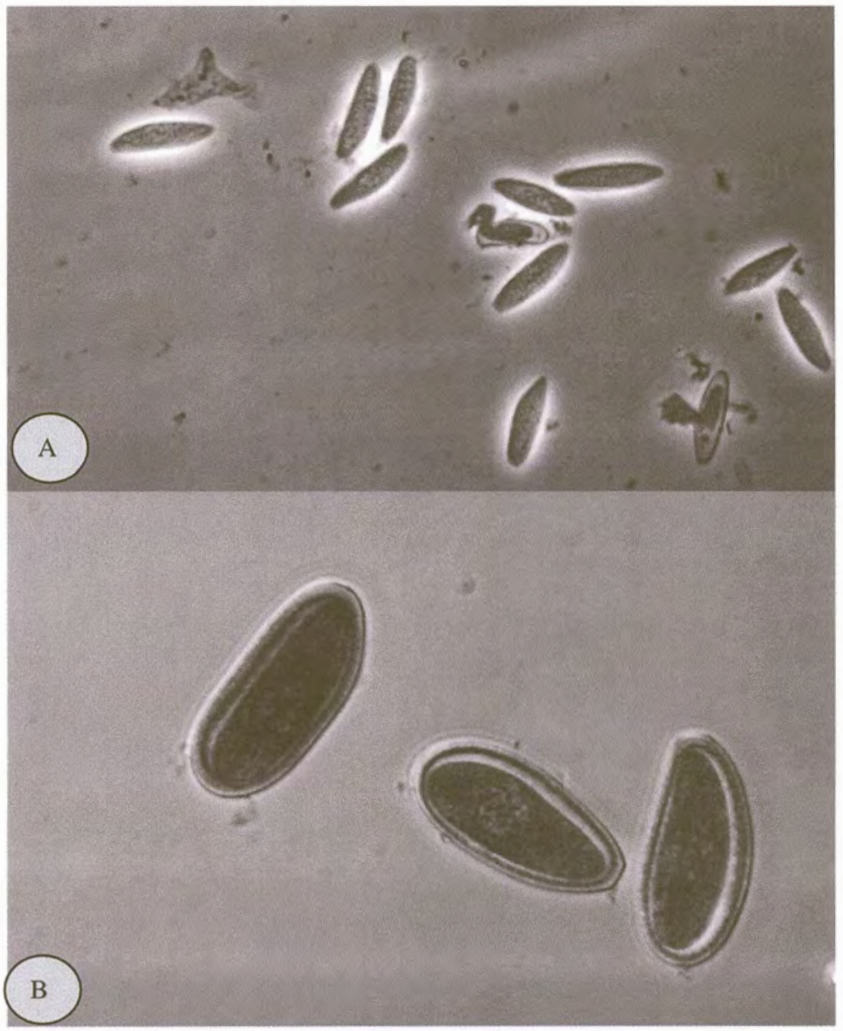



**Figure 1.** Condial morphology for fungal isolates from *P. patula* cones in Ethiopia. (a) *Fusicoccum* sp. (b) *D. pinea.*

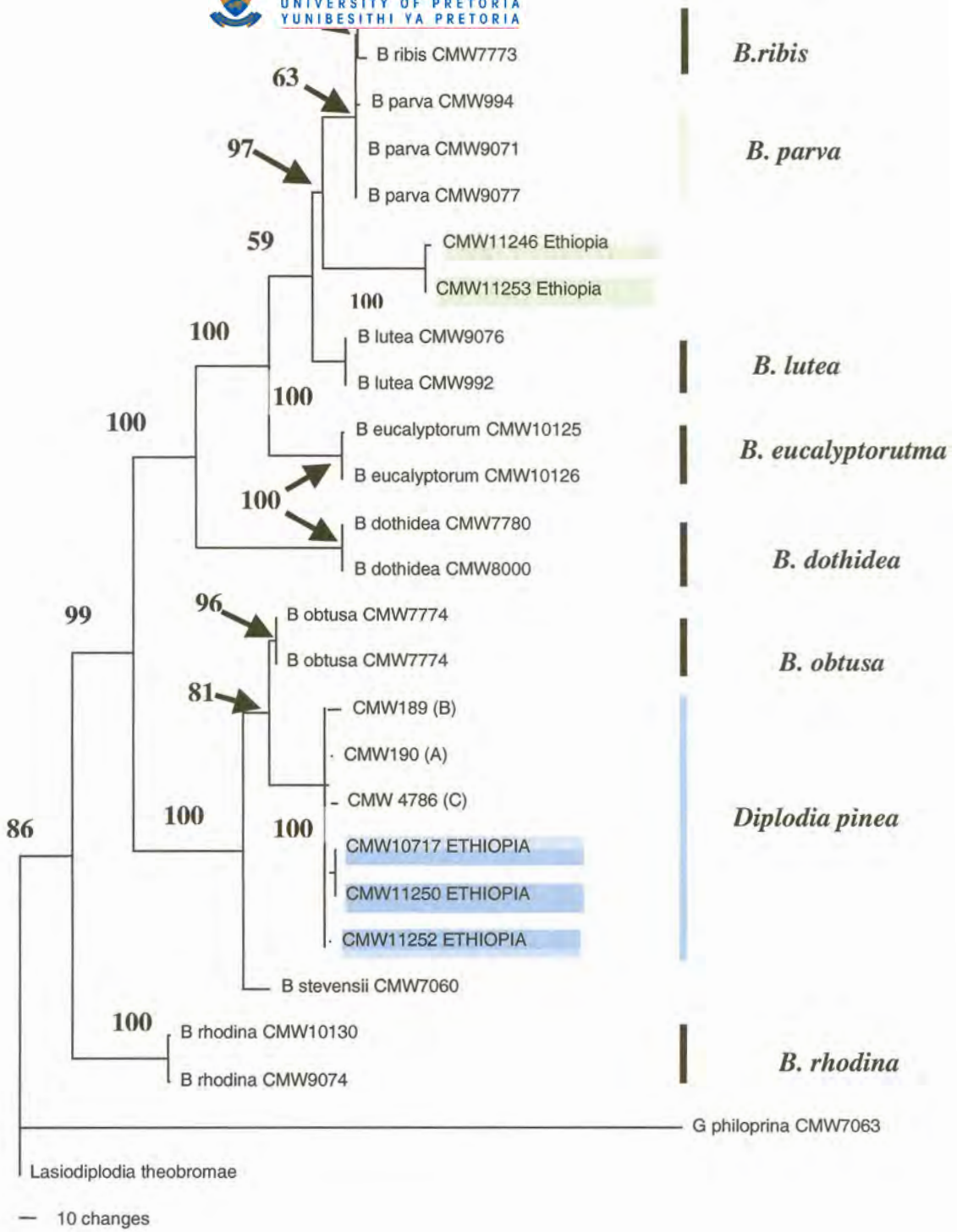



**Figure 2.** Phylogenetic tree of the combined sequences of the ITS rDNA and β-tubulin gene of *Diplodia* and *Fusicoccum* sp. Bootstrap values are shown at each branch.

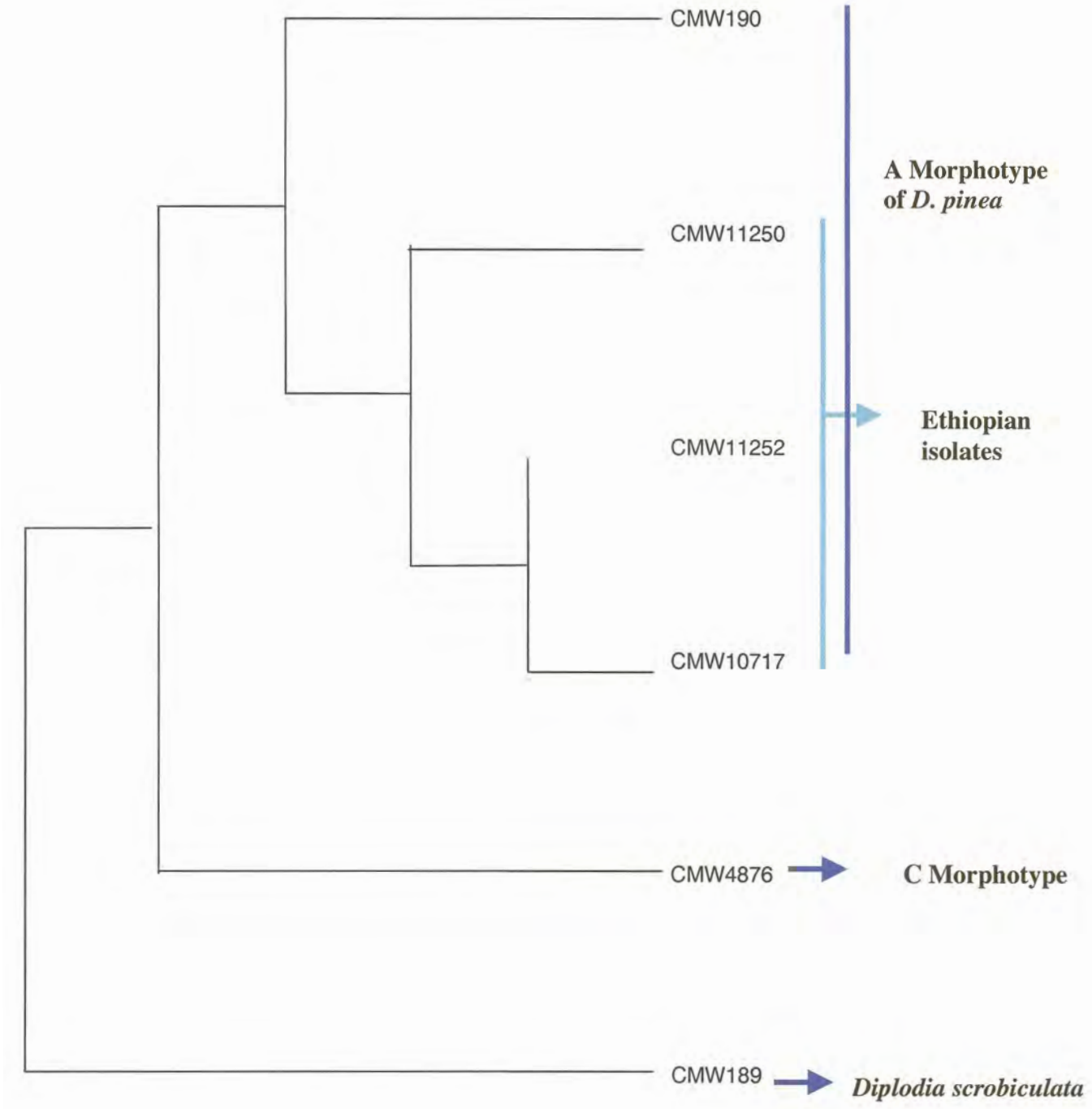



**Figure 3**. Phylogram of sequence data generated using SSR markers separating the A and C morphotypes of *Diplodia pinea*.


**Figure 4.** Lesion development on *Pinus tadea* in the greenhouse inoculation trial (a) Control, (b) Lesion from *Fusicoccum* isolate, and (c) Lesion from *D. pinea*.

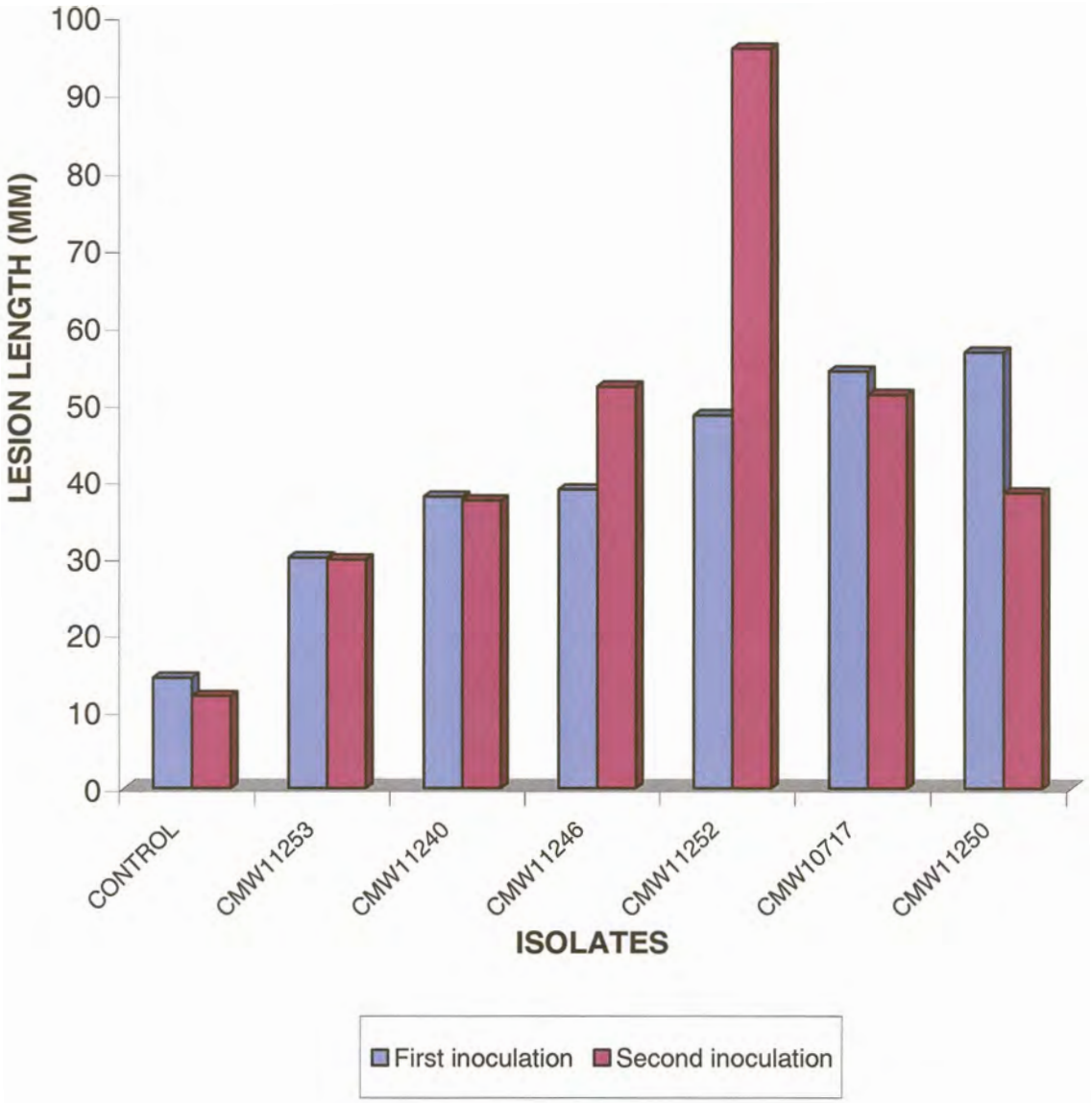



**Figure 5.** Mean lesion lengths of *Diplodia pinea* and *Fusicoccum* sp. in greenhouse inoculation trials. Isolates CMW11240, 11246 and 11253 represent *Fusicoccum* sp. whereas CMW10717, 11250 and 11252 represent *Diplodia pinea* isolates.

**Figure 6**. Alignment of combined sequences of The ITS rDNA and β-tubulin genes of *Fusicoccum* sp. and *D. pinea*. (-)= gaps, (.)= Homologous nucleotides, (N)= Unknown bases.

```
                                  10         20         30         40         50         60         70
B_ribis_CMW7772                   NNNNNACCAA A-TCGGTGCT GCTTTCTGGT TTGTTGCCAA AACACTCCCG CTCCCGCGCC CCC--GCTGA
B_ribis_CMW7773                   NNNNN..... .......... .......... .......... .......... .......... ..........
B_parva_CMW994                    NNNNN..... .......... .......... .......... .......... .......... ..........
B_parva_CMW9071                   NNNNNNNNNN N......... .......... .......... .......... .......... ..........
B_parva_CMW9077                   NNNNNNNNNN .......... .......... .......... .......... .......... ..........
B_eucalyptorum_CMW10125           NNNNN..... .......... .......... .......... .......T.. ....T..... ..........
B_eucalyptorum_CMW10126           NNNNNNNNNN .......... .......... .......... .......T.. ....T..... ..........
B_lutea_CMW9076                   NNNNN..... .......... .......... .......... ......G... .......... ..........
B_lutea_CMW992                    NNNNN..... .......... .......... .......... ......G... .......... ..........
B_dothidea_CMW7780                NNNNN..... .......... .......... .......... .....--... .......... .........A.
B_dothidea_CMW8000                NNNNN..... .......... .......... .......... .....--... .......... .........A.
B_rhodina_CMW10130                NNNNN..... .......... .......... .......... ........T. ....T..... ...C......
B_rhodina_CMW9074                 NNNNN..... .......... .......... .......... ........T. ....T..... ...C......
B_obtusa_CMW7774                  NNNNN..... .......... .......... .......... .......... ..G....... ...C......
B_obtusa_CMW7774                  NNNNN..... .......... .......... .......... .......... ..G....... ...C......
B_stevensii_CMW7060               NNNNN..... .......... .......... .......... .......... .AG....... ...CC.....
CMW11246_Ethiopia                 NNNNN..... .......... .......... .......... .......... .......... ..........
CMW11253_Ethiopia                 NNNNN..... .......... .......... .......... .......... .......... ..........
CMW189 (B)                        TGGTA..... .A........ .......... .......... .......... ..G....... ...C......
CMW190 (A)                        TGGTA..... .......... .......... .......... .......... ..G....... ...C......
CMW4786(C)                        TGGTA..... .......... .......... .......... ........T. ..G....... ...C......
CMW10717_ETHIOPIA                 TGGTA..... .......... .......... .......... .......... ..G....... ...C......
CMW11252_ETHIOPIA                 TGGTA..... .......... .......... .......... .......... ..G....... ...C......
CMW11250_ETHIOPIA                 TGGTA..... .......... .......... .......... .......... ..G....... ...C......
LasioBt2                          NNNNN..... .......... .......... .......... ........T. ..-....... ...C......
G_philoprina_CMW7063              NNNNN..... .......... .........- ---------- ---------- ---------- ---..------
```

```
                                  80         90         100        110        120        130        140
B_ribis_CMW7772          CGCGAATCGA CACCACAGGC AGACCATTTC CGGCGAGCAC GGCCTGGACG GCTCTGGCGT GTGAGTCTGC
B_ribis_CMW7773          .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW994           .......... .......... .......... T......... .......... .......... ..A.......
B_parva_CMW9071          .......... .......... .......... T......... .......... .......... ..A.......
B_parva_CMW9077          .......... .......... .......... T......... .......... .......... ..A.......
B_eucalyptorum_CMW10125  .......... .....T.... .......... T..T..A... .......... .......... ..A.......
B_eucalyptorum_CMW10126  .......... .......... .......... T..T..A... .......... .......... ..A.......
B_lutea_CMW9076          .......... ....G..... .......... T......... .......... .......... ..A.......
B_lutea_CMW992           .......... ....G..... .......... T......... .......... .......... ..A.......
B_dothidea_CMW7780       .......... .......... .......C.. .......... .......... .......... ..A.......
B_dothidea_CMW8000       .......... .......... .......C.. .......... .......... .......... ..A.......
B_rhcdina_CMW10130       ..-...G... .....T.... .......C.. .......... ........T. ....C..T.. ..A...G...
B_rhcdina_CMW9074        ..-...G... .....T.... .......C.. .......... ........T. ....C..T.. ..A...G...
B_obtusa_CMW7774         ...C...... .......... ....T..C.. T......... .......... ....C..... ..A...T...
B_obtusa_CMW7774         ...C...... .......... ....T..C.. T......... .......... ....C..... ..A...T...
B_stevensii_CMW7060      .C.C...... ...-...... .......C.. T......... .......... .......... ..A...G...
CMW11246_Ethiopia        .......... .......... .......... T......... .......... .......A.. ..A.......
CMW11253_Ethiopia        .......... .......... .......... T......... .......... .......... ..A.......
CMW189 (B)               ...C...... .......... ....T..C.. T......... ........T. ....C..... ..A...T...
CMW190 (A)               ...C...... .......... ....T..C.. T......... .......... ....C..... ..A...T...
CMW4786 (C)              ...C...... ..-....... ....T..C.. T......... .......... ....C..T.. ..A...T...
CMW10717_ETHIOPIA        ...C...G.. .......... ....T..C.. T......... .......... ....C..... ..A...T...
CMW11252_ETHIOPIA        ...C...... .......... ....T..C.. T......... .......... ....C..... ..A...T...
CMW11250_ETHIOPIA        ...C...G.. .......... ....T..C.. T......... .......... ....C..... ..A...T...
LasioBt2                 ..G-..G... .....T.... .......C.. .......... ........T. ....C..T.. ..A...G...
G_philoprina_CMW7063     ---------- -------... .......C.. T......... .....C...A ..AA...T.. C.ACA---..
```

```
                                150        160        170        180        190        200        210
B_ribis_CMW7772          GCCGTTTC-- -CCGCGC--- --GAA--TGG CAATGGCTGA CCC-GTAGCA GC------TA CAATGGCACC
B_ribis_CMW7773          .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW994           .......... .......... .......... .......... .....C.... .......... ..........
B_parva_CMW9071          .......... .......... .......... .......... .....C.... .......... ..........
B_parva_CMW9077          .......... .......... .......... .......... .....C.... .......... ..........
B_eucalyptorum_CMW10125  .......... .....T.... .......... .......... .....C.A.. .......... ..........
B_eucalyptorum_CMW10126  .......... .....T.... .......... .......... .....C.A.. .......... ..........
B_lutea_CMW9076          ........TT .--........ .......... .......... .....C.... .......... ..........
B_lutea_CMW992           ........TT .--........ .......... .......... .....C.... .......... ..........
B_dothidea_CMW7780       AT.-A...TC A--...-TGG GA...CA.-- .....A..-. AA.T...... .......... ..........
B_dothidea_CMW8000       AT.-A...TC A--...-TGG GA...CA.-- .....A..-. AA.T...... .......... ..........
B_rhodina_CMW10130       ...-..CTCC G.....-... ..---CA... ....C..... .-.T...... .......... .........T
B_rhodina_CMW9074        ...-..CTCC G.....-... ..---CA... ....C..... .-.T...... .......... .........T
B_obtusa_CMW7774         ..T..C.TT. G......TC. ..---....- ....C..... ...TTG-... .......... ..........
B_obtusa_CMW7774         ..T..C.TT. G......TC. ..---....- ....C..... ...TTG-... .......... ..........
B_stevensii_CMW7060      ..T..C.TT. G......TG. ..---....- ....C..... .T.TCG-... .......... ..........
CMW11246_Ethiopia        ....--.TTC .......... .......... .......--- ---T.ACC.- ..AGCAGC.. .........A
CMW11253_Ethiopia        ....--.TTC .......... .......... .......--- ---T.ACC.- ..AGCAGC.. ..........
CMW189 (B)               ..T..C.TT. G......... ..---TC.-. ....C..... ...TT.-... .......... ..........
CMW190 (A)               ..T..C.TT. G......... ..---TC.-. ....C..... ...TTG-... .......... ..........
CMW4786 C)               ..T..C.TT. G......... ..---TC.-. ....C..... ...TTG-... .......... ..........
CMW10717_ETHIOPIA        ..T..C.TT. G......... ..---TC.-. ....C..... ...TTG-... .......... ..........
CMW11252_ETHIOPIA        ..T..C.TT. G......... ..---TC.-. ....C..... ...TTG-... .......... ..........
CMW11250_ETHIOPIA        ..T..C.TT. G......... ..---TC.-. ....C..... ...TTG-... .......... ..........
LasioBt2                 ...-..CT.. .....CGCGC AT---..-.. ....C..... ..-.TG--T. ..AGC..... .........T
G_philoprina_CMW7063     --------.. .------... ..---..--- ---------- ---.------ --.......-- ----..T...
```

```
                                220        230        240        250        260        270        280
B_ribis_CMW7772                 TCCGACCTGC AGCTCGAGCG CATGAACGTC TACTTCAACG AGGTACTCTC TC-ACTAATT GCACAAACAC
B_ribis_CMW7773                 .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW994                  .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW9071                 .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW9077                 .......... .......... .......... .......... .......... .......... ..........
B_eucalyptorum_CMW10125         ........C. .......... .......... .......... .......... .........C A.........
B_eucalyptorum_CMW10126         ........C. .......... .......... .......... .......... .........C A.........
B_lutea_CMW9076                 ........C. .......... .......... .......... .......... .........C ....G.....
B_lutea_CMW992                  ........C. .......... .......... .......... .......... .........C ....G.....
B_dothidea_CMW7780              ..G.....T. .......... .......... ..T....... .......... .......... AG........
B_dothidea_CMW8000              ..G.....T. .......... .......... ..T....... .......... .......... AG........
B_rhodina_CMW10130              ..G.....C. .A..G..... .......... .......... .......... ..C.-..... AG........
B_rhodina_CMW9074               ..G.....C. .A..G..... .......... .......... .......... ..C.-..... AG........
B_obtusa_CMW7774                ........C. ....G..... .......... .......... .......... .-.....G.. AG........
B_obtusa_CMW7774                ........C. ....G..... .......... .......... .......... .-.....G.. AG........
B_stevensii_CMW7060             ........C. .A..G..... .......... .......... .......... .TG-...... AG........
CMW11246_Ethiopia               .......... .......... .........N .......... .......... .-C....... ..........
CMW11253_Ethiopia               .......... .......... .......... .......... .......... .-C....... ..........
CMW189  (B)                     ........C. ....G..... .......... .......... .......... .-.....G.. AG........
CMW190  (A)                     ........C. ....G..... .......... .......... .......... .-.....G.. AG........
CMW4786 (C)                     ........C. ....G..... .......... .......... .......... .-.....G.. AG........
CMW10717_ETHIOPIA               ........C. ....G..... .......... .......... .......... .-.....G.. AG........
CMW11252_ETHIOPIA               ........C. ....G..... .......... .......... .......... .-.....G.. AG........
CMW11250_ETHIOPIA               ........C. ....G..... .......... .......... .......... .-.....G.. AG........
LasioBt2                        ..G.....C. .A..G..... .......... .......... .......... ..C.-..... AG........
G_philoprina_CMW7063            .....G..C. .......... .......... .......... .....-.G-- ..C.GACCGA ..TTC.CATA
```

```
                                290        300        310        320        330        340        350
B_ribis_CMW7772          GTAAAGTATG GCAATCTTCT GAACG----- -CGCAGCAGG CGTC---C-- AACAACAAGT ACGTTCCTCG
B_ribis_CMW7773          .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW994           .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW9071          .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW9077          .......... .......... .......... .......... .......... .......... ..........
B_eucalyptorum_CMW10125  A......... .......... .......... .......... .......... .......... ..........
B_eucalyptorum_CMW10126  A......... .......... .......... .......... .......... .......... ..........
B_lutea_CMW9076          .......... .......... .......... .......... .......-G. .......... ..........
B_lutea_CMW992           .......... .......... .......... .......... .......-G. .......... ..........
B_dothidea_CMW7780       .......... .......... .......... .......... .......... .......... ..........
B_dothidea_CMW8000       .......... .......... .......... .......... .......... .......... ..........
B_rhodina_CMW10130       .......... .......... .......... .......... .......... .......... ..........
B_rhodina_CMW9074        .......... .......... .......... .......... .......... .......... ..........
B_obtusa_CMW7774         .......... .......... .......... .......... .A........ .....T.... ..........
B_obtusa_CMW7774         .......... .......... .......... .......... .A........ .....T.... ..........
B_stevensii_CMW7060      .......... .......... .......... .......... .T........ .......... ..........
CMW11246_Ethiopia        .......... .......... .......... .......... .......... .......... ..........
CMW11253_Ethiopia        .......... .......... .......... .......... .......... .......... ..........
CMW189  (A)              .C........ .......... .......... .......... .-.T....G. .....T.... ..........
CMW190  (B)              .......... .......... .......... .......... .-.T....G. .....T.... ..........
CMW4786 (C)              .......... .......... .......... .......... .-.T....G. .....T.... ..........
CMW10717_ETHIOPIA        .......... .......... .......... .......... .-.T....G. .....T.... ..........
CMW11252_ETHIOPIA        .......... .......... .......... .......... .-.T....G. .....T.... ..........
CMW11250_ETHIOPIA        .......... .......... .......... .......... .-.T....G. .....T.... ..........
LasioBt2                 .......... .......... .......... .......... .......... .......... ..........
G_philoprina_CMW7063     T.CTG..GAT TTTCATC.TC TG..-CGAGA TTTGG.T.TA G.C.TCCGGC .....---.. .T........
```

```
                                360        370        380        390        400        410        420
B_ribis_CMW7772          TGCCGTCCTC GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG CCAGCTCTTC
B_ribis_CMW7773          .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW994           .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW9071          .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW9077          .......... .......... .......... .......... .......... .......... ..........
B_eucalyptorum_CMW10125  .......... ..T....... .......... .......... .......... .......... ..........
B_eucalyptorum_CMW10126  .......... ..T....... .......... .......... .......... .......... ..........
B_lutea_CMW9076          .......... .......... .......... .......... .......... .......... ..........
B_lutea_CMW992           .......... .......... .......... .......... .......... .......... ..........
B_dothidea_CMW7780       .......... .......... .......... G......... .......... .......... ......T...
B_dothidea_CMW8000       .......... .......... .......... G......... .......... .......... ......T...
B_rhodina_CMW10130       ...T...... .......... .......... .......... .......... .......... ..........
B_rhodina_CMW9074        ...T...... .......... .......... .......... .......... .......... ..........
B_obtusa_CMW7774         ...T...... ..T....... .......... .......... .......... .......... ..........
B_obtusa_CMW7774         ...T...... ..T....... .......... .......... .......... .......... ..........
B_stevensii_CMW7060      ...T...... ..T....... .......T.. .......... .....T.... .......... ..........
CMW11246_Ethiopia        .......... .......... .......... ........T. .......... .......... ..........
CMW11253_Ethiopia        .......... .......... .......... .......... .......... .......... ..........
CMW189  (A)              ...T...... ..T....... .......... .......... .......... .......... ..........
CMW190  (B)              ...T...... ..T....... .......... .......... .......... .......... ..........
CMW4786 (C)              ...T...... ..T....... .......... .......... .......... .......... ..........
CMW10717_ETHIOPIA        ...T...... ..T....... .......... .......... .......... .......NNN NNNNNNNNNN
CMW11252_ETHIOPIA        ...T...... ..T....... .......... .......... .......... .......... ..........
CMW11250_ETHIOPIA        ...T...... ..T....... .......... .......... .......... .......... ..........
LasioBt2                 ...T...... .......... .......... .......... .......... .......... ..........
G_philoprina_CMW7063     C..T...... .....T..T. .......T.. .......... .....T..T. .A..T..... ..........
```

```
                                430        440        450        460        470        480        490
B_ribis_CMW7772                 CGCCCTGACA ACTTCGTCTT CGGTCAGTCT GGCGCCGGTA ACAACTGGGA AGGATCATTA CCGAGTTGAT
B_ribis_CMW7773                 .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW994                  .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW9071                 .......... .......... .......NNN NNNNNNNNNN NNNNNNN... .......... ..........
B_parva_CMW9077                 .......... .......... .......... .......... .......... .......... ..........
B_eucalyptorum_CMW10125         .....C.... ....T..... ...C...... ..T....... ....T..... .......... .........C
B_eucalyptorum_CMW10126         .....C.... ....T..... ...C...... ..T....... ....T..... .......... .........C
B_lutea_CMW9076                 .....C.... ....T..... ...C...... ..T....... .......... .......... ..........
B_lutea_CMW992                  .....C.... ....T..... ...C...... ..T....... .......... .......... ..........
B_dothidea_CMW7780              .....C.... .......... .........C ..T....... .......... .......... ..........
B_dothidea_CMW8000              .....C.... .......... .........C ..T....... .......... .......... ..........
B_rhodina_CMW10130              .....C.... .......... ...C...... ..T....... .......... .......... .......--.
B_rhodina_CMW9074               .....C.... .......... ...C...... ..T....... .......... .......... .......--.
B_obtusa_CMW7774                ..T..C.... .......T.. ...C...... ..T....... .......... ..N....... .......--C
B_obtusa_CMW7774                ..T..C.... .......T.. ...C...... ..T....... .......... .......... .......--C
B_stevensii_CMW7060             ..T..C.... .......T.. ...C...... ..T....... .......... .......... .......--C
CMW11246_Ethiopia               .......... .......... .......... .......... .......... .......... ..........
CMW11253_Ethiopia               .......... .......... .......... .......... .......... .......... ..........
CMW189  (A)                     ..T..C.... .......T.. ...C...... ..T.....NN NNNNNNNNNN NNNNN..... .......--C
CMW190  (B)                     ..T..C.... .......T.. ...C...... ..T.....NN NNNNNNNNNN NNNNN..... .......--C
CMW4786 (C)                     ..T..C.... .......T.. ...C...... ..T.....NN NNNNNNNNNN NNNNN..... .......--C
CMW10717_ETHIOPIA               NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNN..... .......--C
CMW11252_ETHIOPIA               ..T..C.... .......T.. ...C...... ..T.....NN NNNNNNNNNN NNNNN..... .......--C
CMW11250_ETHIOPIA               ..T..C.... .......T.. ...C...... ..T.....NN NNNNNNNNNN NNNNN..... .......--C
LasioBt2                        .....C.... .......... ...C...... ..T.....NN NNNNNNNNNN NNNNN..... .......---
G_philoprina_CMW7063            .....C.... .......T.. .........C ..T..T..C. .......... .......... .......---
```

```
                                500        510        520        530        540        550        560
B_ribis_CMW7772                 TCGAGCTCCG -GCTC-GAC- TC--TC--CC ACCCAA--TG TGTACCTACC ----TCTGTT GCTTTGGCGG
B_ribis_CMW7773                 .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW994                  .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW9071                 .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW9077                 .......... .......... .......... .......... .......... .......... ..........
B_eucalyptorum_CMW10125         .......... .......... .......... ....T..... .......... .......... ..........
B_eucalyptorum_CMW10126         .......... .......... .......... ....T..... .......... .......... ..........
B_lutea_CMW9076                 .......... .......... .......... ....C..... .......... .......... ..........
B_lutea_CMW992                  .......... .......... .......... ....C..... .......... .......... ..........
B_dothidea_CMW7780              ...G...... ...C....-. ...C...... ....--TT.. .......... .......... ..........
B_dothidea_CMW8000              ...G...... ...C....-. ...C...... ....--TT.. .......... .......... ..........
B_rhodina_CMW10130              ...G...T.. .......... .....N.... ....--TT.. ..A..G.... .......... ..........
B_rhodina_CMW9074               .......... .......... .......... ....--TT.. ..A..G.... .......... ..........
B_obtusa_CMW7774                ...G...T.. ........A. .......... ....--TT.. ..A..A.... .......... ..........
B_obtusa_CMW7774                ...G...T.. ........A. .......... ....--TT.. ..A..A.... .......... ..........
B_stevensii_CMW7060             .......T.. ........A. .......... ....--TT.. ..A..A.... .......... ..........
CMW11246_Ethiopia               .......... .......... .......... .......... ..----.... TACC...... ..........
CMW11253_Ethiopia               .......... .......... .......... .......... ..----.... TACC...... ..........
CMW189 (B)                      ...G...T.. ........A. --....TC.. ....--TT.. ..A..A.... TCTG.TGC.. ----......
CMW190 (A)                      ...G...T.. ........A. --....TC.. ....--TT.. ..A..A.... TCTG.TGC.. ----......
CMW4786(C)                      ...G...T.. ........A. --....TC.. ....--TT.. ..A..A.... TCTG.TGC.. ----......
CMW10717_ETHIOPIA               ...G...T.. ........A. --....TC.. ....--TT.. ..A..A.... TCTG.TG-.. ---C......
CMW11252_ETHIOPIA               ...G...T.. ........A. --....TC.. ....--TT.. ..A..A.... TCTG.TGC.. ----......
CMW11250_ETHIOPIA               ...G...T.. ........A. --....TC.. ....--TT.. ..A..A.... TCTG.TG-.. ---C......
LasioBt2                        ------.T.. A....C.G.T CGAC..TC.. ....--TT.. ..A..G.... TCTG.TGC.. ---.G.CG.C
G_philoprina_CMW7063            ---------- .--.ACA..T C-..--CCAA .....-.... ..A..A.... TA...TGT.G CT.CG.CG..
```

```
                                570        580        590        600        610        620        630
B_ribis_CMW7772                 GCCGCGGTCC T--CCGC-AC CGG-CGCCC- TT--CG-GGG GGGCTGGCCA GCGC-----C CGCCAGAGGA
B_ribis_CMW7773                 .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW994                  .......... .......... .......... .......A.. ..-....... .......... ..........
B_parva_CMW9071                 .......... .......... .......... .......... ..-....... .......... ..........
B_parva_CMW9077                 .......... .......... .......... .......... ..-....... .......... ..........
B_eucalyptorum_CMW10125         .......... .......... .....T...T ....-..... .--....... ...T...... ..........
B_eucalyptorum_CMW10126         .......... .......... .....T...T ....-..... .--....... ...T...... ..........
B_lutea_CMW9076                 .......... .......... ..AC.C..G. .......... ..-.C..... .......... ..........
B_lutea_CMW992                  .......... .......... ..AC.C..G. .......... ..-.C..... .......... ..........
B_dothidea_CMW7780              .......... .......GG. ...C.C.... .CCC...... ..--...... .......... ..........
B_dothidea_CMW8000              .......... .......GG. ...C.C.... .CCC...... ..--...... .......... ..........
B_rhodina_CMW10130              -.-----..- -..---.NG- ---.-----. --..--.--- ---------- ----...... .....A....
B_rhodina_CMW9074               -.-----..- -..---.GG- ---.-----. --..--.--- ---------- ----...... .....A....
B_obtusa_CMW7774                -.-----..T .TG....G.- -..AG..... .-....C... CC--CCC..- ....GCTTT. ..........
B_obtusa_CMW7774                -.-----..T .TG....G.- -..AG..... .-....C... CC--CCC..- ....GCTTT. ..........
B_stevensii_CMW7060             -.-----..T ..G....GT- -..AG..... .-...AAAAA .C--CCC..C .T..GCT.T. ..........
CMW11246_Ethiopia               .......... .......... ...C-....T .-....G... .C--...... .......... ..........
CMW11253_Ethiopia               .......... .......... ...C-....T .-....G... .C--...... .......... ..........
CMW189 (B)                      C------..T .TG....G.- -..AG....T --....C... CCC.CCA.GC ......TTT. ..........
CMW190 (A)                      C------..T .TG....G.- -..AG....T --....C... CCC.CC-.GC ......TTT. ..........
CMW4786(C)                      C------..T .TG....G.- -..AG....T --....C... CCC.CC-.GC ......TTT. ..........
CMW10717_ETHIOPIA               C------..T .TG....G.- -..AG....T --....C... CCC.CC-.GC ......TTT. ..........
CMW11252_ETHIOPIA               C------..T .TG....G.- -..AG....T --....C... CCC.CC-.GC ......TTT. ..........
CMW11250_ETHIOPIA               C------..T .TG....G.- -..AG....T --....C... CCC.CC-.GC ......TTT. ..........
LasioBt2                        T...------ -.G....C.A -..G-----. --..--.--- ---------- ----.....- ----------
G_philoprina_CMW7063            ------A.T- -.G..C.G-- -..CGC.--T CGTGT.CCCC ..ATCA.G.G C---...... ....TAG.A.
```

```
                                640        650        660        670        680        690        700
B_ribis_CMW7772          CCAT-AAAAC TCCAGTCAGT GAAC-TTCGC AGTCTGAAAA AC-AAGTTAA TAAACTAAAA -CTTTCAACA
B_ribis_CMW7773          ..........  ..........  ..........  ..........  ..........  ..........  ..........
B_parva_CMW994           ..........  ..........  ..........  ..........  ..........  ..........  ..........
B_parva_CMW9071          ..........  ..........  ..........  ..........  ..........  ..........  ..........
B_parva_CMW9077          ..........  ..........  ..........  ..........  ..........  ..........  ..........
B_eucalyptorum_CMW10125  ...-C.....  ..........  A...G..-..  ..........  ..........  ..........  ..........
B_eucalyptorum_CMW10126  ...-C.....  ..........  A...G..-..  ..........  ..........  ..........  ..........
B_lutea_CMW9076          ...-C.....  ..........  A...G-....  .......G..  ..........  ..........  ..........
B_lutea_CMW992           ...-C.....  ..........  A...G-....  .......G..  ..........  ..........  ..........
B_dothidea_CMW7780       ....C...-.  ..........  A...GA.-..  ..........  ....T-....  ..........  ..........
B_dothidea_CMW8000       ....C...-.  ..........  A...GA.-..  ..........  ....T-....  ..........  ..........
B_rhodina_CMW10130       ..T.C...-.  ..........  A...GCA-.A  C......T..  ..........  ..........  ..........
B_rhodina_CMW9074        ..T.C...-.  ..........  A...GCA-.A  C......T..  ..........  ..........  ..........
B_obtusa_CMW7774         ..T.C...-.  ..........  A...G-...A  C......T..  ..........  ..........  ..........
B_obtusa_CMW7774         ..T.C...-.  ..........  A...G-...A  C......T..  ..........  ..........  ..........
B_stevensii_CMW7060      ..T.C...-.  ..........  A...G-...A  C..........  ..........  ..........  ..........
CMW11246_Ethiopia        ..........  ..........  ..........  ..........  ..........  ..........  ..........
CMW11253_Ethiopia        ..........  ..........  ..........  ..........  ..........  ..........  ..........
CMW189 (B)               ..T.C..-T.  ..........  A...G.CGA-  C......T..  ..T.......  ...G......  ..........
CMW190 (A)               ..T.C...-.  ..........  A...G.CGA-  C......T..  ..........  ..........  ..........
CMW4786(C)               ..T.C...-.  ..........  A...G.CGA-  C......T..  ..........  ..........  ..........
CMW10717_ETHIOPIA        ..T.C...-.  ..........  A...G.CGA-  C......T..  ..........  ..........  ..........
CMW11252_ETHIOPIA        ..T.C...-.  ..........  A...G.CGA-  C......T..  ..........  ..........  ..........
CMW11250_ETHIOPIA        ..T.C...-.  ..........  A...G.CGA-  C......T..  ..........  ..........  ..........
LasioBt2                 ..T.C...-.  ..........  A.-.G-C-AG  C......T..  ..........  ..........  ..........
G_philoprina_CMW7063     A.T..-..-.  ..TT..TTTA  TTTTG-GAAT  CT.....GT.  GTTTTTAC..  AT..A.....  A.........
```

```
                                710        720        730        740        750        760        770
B_ribis_CMW7772          ACGGATCTCT TGGTTCTGGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT
B_ribis_CMW7773          .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW994           .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW9071          .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW9077          .......... .......... .......... .......... .......... .......... ..........
B_eucalyptorum_CMW10125  .......... .......... .......... .......... .......... .......... ..........
B_eucalyptorum_CMW10126  .......... .......... .......... .......... .......... .......... ..........
B_lutea_CMW9076          .......... .......... .......... .......... .......... .......... ..........
B_lutea_CMW992           .......... .......... .......... .......... .......... .......... ..........
B_dothidea_CMW7780       .......... .......... .......... .......... .......... .......... ..........
B_dothidea_CMW8000       .......... .......... .......... .......... .......... .......... ..........
B_rhodina_CMW10130       .......... .......... .......... .......... .......... .......... ..........
B_rhodina_CMW9074        .......... .......... .......... .......... .......... .......... ..........
B_obtusa_CMW7774         .......... .......... .......... .......... .......... .......... ..........
B_obtusa_CMW7774         .......... .......... .......... .......... .......... .......... ..........
B_stevensii_CMW7060      .......... .......... .......... .......... .......... .......... ..........
CMW11246_Ethiopia        .......... .......... .......... .......... .......... .......... ..........
CMW11253_Ethiopia        .......... .......... .......... .......... .......... .......... ..........
CMW189  (B)              .......... .......... .......... .......... .......... .......... ..........
CMW190  (A)              .......... .......... .......... .......... .......... .......... ..........
CMW4786 (C)              .......... .......... .......... .......... .......... .......... ..........
CMW10717_ETHIOPIA        .......... .......... .......... .......... .......... .......... ..........
CMW11252_ETHIOPIA        .......... .......... .......... .......... .......... .......... ..........
CMW11250_ETHIOPIA        .......... .......... .......... .......... .......... .......... ..........
LasioBt2                 .......... .......... .......... .......... .......... .......... ..........
G_philoprina_CMW7063     .......... .......... .......... .......... .......... .......... ..........
```

```
                                780        790        800        810        820        830        840
B_ribis_CMW7772          CAGTGAATCA TCGAATCTTT GAACGCACAT TGCGCCCCTT GGTATTCCGA GGGG-CATGC CTGTTCGAGC
B_ribis_CMW7773          .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW994           .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW9071          .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW9077          .......... .......... .......... .......... .......... .......... ..........
B_eucalyptorum_CMW10125  .......... .......... .......... .......... .......... .......... ..........
B_eucalyptorum_CMW10126  .......... .......... .......... .......... .......... .......... ..........
B_lutea_CMW9076          .......... .......... .......... .......... .......... .......... ..........
B_lutea_CMW992           .......... .......... .......... .......... .......... .......... ..........
B_dothidea_CMW7780       .......... .......... .......... .......T.. .......... A......... ..........
B_dothidea_CMW8000       .......... .......... .......... .......T.. .......... A......... ..........
B_rhodina_CMW10130       .......... .......... .......... .......... .........G .......... ..........
B_rhodina_CMW9074        .......... .......... .......... .......... .........G .......... ..........
B_obtusa_CMW7774         .......... .......... .......... ........C. ..C......G .......... ..........
B_obtusa_CMW7774         .......... .......... .......... ........C. ..C......G .......... ..........
B_stevensii_CMW7060      .......... .......... .......... .......... ..C....... .......... ..........
CMW11246_Ethiopia        .......... .......... .......... .......... .......... .......... ..........
CMW11253_Ethiopia        .......... .......... .......... .......... .......... .......... ..........
CMW189  (B)              .......... .......... .......... .......... ..C....... .......... ..........
CMW190  (A)              .......... .......... ....C..... .......... ..C....... .......... ..........
CMW4786 (C)              .......... .......... ....C..... .......... ..C....... .......... ..........
CMW10717_ETHIOPIA        .......... .......... .......... .......... ..C....... .......... ..........
CMW11252_ETHIOPIA        .......... .......... .......... .......... ..C....... .......... ..........
CMW11250_ETHIOPIA        .......... .......... .......... .......... ..C....... .......... ..........
LasioBt2                 .......... .......... .......... .......... .........- ....G..... ..........
G_philoprina_CMW7063     .......... .......... .......... .......GCC A......T.- .C..G..... ....CT....
```

```
                                850        860        870        880        890        900        910
B_ribis_CMW7772          GTCATTTCAA CCCTCAAGCT CT----GCTT GGTATTGGGC TCCGTCCTCC A----CGGAC GCGCCTTAAA
B_ribis_CMW7773          .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW994           .......... .......... .......... .......... C......... .......... ..........
B_parva_CMW9071          .......... .......... .......... .......... C......... .......... ......C...
B_parva_CMW9077          .......... .......... .......... .......... C......... .......... ......C...
B_eucalyptorum_CMW10125  .......... .......... T......... .......... C.........- -..TGT.... ......C...
B_eucalyptorum_CMW10126  .......... .......... T......... .......... C.........- -..TGT.... ......C...
B_lutea_CMW9076          .......... .......... .......... .......... ..........- -..TGT.... ......CG..
B_lutea_CMW992           .......... .......... .......... .......... ..........- -..TGT.... ......CG..
B_dothidea_CMW7780       ......A... .......... .......... .......... A.......T- -..TG...G. ......C...
B_dothidea_CMW8000       ......A... .......... .......... .......... A.......T- -..TG...G. ......C...
B_rhodina_CMW10130       ......A... .......... .......... ..A....... A.........A -.CTG..... ......C...
B_rhodina_CMW9074        ......A... .......... .......... ..A....... A.........A -.CTG..... ......C...
B_obtusa_CMW7774         ......A... .......... .......... .......... G.........- -TCTG..... ..........
B_obtusa_CMW7774         ......A... .......... .......... .......... G.........- -TCTG..... ..........
B_stevensii_CMW7060      ......A... .......... .......... .......... GA........- -TCTG..... ......C...
CMW11246_Ethiopia        .......... .......... .......... .......... C......... .......... ..........
CMW11253_Ethiopia        .......... .......... .......... .......... C......... .......... ..........
CMW189  (B)              ......A... .......... .......... .......... G.........- -TCTG..... ..........
CMW190  (A)              ......A... .......... .......... .......... G.........- -TCTG..... ..........
CMW4786 (C)              ......A... .......... .......... .......... G.........- -TCTG..... ..........
CMW10717_ETHIOPIA        ......A... .......... .......... .......... G.........- -TCTG..... ..........
CMW11252_ETHIOPIA        ......A... .......... .......... .......... G.........- -TCTG..... ..........
CMW11250_ETHIOPIA        ......A... .......... .......... .......... G.........- -TCTG..... ..........
LasioBt2                 ......A... .......... .......... ..A....... A.........A -.CTG..... ..........
G_philoprina_CMW7063     .......... ......T..C .CTAGG..G. ...G.....G AT..G..AAA GCCCG..AGG .ACGGCCGGC
```

```
                          920        930        940        950        960        970        980
B_ribis_CMW7772           GACCTCGGCG GTGGC-GTCT TGCC-TCAAG CGTAGTAGAA AA--CACCTC GCTTTGGAGC GCACGGCGTC
B_ribis_CMW7773           .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW994            .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW9071           .......... .......... .......... .......... .......... .......... ..........
B_parva_CMW9077           .......... .......... .......... .......... .......... .......... ..........
B_eucalyptorum_CMW10125   .......... .......... .......... .......... -.T....... .......... ...T......
B_eucalyptorum_CMW10126   .......... .......... .......... .......... -.T....... .......... ...T......
B_lutea_CMW9076           .......... .......... .......... .......... .......... .......... ..........
B_lutea_CMW992            .......... .......... .......... .......... .......... .......... ..........
B_dothidea_CMW7780        .......... .......... .......... .......... C.TA..T... ....C..... ...G......
B_dothidea_CMW8000        .......... .......... .......... .......... C.TA..T... ....C..... ...G......
B_rhodina_CMW10130        .......... .....T..TC A...C..... .......... --TA...... .......... .GTT......
B_rhodina_CMW9074         .......... .....T..TC A...C..... .......... --TA...... .......... .GTT......
B_obtusa_CMW7774          .......... .....T..TC A...C..... .......... --TA...... .......... .GTT......
B_obtusa_CMW7774          .......... .....T..TC A...C..... .......... --TA...... .......... .GTT......
B_stevensii_CMW7060       .......... .....T..TC A...C..... ....A..... --TA...... .......... .GTT......
CMW11246_Ethiopia         .......... .....GTCT. GC.-...... .......... .-.A...... .......... .......C..
CMW11253_Ethiopia         .......... .....GTCT. GC.-...... .......... .-.A...... .......... ..........
CMW189  (B)               .......... .....T..TC A...C..... .......... --TA...... .......... .GTT......
CMW190  (A)               .......... .....T..TC A...C..... .......... --TA...... .......... .GTT......
CMW4786 (c)               .......... .....T..TC A...C..... .......... --TA...... .......... .GTT......
CMW10717_ETHIOPIA         .......... .....T..TC A...C..... .......... --TA...... .......... .GTT......
CMW11252_ETHIOPIA         .......... .....T..TC A...C..... .......... --TA...... .......... .GTT......
CMW11250_ETHIOPIA         .......... .....T..TC A...C..... .......... --TA...... .......... .GTT......
LasioBt2                  .......... .....T..TC A...C..... .......... --TA...... .......... .GTT......
G_philoprina_CMW7063      -C...AAATC TA.TGGCGG- AC..G..GT. GCCTCCTCTG CGAAGTAG.G ATA..CCGCA T.GGA.A.CG
```

```
                                990       1000       1010       1020       1029
B_ribis_CMW7772                 GCCCGCCGGA CGAACCTT-T GAATTATTT- CTCAAGGTTG ACCTCGGAT
B_ribis_CMW7773                 .......... .......... .......... .......... .........
B_parva_CMW994                  .......... .......... .......... .......... .........
B_parva_CMW9071                 .......... .......... .......... .......... .........
B_parva_CMW9077                 .......... .......... .......... .......... .........
B_eucalyptorum_CMW10125         .......... .......... .....-.... .......... .........
B_eucalyptorum_CMW10126         .......... .......... .....-.... .......... .........
B_lutea_CMW9076                 .......... .......... .....-.... .......... .........
B_lutea_CMW992                  .......... .......... .....-.... .......... .........
B_dothidea_CMW7780              .......... .........C. ...C.-.... .......... .........
B_dothidea_CMW8000              .......... .........C. ...C.-.... .......... .........
B_rhodina_CMW10130              .......... .........C. ...C.-.... .......... .........
B_rhodina_CMW9074               .......... .........C. ...C.-.... .......... .........
B_obtusa_CMW7774                .......... .........C. ...C.-.... .......... .........
B_obtusa_CMW7774                .......... .........C. ...C.-.... .......... .........
B_stevensii_CMW7060             .......... .........C. ...C.-.... .......... .........
CMW11246_Ethiopia               .......... .......... .......... .......... .........
CMW11253_Ethiopia               .......... .......... .......... .......... .........
CMW189  (B)                     .......... .........C. ...C.-.... .......NNN NNNNNNNNN
CMW190  (A)                     .......... .........C. ...C.-.... .......NNN NNNNNNNNN
CMW4786 (C)                     .......... .........C. ...C.-.... .......NNN NNNNNNNNN
CMW10717_ETHIOPIA               .......... .........C. ...C.-.... .......NNN NNNNNNNNN
CMW11252_ETHIOPIA               .......... .........C. ...C.-.... .......NNN NNNNNNNNN
CMW11250_ETHIOPIA               .......... .........C. ...C.-.... .......NNN NNNNNNNNN
LasioBt2                        .......... .........C. ...C.-.... .......NNN NNNNNNNNN
G_philoprina_CMW7063            A.GA...CCT GCCGTTAAAC CCCCA.C..T .-........ .....A...
```

**Figure 7**. Alignment of combined SSR sequences of *D. pinea* sequenced with SS7, SS9 and SS10 markers. (-)= gaps, (.)= Homologous nucleotides, (N)= Unknown bases.

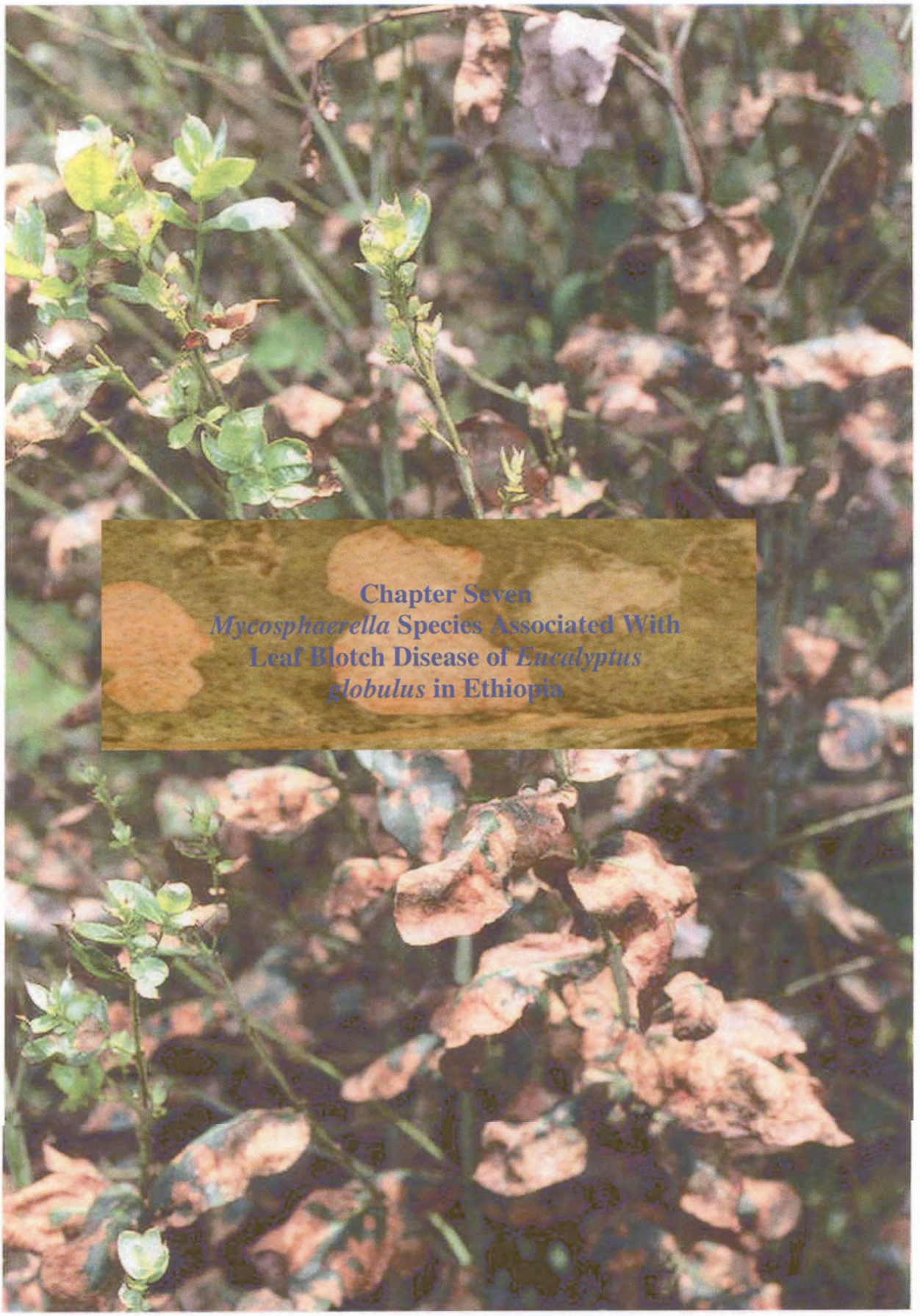
```
                    10         20         30         40         50         60         70
CMW190A         GACAAGACAT CTAGGCCCTG CCGGTCCCG- TCCCCGTCTC CAGGCTCACA TGGAAACAAA -CTGTACAGG
CMW11250        ....-..... .......... .........- .......... .......... .......... -.........
CMW11252        ....-..... .......... .........- .......... .......... .......... -.........
CMW10717        ....-..... .......... .........- .......... .......... .......... -.........
CMW4786         NNNNNNNNN  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
CMW189B         ....-..... T......... ....C...CA .......T.. .G..G..... A......... A...C..T..

                    80         90         100        110        120        130        140
CMW190A         CAAAAGCAGT GAAGTCGTAA GGCCCGGACC CTA-GAAGAG GCGCTTTCCT CTCCACGGAG TAACCACCGG
CMW11250        .......... .......... .......... ...-...... .......... .......... ..........
CMW11252        .......... .......... .......... ...-...... .......... .......... ..........
CMW10717        .......... .......... .......... ...-...... .......... .......... ..........
CMW4786         NNNN...... .......... .......... ...-...... .......... .......... ..........
CMW189B         GG...A.... .....T.... .......... ..-GA..... .......G.. T......... C.........

                    150        160        170        180        190        200        210
CMW190A         CTCGGCTACG CTAGAAAGCA AATTCCCCGA TCTTAGTGGC ATTTTTTCTT TTGCATCATT CCCGGGCCTC
CMW11250        .........C .......... .......... .......... .......... .......... ..........
CMW11252        .......... .......... .......... .......... .......... .......... ..........
CMW10717        .......... .......... .......... .......... .......... .......... ..........
CMW4786         .......... .......... ...C...... .......... .......... .......... ..........
CMW189B         G....G.... G..A...... ...C...... .......... .......... ........A. ..........
```

```
                220        230        240        250        260        270        280

CMW190A         TTTGGAAATT GCTTTTTTTT -----GATTT TGATTTT--- CTTCTTTTCC TCCTCCTCCT CC--------
CMW11250        .......... .......... -----..... .......--- .......... .......... ..--------
CMW11252        .......... .......... -----..... .......--- .......... .......... ..--------
CMW10717        .......... .......... -----..... .......--- .......... .......... ..--------
CMW4786         .......... .......... T-----..... .......--- .......... .......... ..TCC-----
CMW189B         ........A. .G........ TTTTG.T... .......TCT .......... .......... ..TCCTCCTC


                290        300        310        320        330        340        350

CMW190A         -TCTCTTTCT CAACACGAGG CTCACCAATC ACGATGACGA CGACGACGCC GCTGAGAATG AGCGGAAAAT
CMW11250        -.......... .......... .......... .......... .......... .......... ..........
CMW11252        -.......... .......... .......... .......... .......... .......... ..........
CMW10717        -.......... .......... .......... .......... .......... .......... ..........
CMW4786         -.......... .......... .......... .......... .......... .......... ..........
CMW189B         C.......... .......... .......... .......... .......... ......T... .........C


                360        370        380        390        400        410        420

CMW190A         TATCCGAGAA TCATTCCAC- TTCACCGNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNCA GCGGTTTCAT
CMW11250        .......... .........C .......GAT GGGCCCCTCG TCTCGACCCG ACTGATCANN NN....C...
CMW11252        .......... .........C .......GAT GGGCCCCTCG TCTCGACCCG ACTGATCANN NN....C...
CMW10717        .......... .........C .......GAT GGGCCCCTCG TCTCGACCCG ACTGATCANN NN....C...
CMW4786         .......... .........C .......GAT GGGCCCCNNN NNNNNNNNNN NNNNNNNNNN N.........
CMW189B         .......... ...C...G.C .......GAN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN N.........
```

```
                    430        440        450        460        470        480        490

CMW190A         TGAAATGCCA TCTTCAGTAT CTTGGATATC TTTTTTTTTT TTTTTGATGA GTGCGCGCGC ACACTGCGTT
CMW11250        .A...A...C .T...CAG.. T.....A..T .......... .......... .......... ..........
CMW11252        GA...A...C .T...CAG.. T.....A..T .......... .......... .......... ..........
CMW10717        .A...A...C .T...CAG.. T.....A..T .......... .......... .......... ..........
CMW4786         .......... .......... .......... .......... ..----.... .......... ..........
CMW189B         .......... .G........ .......... ......---- -----....C .......... ....C.T...


                    500        510        520        530        540        550        560

CMW190A         GAGTGAGGAC GGTGTGCTGG TGGCGG---T GATGTATGTG TGTTGTTGGT GGTG---TGG GTAGTGTGTG
CMW11250        .......... .......... ......---. .......... .......... ....---... ..........
CMW11252        .......... .......... ......---. .......... .......... ....---... ..........
CMW10717        .......... .......... ......---. .......... .......... ....---... ..........
CMW4786         .......... .......... ......---. .......... .......... ....---... ..........
CMW189B         .......... ...A...... ...T..TGG. ....----.. .......... ....TGG... ..--------


                    570        580        590        600        610        620        630

CMW190A         GATGGAGTGG ATGGAGGAAG GGGTCCGGGA GTGTTGGTTG TTGTATCTGC TCTTCGGGCG AGAGAGAGTC
CMW11250        .......... .......... ..A....... .......... ..CN-..... .AGNGAN... ..........
CMW11252        .......... .......... ..A....... .......... ..CN-..... .AGNGAN... ..........
CMW10717        .......... .......... ..A....... .......... CGACTG.... .AGNGAN... ..........
CMW4786         .......... .......... .......... .......... .......... .......... ..........
CMW189B         ---..T.... .......... .......... .......... .......... .......... ..........
```

```
                640        650        660        670        680        690        700
CMW190A         CAAGGAAGAA G-GAAG-TGG GAATCGGTAG GAGACAAGTC GCCAACCCTA ATGCTTCCAT AGAAACCAAT
CMW11250        .......... .AA.NNNNNN NNNNNNNNNN NNNNNNNNNN .......... .......... ......G...
CMW11252        .......... .AA.NNNNNN NNNNNNNNNN NNNNNNNNNN .......... .......... ..........
CMW10717        .......... .AA.NNNNNN NNNNNNNNNN NNNNNNNNNN .......... .......... ..........
CMW4786         .......... ANNNNNNNNN NNNNNNNNNN NNNNNNNNNN .......... .......... C.........
CMW189B         ...A...... AG.NNNNNNN NNNNNNNNNN NNNNNNNNNN .G........ .......... C.........

                710        720        730        740        750        760        770
CMW190A         TGACGGCGGA AAGACAAAGG AGCTTACACC GCAGCACCAT TCCCTCCCAC AATCCCTGGT CACAAGACAC
CMW11250        A......... .......... .......... .......... .......... .......... ..........
CMW11252        .......... .......... .......... .......... .......... .......... ..........
CMW10717        .......... .......... .......... .......... .......... .......... ..........
CMW4786         C......... .......... .......... -......... .......... .........C ..........
CMW189B         C......... ...T...... .........A .......... .A......G. .........C ..........

                780        790        800        810        820        830        840
CMW190A         ATACAGACAC ACACACACAC ACACACACAC ACCCAACACA CACATACAAC CTCTCCAACT CACCACCACG
CMW11250        .......... .......... .......... .......... .......... .......... ..........
CMW11252        .......... .......... .......... .......... .......... .......... ..........
CMW10717        .......... .......... .......... .......... .......... .......... ..........
CMW4786         .......T.. .......... .......... ..---..... .........T .......... ..........
CMW189B         .......... .....----- ---------- ---------- ----....G. .......... ..........
```

```
                    850        860        870        880        890        900        910
CMW190A       GCGCCTTCAA CGCCCCGATC TGTTCCCTCG GACCACCCAG CAGCAGCATG AACTCCCGCG CACCGTCACT
CMW11250      .......... .......... .......... .......... .......T.. .......... .........T.
CMW11252      .......... .......... .......... .......... .......T.. .......... .....G....
CMW10717      .......... .......... .......... .......... .......T.. .......... .....G....
CMW4786       .......... .......... .......... .......... .......... .......... ..........
CMW189B       .......... T......... .......... .......... .......... .G........ ..........

                    920        930        940        950        960        970        980
CMW190A       AACCTCCCTT CCTTCATCGA CTCCTGGCGC TTCCACCGCC GCCGAAGTGG CAGAACCCTC CAGACCGCAA
CMW11250      .......... ........A. T...G..G.T .......... ...A.G.G.. ..A....... ..T....G.G
CMW11252      .......... .A.....NNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
CMW10717      .......... .A..G..NNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
CMW4786       .......... .......... .......... .......... .......... .......... ..........
CMW189B       .......... .......... .......... .......... ..NNNNNNNN NNNNNNNNNN NNNNNNNNNN

                   989
CMW190A       TCGCGGTTG
CMW11250      ....NNNNN
CMW11252      NNNNNNNNN
CMW10717      NNNNNNNNN
CMW4786       ....C.C..
CMW189B       NNNNNNNNN
```



# Chapter Seven
# *Mycosphaerella* Species Associated With Leaf Blotch Disease of *Eucalyptus globulus* in Ethiopia

## ABSTRACT

Several damaging leaf pathogens are known from *Eucalyptus* spp., worldwide. Of these, *Mycosphaerella* spp. are among the most important. Characteristic symptoms of Mycosphaerella leaf blotch disease (MLD) include leaf spot, premature defoliation, stunting, shoot and twig die-back as well as twig and stem cankers. Recent disease surveys conducted in Ethiopian *Eucalyptus* plantations have revealed disease symptoms similar to those caused by *Mycosphaerella* spp. These symptoms were restricted to *E. globulus* trees growing in several localities in South, South Western and Western Ethiopia. The aim of this study was to identify the fungi associated with this disease. This was achieved by examining the germination patterns of the ascospores and by sequencing the ITS region of the rRNA operon, for representative isolates. Several different ascospore germination patterns were observed, suggesting that more than one *Mycosphaerella* sp. is responsible for MLD on *E. globulus,* in Ethiopia. Analysis of sequence data showed that three *Mycosphaerella* spp., *M. marksii, M. nubilosa* and *M. grandis* were present. This is the first report of these three species from Ethiopia and it is also the first report of *M. grandis* from a country other than Australia. *M. grandis* and *M. nubilosa* were the most common species associated with leaf blotch in Ethiopia. Given the fact that these fungi are well-recognised pathogens of *Eucalyptus*, we assume that they are the most important cause of MLD on *E. globulus* in Ethiopia.

## INTRODUCTION

Plantations of exotic tree species are widely utilised in the tropics and sub-tropics for the production of solid timber products and pulp. *Pinus*, *Eucalyptus*, *Cupressus* and Australian *Acacia* spp. are among the most widely planted exotic species in these situations. Plantations of *Eucalyptus* spp. alone cover approximately 10 million ha of land world-wide (Eldridge *et al.* 1997). In Ethiopia, planting of exotic species commenced with the introduction of *Eucalyptus globulus* Labill. about 110 years ago (Persson 1995). Thereafter, several *Eucalyptus* spp. including *E. camaldulensis* Dhen., *E. saligna* Sm., *E. grandis* Hill ex Maid and *E. citriodora* Hook were introduced. It has been estimated that plantations of *Eucalyptus* spp. constitute about one third of the total plantation area in the country (Anonymous 1994). The wood from plantations of *Eucalyptus* species is commonly used for construction purposes, for fuel, poles and posts and is an important resource for subsistence farmers.

Plantations of *Eucalyptus* spp., though displaying tremendous promise in areas where they have been planted as exotics, are threatened by various pathogens (Wingfield 1990, Persson 1995). Several foliage diseases have been recorded on *Eucalyptus* spp., both in their areas of origin and also in several areas where they have been introduced as plantation species. These include, for example, foliage diseases caused by *Pseudocercospora eucalyptorum* Crous, Wingfield, Marasas & Sutton (Crous *et al.* 1989), *Phaeoseptoria eucalypti* Hansf. emend. Walker (Chipompha 1987), diseases caused by *Cylindrocladium* spp. (Sharma & Mohanan 1982, Crous, Phillips & Wingfield 1991, Schoch *et al.* 1999) and leaf blotch caused by several *Mycosphaerella* spp. (Park & Keane 1982a, Crous 1998).

*Mycosphaerella* spp. are important leaf pathogens of *Eucalyptus* spp. and they are distributed world-wide (Corlett 1991, Crous 1998). They include both saprobes and aggressive pathogens (Von Arx 1983). Thirty-Two *Mycosphaerella* spp. have been described associated with diseases of *Eucalyptus* spp. (Crous 1998, Carnegie 2000, Milgate *et al.* 2001, Hunter *et al.* 2003). Of these, 12 have been described associated with *Eucalyptus* spp. in different African countries (Crous 1998, Hunter *et al.* 2003). For example, in South Africa nine *Mycosphaerella* species have been reported associated with different *Eucalyptus* species (Crous 1998, Hunter *et al.* 2003) and

thirteen species have been recorded from Australia (Carnegie 2000, Milgate *et al.* 2001). Similarly five *Mycosphaerella* spp. have been identified from *E. globulus* and *E. nitens* (Deane & Maid.) Maid. plantations in Chile (Ahumada 2002).

The most important symptoms of Mycosphaerella leaf disease (MLD) include leaf spot, defoliation, stunting, stem canker, twig and shoot die-back (Beresford 1978, Dick & Gadgil 1983, Lundquist & Purnell 1987, Crous 1998). MLD reduces the photosynthetic capacity of the plant, causes shoot die-back, resulting in multi-stemmed trees and reduces growth and yield of trees (Park & Keane 1982b, Dick 1982, Carnegie 2000). Lundquist & Purnell (1987) showed that MLD causes a reduction in growth of *E. nitens* trees in South Africa. Similarly, a positive correlation between severity of *M. nubilosa* infections and growth of *E. grandis* was observed in Australia (Carnegie *et al.* 1994). It has also been shown that the provenances of some *Eucalyptus* spp. such as *E. globulus*, *E. nitens* and *E. regnans* F. Muell. vary in resistance to *Mycosphaerella* infection (Dick & Gadgil 1983, Purnell & Lundquist 1986, Carnegie *et al.* 1994). In South Africa, for example, it is recommended that the New South Wales provenances of *E. nitens* are planted, as they are considerably more tolerant to infection than those from areas such as Victoria (Purnell & Lundquist 1986, Wingfield & Roux 2000).

Several different *Mycosphaerella* spp. can infect individual *Eucalyptus* trees. Similarly, more than one *Mycosphaerella* sp. can be found on a single leaf and even on the same lesion (Crous & Wingfield 1996). Milgate *et al.* (2001), for example, showed that *M. grandis* Carnegie & Keane was found associated with older lesions of *M. tasmaniensis* Crous & M.J. Wingfi., *M. nubilosa* (Cooke) Hansf. and *M. cryptica* (Cooke) Hansf. It has also been shown that lesions of *M. marksii* Carnegie & Keane coalesce with those of *M. cryptica* and *M. molleriana* (Thum.) Lindau. Park & Keane (1984) also indicated the association of *M. parva* R. F. Park & Keane, a saprophytic species, with *M. nubilosa* (Park & Keane 1982b, Crous *et al.* 1993, Carnegie & Keane 1994). In this manner, multiple infections of trees can take place, compounding the impact of MLB on susceptible trees. Such, multiple infections often result in defoliation (Park & Keane 1982b) and they also complicate identification of the causal agents.

The occurrence of *Mycosphaerella* spp. on *Eucalyptus* leaves can vary with the age of the leaves. Some *Mycosphaerella* spp. infect both juvenile as well as mature leaves and others even infect twigs and branches (Park 1988, Crous 1998). Some *Mycosphaerella* spp., including *M. nubilosa*, *M. molleriana* and *M. juvenis* Crous & M. J. Wingf. are commonly associated with severe defoliation of juvenile leaves (Crous & Wingfield 1996, Carnegie & Keane 1998), whereas *M. cryptica* and *M. suberosa* Crous, F. A. Ferreira, Alfenas & M. J. Wingf. are found mainly on mature leaves (Park & Keane 1982a, Crous *et al.* 1993, Carnegie *et al.* 1994). Succession of infections by different *Mycosphaerella* spp. thus results in susceptible trees being affected at all stages of their rotation. In cases where only juvenile leaves are attacked, for example, *M. nubilosa* on *E. nitens* in South Africa, trees can outgrow the problem as they change to their mature leaf stage, normally during their second year of growth (Lundquist & Purnell 1987).

In Ethiopia, symptoms of MLD have been reported from several plantations of *E. globulus*. It has been observed that the disease causes severe damage on juvenile *E. globulus* leaves, in most areas where this tree species is planted (Alemu, Roux & Wingfield 2003). The *Mycosphaerella* spp. involved in causing the disease have, however, not been identified. This study was, therefore, conducted to identify the fungi associated with MLD on *E. globulus* in Ethiopia. To accomplish this, a suite of identification techniques, including examination of ascospore germination patterns, cultural characteristics as well as sequencing of the Internal Transcribed Spacer (ITS) regions of the ribosomal RNA operon, were used.

## MATERIALS AND METHODS

### *Sample collection and isolations*

In a previous survey conducted in *Eucalyptus* plantations in Ethiopia, symptoms similar to those of MLD were observed in most *E. globulus* plantations investigated (Alemu *et al.* 2003). The samples used in the current study were thus collected from *E. globulus* plantations in South, South Western and Western Ethiopia (Table 1,

Figure 1). At each locality where trees showed leaf blotch symptoms, five to ten symptomatic leaves per tree were colleted from three to ten trees, depending on the size of the stand of trees.

The method described by Crous (1998), was used to isolate the *Mycosphaerella* spp. Two to four leaves were selected from each sample and four leaf discs containing lesions were excised from each leaf. These discs were then immersed in water for two hours to moisten the pseudothecia, facilitating spore release. The discs were then attached to the insides of Petri dish lids with the pseudothecia facing downwards over malt extract agar (MEA) (2% Biolab malt extract, 1.5% Biolab agar). The Petri dishes were kept in the dark at room temperature for 24 hours. After 24 hr, plates were examined for the germination of ascospores. Single germinating spores were picked up and transferred to 2% MEA plates and incubated at 25 °C in the dark. Cultures resulting from germinated ascospores were incubated at 25 °C under continuous light. Isolates obtained in this study are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

***Morphological characterisation***

Growth of the fungi on MEA, germination patterns and anamorph associations were used to differentiate the *Mycosphaerella* spp. associated with MLD in Ethiopia. Colony colour was determined using Rayner's (1970) colour charts. Germinating ascospores for each sample were mounted in lactophenol on microscope slides and the germination patterns noted. Ascospore germination patterns were studied using a light microscope (Zeiss Axioskope) and compared with those described for *Mycosphaerella* spp. on *Eucalyptus* (Crous 1998). To identify the anamorph states of the *Mycosphaerella* spp., isolates were grown on water agar (1.5% Biolab agar) containing sterilised carnation leaves at 25 °C under near ultra violate light (NUV) 250 nm.

***DNA extraction***

Isolates for DNA extraction were selected based on differences detected in culture morphology and ascospore germination patterns. Mycelium used for DNA extraction was scraped directly from the surface of cultures on agar plates. Mycelium was placed in Eppendorf tubes and freeze dried under vacuum. A modified version of the DNA extraction method described by Raeder and Broda (1985) was used to isolate DNA. A repeated Phenol:chlorophorm purification step was conducted to remove cell debris. Sodium Acetate (3M NaAc, pH 5.5) (0.1v/v) and two volumes of absolute ethanol were added to the clean aqueous phase to precipitate the nucleic acids. The precipitated DNA was washed with 70% ethanol. The DNA pellets obtained were vacuum dried to remove the remaining ethanol and the pellets were re-suspended in 50 µl sterile water. The contaminating RNA was removed by digesting the isolated DNA with RNase A (Roche, South Africa) in a 37 °C water bath overnight. The DNA in each sample was visualised under ultra-violate light after electrophoresis on a 1% agarose gel containing ethidium bromide.

***PCR amplification***

Specific DNA fragments from isolates included in this study were amplified using the Polymerase Chain Reaction (PCR). The Internal Transcribed Spacer (ITS) region and 5.8S genes of the ribosomal RNA operon were amplified using Primers ITS 1 (5'-TCC GTA GGT GAA CCT GCG G -3') (White *et al.* 1990) and LR 1 (5'- GGT TGG TTT CTT TTC CT-3') (Vilgalys & Hester 1990). The PCR mix consisted of 1 µL DNA, 0.25 mM dNTP's, PCR Buffer (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, pH 8.3) (Roche, South Africa), 0.2 mM of each primer, 2.5 U *Taq* DNA polymerase (Roche Diagnostic, South Africa) and 37 µl sterilised water. The PCR reactions consisted of an initial denaturation step at a temperature of 96 °C for 2 min, followed by 40 cycles of template denaturation at 94 °C for 30 s, primer annealing for 30 s at 55 °C and chain elongation for 2 min at 75 °C. This was followed by a final elongation at 75 °C for 7 min. PCR amplicons were electrophoresed on a 1% agarose gel, stained with ethidium bromide and viewed under UV illumination. Sizes of he PCR fragments were estimated using a 100 bp molecular weight marker (XIV)

(Roche). Prior to sequencing the PCR products were cleaned with the High Pure PCR product purification kit (Roche, South Africa).

***DNA sequencing and phylogenetic analysis***

The PCR products obtained were used as templates for DNA sequencing using an ABI Prism, Big Dye Terminator Cycle sequencing reaction kit (Perkin Elmer Biosystems, USA) according to the manufacturers protocol. Primers ITS 1 and LR 1 were used to sequence both strands of the amplicons. Sequencing reactions were analysed using an ABI PRISM™ 3100 automated DNA sequencer (Perkin Elmer, Norwalk, Con).

DNA sequences of the Ethiopian isolates used in this study were compared with sequences deposited in GenBank [National Centre for Biotechnology Information (NCBI), US National Institute of Health Bethesda, http:/www.ncbi.nlm.nih.gov/BLAST] for preliminary identification. Sequence Navigator (Version 1.0.1) was used to align sequences and gaps were inserted manually and treated as missing data. Sequences were aligned against those of *Mycosphaerella* spp. from and extensive in-house database emerging from previous studies (Hunter *et al.* 2003, Crous *et al.* 2001) and those obtained from GenBank (Table 1). Phylogenetic analysis of the aligned sequences was conducted using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b (Swofford 1998). The sequences were analysed using parsimony, with trees generated by heuristic searches, simple addition and Tree Bisection Reconstruction (TBR) branch swapping. Bootstrap values for the branching points were calculated using 1000 replicates (Felsenstein 1993). In the phylogenetic analysis, *Ramulispora anguoides* (Nirenberg) Crous was used as the outgroup taxon.

**RESULTS**

***Sample collection and isolation***

Symptoms of MLB were found on *E. globulus* at several localities, including Wondo Genet, Hossana, Endibir, Bedele, Menagesha, Holeta and Addis Alem (Table 1).

Disease symptoms, including shoot die-back and leaf blotch (Figure 2a-2d) were common. In some cases, nearly 100% of the leaves on a tree and nearly 100% of the leaf surfaces of these trees were affected (Figure 2c). Lesions varied in size from small to large spots spreading over the whole leaf surface. Some lesions coalesced to form larger lesions (Figure 2a, 2b). The lesions were light brown in colour and had raised brown margins. On some leaves, lesions were confined to the margins of the leaves. Other samples had leaf spots that extended through the leaf laminas, with lesions visible on both leaf surfaces with a light brown colour and a faint red margin (Figure 2).

Ascospores germinated within 24 hours. *Mycosphaerella* spp. were successfully isolated from samples collected from 16 trees. Ascospores from a number of samples failed to germinate, while some isolates died shortly after germination. Representative isolates were, however, obtained from all areas sampled.

***Morphological characterisation***

When the growth of the fungi on MEA was considered, three culture morphologies were found (Figure 3a, b, c). Four *Mycosphaerella* isolates (CMW10186, CMW10189, CMW10376, CMW10187) obtained from *E. globulus* leaves collected from Addis Alem, Endibir and Hossana (Table 1) had similar colony morphology and constituted one group designated as Group I (Figure 3a). The colony colour of this group is olivaceous black 27''''m. Group II isolates (CMW11148, CMW11149, CMW10377, CMW11150), obtained from Hossana, Endibir, Holeta, and Bedele (Table 1) showed a dark olivaceous grey colour, 23''''i (Figure 3b). The third group included only one isolate (CMW10190) obtained near Hossana. This isolate had a pale olivaceous grey colour (23''''f ) (Figure 3c).

Examination of the ascospore germination of *Mycosphaerella* isolates obtained from Ethiopia showed three different germination patterns. These germination patterns could be directly correlated to the morphological groups defined based on culture morphology (Figure 4). Isolates belonging to morphotype I (CMW10186, CMW10189, CMW10376 and CMW10187) had an ascospore germination pattern

closely resembling a Type F pattern (Figure 4a). This pattern is characteristics of *M. juvenis*. The four isolates in morphotype Group II (CMW11148, CMW11149, CMW10377, CMW11150) had Type C (Crous 1998) germination patterns (Figure 4b). This type of germination is characteristic of *M. heimii, M. gregaria* Carnegie & Keane, *M. molleriana* and *M. nubilosa* (Crous 1998). The isolate obtained from Hossana (CMW10190) had a Type B germination pattern (Figure 4c) which is associated with *M. gracilis* Crous & Alfenas and *M. marksii* (Crous 1998). No anamorph structures were found for any of the Ethiopian *Mycosphaerella* isolates.

***DNA sequencing and phylogenetic analysis***

Amplification of the ITS region of the rRNA operon produced a similar sized fragment of approximately 600 bp for all *Mycosphaerella* isolates obtained from Ethiopia. A BLAST search using sequences of Ethiopian *Mycosphaerella* isolates showed that these isolates were closely related to three different *Mycosphaerella* species. When the sequence data were incorporated into a larger data base of sequences from previous studies including those in GenBank and analysed, 12 trees were generated. These trees had the same topology. The number of characters in the analysed data set was 705 bp's, of which 267 characters were constant, 131 variable characters were parsimony uninformative and 307 characters were parsimony informative. The phylogenetic tree generated using a heuristic search had CI and RI values of 0.698 and 0.861 respectively. In all parsimonious phylogenetic trees (Figure 5) one of the Ethiopian *Mycosphaerella* isolates (CMW10190), grouped with *M. marksii* with 100% bootstrap support. Four of the isolates (CMW10186, CMW10189, CMW10376 and CMW10187) grouped with *M. grandis* (100% bootstrap support) and the remaining four isolates, (CMW11148, CMW11149, CMW10377 and CMW11150) resided in the *M. nubilosa* clade (100% bootstrap support).

**DISCUSSION**

Mycosphaerella leaf blotch was the most common foliage disease observed on *E. globulus* in Ethiopia, during surveys in 2000 and 2001 (Alemu *et al.* 2003). Results

of the present study provide the first identification of this group of fungi on *Eucalyptus* in Ethiopia. Three *Mycosphaerella* spp., namely *M. grandis, M. nubilosa* and *M. marksii* were thus identified and this study represents the first report of these *Mycosphaerella* spp. on *Eucalyptus* spp. from Ethiopia. This study also represents the first report of *M. grandis* from a country other than Australia.

Ascospore germination patterns present a useful method to differentiate between *Mycosphaerella* spp. (Park & Keane 1982a). Crous (1998) described 14 types of ascospore germination patterns for *Mycosphaerella* spp. Examination of the ascospore germination patterns revealed that three different species of *Mycosphaerella* were linked to MLD in Ethiopia. The occurrence of 3 different species was supported by DNA sequence data confirming the value that germination patterns have when identifying *Mycosphaerella* spp.

*Mycosphaerella marksii* was found only from a single leaf sample collected from *E. globulus* near Hossana. Previous studies have shown that *M. marksii* occurs on several *Eucalyptus* spp., including *E. globulus, E. grandis, E. nitens* and *E. saligna* (Carnegie *et al.* 1994). This fungus was first described in Australia and it is now known to occur in South Africa, Indonesia, Portugal and Uruguay (Carnegie *et al.* 1994, Crous & Wingfield 1996, Carnegie & Keane 1997, Crous 1998). This fungus is common in Australia and South Africa, but has not been reported to cause significant damage (Carnegie 2000, Hunter *et al.* 2003). Because the fungus was collected only from a single leaf, it is probably not an important component of the MLD problem in Ethiopia.

*Mycosphaerella grandis* was found on samples collected from Addis Alem, Endibir and Hossana. This fungus was first described from Australia on *E. grandis*, *E. globulus* and *E. nitens* (Carnegie & Keane 1994, Carnegie 2000). According to Carnegie & Keane (1994), this pathogen is a common cause of necrotic lesions at the margins of leaves. This type of symptom was common in Ethiopia, suggesting that *M. grandis* is one of the more important components of MLD in the country. This is the first report of this species outside of Australia, and given its occurrence in Ethiopia, it might be expected to be found in neighbouring countries in the future.

*Mycosphaerella nubilosa* was found in several areas including Endibir, Holeta, Hossana, and Bedele. This species mostly affects juvenile leaves of *E. globulus* (Park & Keane 1982a, Purnell & Lundquist 1986, Carnegie *et al.* 1994). This is also one of the most common and destructive foliage pathogens of *Eucalyptus* in Australia, New Zealand and South Africa (Park & Keane 1982a, Dick & Gadgil 1983, Purnell and Lundquist 1986, Hunter *et al.* 2003). *M. nubilosa* and *M. molleriana* were once regarded as the same fungus (Crous, Wingfield & Park 1990), but it has been shown that they represent distinct species (Crous & Wingfield 1997, Crous 1998, Crous *et al.* 1999). The presence of *M. nubilosa* in Ethiopia explains the serious defoliation of *E. globulus* in this country. This fungus should be placed on the list of more important constraints to *E. globulus* propagation in the future.

This study has shown that MLD is common, wherever *E. globulus* is grown in Ethiopia. Previous studies have shown that infection by *Mycosphaerella* spp. not only causes premature defoliation and retarded growth, but can also lead to the abandonment of planting certain *Eucalyptus* spp. (Lundquist & Purnell 1987). In South Africa, for example, the planting of *E. globulus* was terminated in the 1940's as a result of MLB (Lundquist & Purnell 1987). As *E. globulus* is a widely planted species in Ethiopia, the discovery of *M. nubilosa* is of concern, especially given the fact that plantations of this species are likely to be expanded in the future. Selection of species and provenances with tolerance to MLB might help to minimise loss of yield caused by *Mycosphaerella* species in the future.

## REFERENCES

Ahumada, R (2002) *Diseases in commercial Eucalyptus plantations in Chile, with special reference to Mycosphaerella and Botryosphaeria species.* M. Sc. Thesis. The Faculty of Natural and Agricultural Science. University of Pretoria, South Africa.

Alemu Gezahgne, Roux, J. & Wingfield, M. J. (2003) Diseases of exotic plantation *Eucalyptus* and *Pinus* species in Ethiopia. *South African Journal of Science* **99**:29-33.

Anonymous (1994) *Ethiopian Forestry Action Program*. Final Report, Vol. I, pp14 and vol. II, pp34. Addis Ababa Ethiopia.

Beresford, R. M. (1978) *Mycosphaerella nubilosa (Cooke) Hansf. on Eucalyptus delegatensis R. T. Baker: Further studies of epidemiology in the northern island of New Zealand.*, Ph.D Thesis. University of Auckland, New Zealand.

Carnegie, A. J. & Keane, P. J. (1994) Further *Mycosphaerella* species associated with leaf diseases of *Eucalyptus*. *Mycological Research* **98**: 413-418.

Carnegie, A. J., Keane, P. J., Ades, P. K. & Smith, I. W. (1994) Variation in susceptibility of *Eucalyptus globulus* provenances to Mycosphaerella leaf disease. *Canadian Journal of Forestry Research* **24**: 1751-1757.

Carnegie, A. J. & Keane, P. J. (1997) A revised *Mycosphaerella gregaria* nom. nov. for *M. aggregata* on *Eucalyptus*. *Mycological Research* **101**:843-844.

Carnegie, A. J. & Keane, P. J. (1998) *Mycosphaerella vespa* sp. nov. from diseased *Eucalyptus* leaves in Australia. *Mycological Research* **102**:1274-1276.

Carnegie, A. J. (2000) *A study of the species of Mycosphaerella on Eucalyptus in Australia and the impact of the Mycosphaerella leaf diseases on Eucalyptus globulus Labill.* Ph. D Thesis. The University of Melbourne, Australia.

Chipompha, N. W. S. (1987) *Phaeoseptoria eucalypti*: A new pathogen of *Eucalyptus* in Malawi. *South African Journal of Science* **142**: 10-12.

Corlett, M. (1991) An annotated list of the published names of *Mycosphaerella* and *Sphaerella*. *Mycologia Memoir* **18**: 1-328.

Crous, P. W., Wingfield, M. J., Marasas, W. F. O. & Sutton, B. C. (1989) *Pseudocercospora eucalyptorum* sp. nov. on *Eucalyptus* leaves. *Mycological Research* **93**: 394-398.

Crous, P. W., Wingfield, M. J. & Park, R. F. (1990) *Mycosphaerella nubilosa*, a synonym of *M. molleriana*. *Mycological Research* **95**: 628-632.

Crous, P.W., Phillips, A. J. L. & Wingfield, M. J. (1991) The genera *Cylindrocladium* and *Cylindrocladiella* in South Africa, with special reference to forestry nurseries. *South African Forestry Journal* **157**: 68-85.

Crous, P. W., Ferreria, F. A., Alfenas, A. C. & Wingfield, M. J. (1993) *Mycosphaerella suberosa* associated with corky leaf spots on *Eucalyptus* in Brazil. *Mycologia* **85**: 705-710.

Crous, P. W. & Wingfield, M. J. (1996) Species of *Mycosphaerella* and their

anamorphs associated with leaf blotch disease of *Eucalyptus* in South Africa. *Mycologia* **88**: 441-458.

Crous, P. W. & Wingfield, M. J. (1997) *Colletogloeopsis*, a new Coelomycetes genus to accommodate anamorphs of two species of *Mycosphaerella* on *Eucalyptus*. *Canadian Journal of Botany* **75**: 781-790.

Crous, P. W. (1998) *Mycosphaerella* spp. and their anamorphs associated with leaf spot diseases of *Eucalyptus*. *Mycologia Memoir* **21**: 1-170.

Crous, P. W., Hong, L., Wingfield, M. J., Wingfield, B. D. & Kang, J. C. (1999) *Uwebraunia* and *Dissoconium*, two morphologically similar anamorph genera with different teleomorph affinity. *Sydowia* **51**: 155-166.

Crous, P. W., Hong, L., Wingfield, B. D. & Wingfield, M. J. (2001) ITS rDNA phylogeny of selected *Mycosphaerella* species and their anamorphs occurring on *Myrtaceae*. *Mycological Research* **105**: 425-431.

Dick, M. (1982) Leaf inhabiting fungi of *Eucalyptus* in New Zealand. *Journal of Forest Science* **12**: 525-537.

Dick, M. & Gadgil, P. D. (1983) *Eucalyptus* leaf spots. *Forest Pathology in New Zealand* **1**: 1-7.

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

Felsentein, J. (1993) DNABOOT-Bootstrap Confidence Interval on DNA parsimony 3.1. Seattle, University of Washington DC.

Hunter, G. C., Crous, P. W., Roux, J., Coutinho, T. A., Wingfield, B. D. & Wingfield M. J. (2003) Identification of *Mycosphaerella* spp. associated with leaf disease of *Eucalyptus nitens* in South Africa. *Australian Journal of Pathology* (Submitted).

Lundquist, J. E. & Purnell, R. C. (1987) Effects of Mycosphaerella leaf spot on growth of *Eucalyptus nitens*. *Plant Disease* **71**: 1025-1029.

Milgate, A. W., Yuan, Z. Q., Vailancourt, R. E. & Mohammed C. (2001) *Mycosphaerella* species occurring on *Eucalyptus globulus* and *Eucalyptus nitens* plantations of Tasmania, Australia. *Forest Pathology* **31**: 53-63.

Park, R. F. & Keane, P. J. (1982a) Three *Mycosphaerella* species from leaf diseases of *Eucalyptus*. *Transactions of the British Mycological Society* **79**: 95-100.

Park, R. F. & Keane, P. J. (1982b) Leaf disease of *Eucalyptus* associated with

*Mycosphaerella* species. *Transactions of the British Mycological Society* **79**: 101-115.

Park, R. F. & Keane, P. J. (1984) Further *Mycosphaerella* species causing leaf diseases of *Eucalyptus*. *Transactions of the British Mycological Society* **83**: 93-105.

Park, R. F. (1988) Effects of certain hosts, inoculum and environmental factors on infections of *Eucalyptus* species by two *Mycosphaerella* species. *Transactions of the British Mycological Society* **91**: 261-266.

Persson, A. (1995) Exotics-prospects and risks from European and African view point. *Agricultural Science* **9**: 47-62.

Purnell, R. C. & Lundquist, J. E. (1986) Provenance variation of *Eucalyptus nitens* on the Eastern Transvaal Highveld in South Africa. *South African Forestry Journal* **138**: 23-31.

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

Rayner, R. W. (1970) *A mycological colour chart*. Commonwealth Mycological Institute, Kew.

Schoch, C. L., Crous, P. W., Wingfield, B. D. & Wingfield, M. J. (1999) The *Cylindrocladium candelabrum* species complex includes distinct mating populations. *Mycologia* **91**: 286-298.

Sharma, J. K. & Mohanan, C. (1982) *Cylindrocladium* spp. associated with various diseases of *Eucalyptus* in Kerala. *European Journal of Forest Pathology* **12**: 129-136

Swofford, D. L. (1998) PAUP*. *Phylogenetic Analysis Using Parsimony* (*and other methods). Version 4.0. Beta version. Sinauer Associates. Sunderland, Massachusetts.

Vilgalys, R. & Hester, M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238-4246.

Von Arx, J. A. (1983) *Mycosphaerella* and its anamorphs. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Series* C **86**: 15-54.

White, T. J., 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*. San Diego, Academic Press.

Wingfield, M. J. (1990) Pathogens in exotic plantation forestry. *International Forestry Review* **1**: 163-168.

Wingfield, M. J. & Roux, J. (2000) Plant disease of South African forest plantations. In *South African Forestry Handbook* (D. L. Owen ed):pp 241-252. The South African Forestry Institute. Pretoria, South Africa.

Table 1. *Mycosphaerella* isolates used in this study.

| Isolates | Species | Host | Origin | Collector | Accession No. |
|---|---|---|---|---|---|
| CMW10190[a] | *M. marksii* | *Eucalyptus globulus* | Ethiopia | Alemu Gezahgne & J. Roux | AY244404 |
| CMW10186[a] | *M. grandis* | ,, | Ethiopia | Alemu Gezahgne & J. Roux | AY244405 |
| CMW10187[a] | *M. grandis* | ,, | Ethiopia | Alemu Gezahgne & J. Roux | AY244406 |
| CMW10377[a] | *M. nubilosa* | ,, | Ethiopia | Alemu Gezahgne & J. Roux | AY244408 |
| CMW10189[a] | *M.grandis* | ,, | Ethiopia | Alemu Gezahgne & J. Roux | AY244412 |
| CMW10376[a] | *M. grandis* | ,, | Ethiopia | Alemu Gezahgne & J. Roux | AY244407 |
| CMW11148[a] | *M. nubilosa* | ,, | Ethiopia | Alemu Gezahgne & J. Roux | AY244409 |
| CMW11149[a] | *M. nubilosa* | ,, | Ethiopia | Alemu Gezahgne & J. Roux | AY244411 |
| CMW11150[a] | *M. nubilosa* | ,, | Ethiopia | Alemu Gezahgne & J. Roux | AY244410 |
| CMW9090 | *M. marksii* | *E. grandis* | South Africa | M. J. Wingfield | AF468870 |
| CMW9091 | *M. marksii* | ,, | ,, | M. J. Wingfield | AF468871 |
| CMW9092 | *M. marksii* | ,, | ,, | M. J. Wingfield | AF468872 |
| CMW3358 | *M. parkii* | *E. grandis* | Australia | A. J. Carnegie | AF309590 |
| CMW4945 | *M. africana* | *E. viminalis* | South Africa | P. W. Crous | AF309602 |
| CMW4942 | *M. heimii* | *Eucalyptus* spp. | Madagascar | P. W. Crous | AF309606 |
| CMW5705 | *M. heimii* | *Eucalyptus* spp. | Brazil | P. W. Crous | AF452508 |
| CMW5224 | *M. flexuosa* | *E. globulus* | Colombia | M. J. Wingfield | AF309603 |
| CMW4937 | *M. juvenis* | *E. grandis* | South Africa | M. J. Wingfield | AF309604 |
| CMW4036 | *M. juvenis* | ,, | ,, | M. J. Wingfield | AF309605 |
| CMW3282 | *M . nubilosa* | *E. globulus* | Australia | A. J. Carnegie | AF309618 |
| CMW4940 | *M. molleriana* | *E. globulus* | Portugal | S. McCare | AF309620 |
| CMW2734 | *M. molleriana* | *E. globulus* | California (USA) | M. J. Wingfield | AF309619 |

[a]Isolates collected from *E. globulus* in Ethiopia and sequenced in this study. All isolates are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria. All other sequences are obtained from Crous *et al.*, (2001) and Hunter *et al.* (2003).


**Figure 1**. Map of Ethiopia showing the plantation areas where samples were collected.


**Figure 2.** Symptoms of Mycosphaerella leaf blotch. (A) Extensive leaf spotting on leaf surfaces, (B) Necrotic leaf lesions especially on the edges of young leaves (C) senescing leaves as a result of severe infection, (D) small round leaf spots showing pseudothecia.

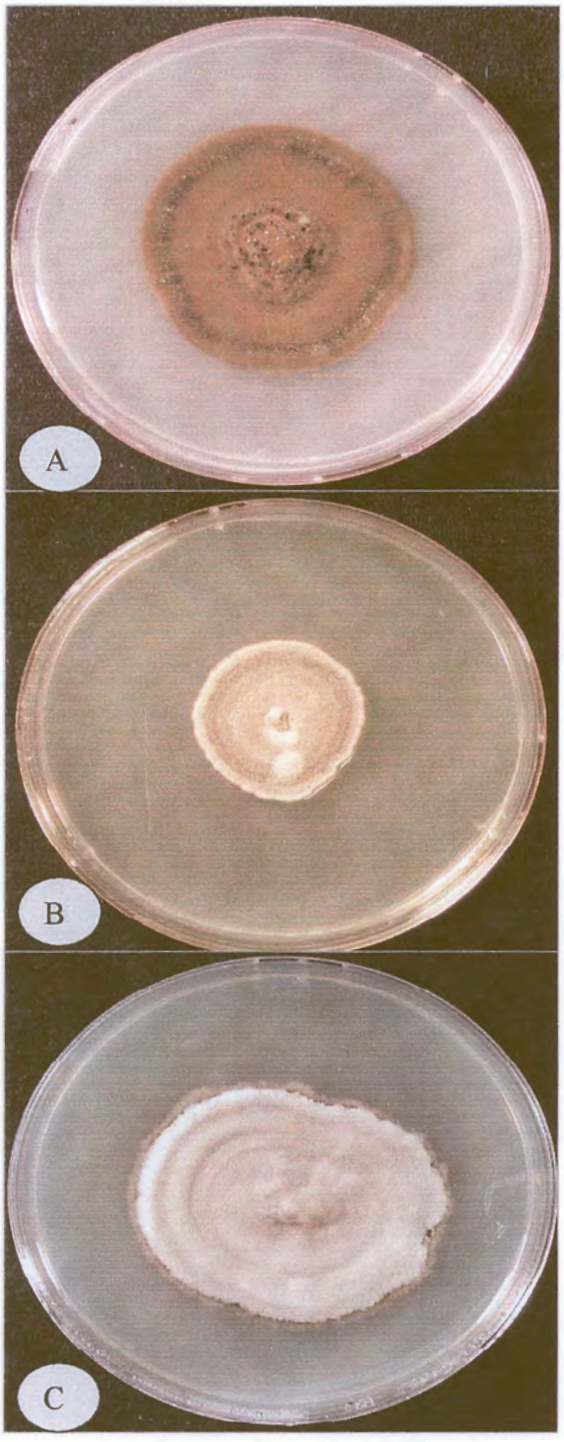



**Figure 3**. Cultural characteristics of *Mycosphaerella* spp. (A) Culture of *Mycosphaerella sp.* of group I on MEA, (B) Culture of *Mycosphaerella* spp. of group II on MEA and (C) Culture of *Mycosphaerella sp.* of group III. on MEA.

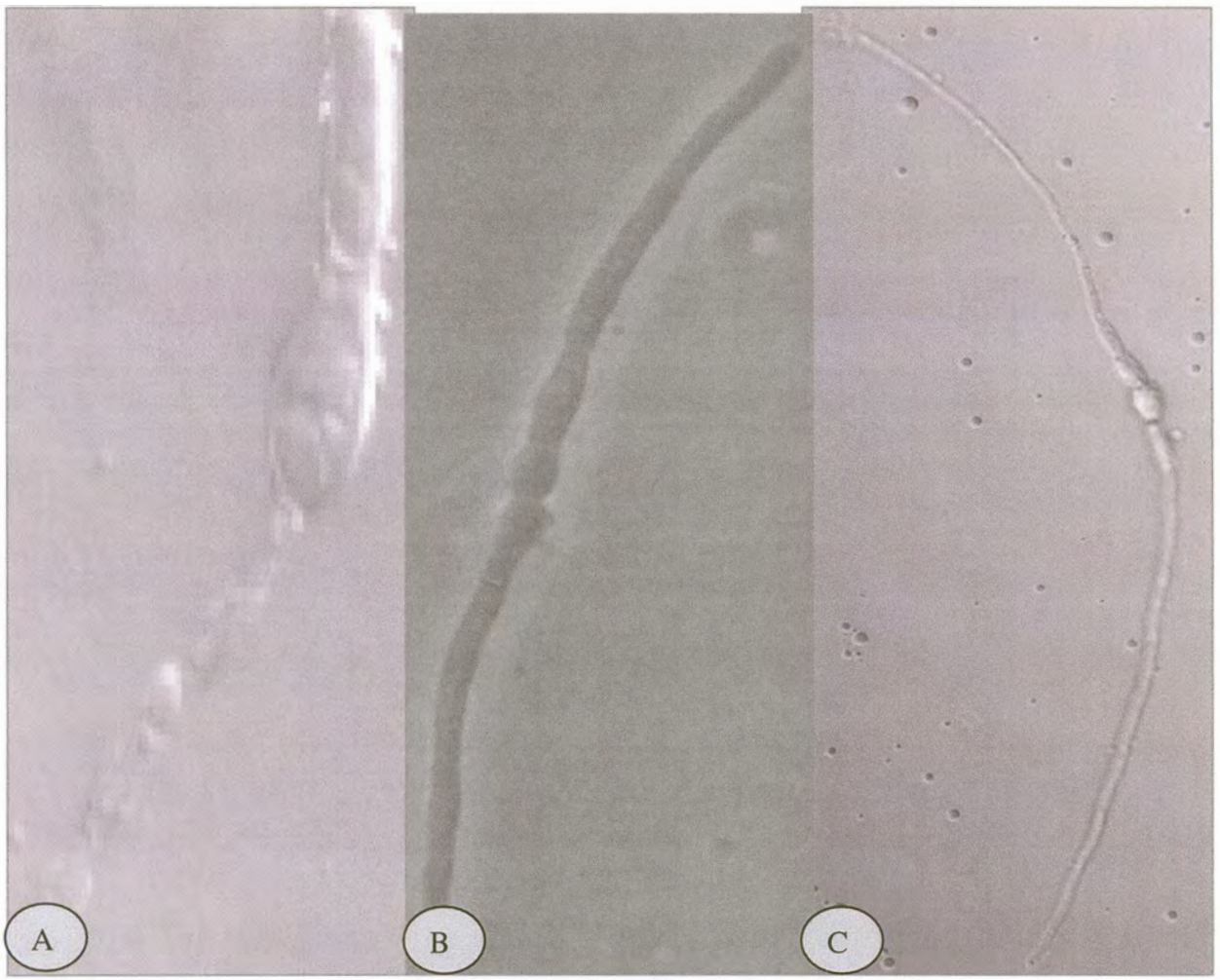



**Figure 4**. Ascospore germination patterns of *Mycosphaerella* spp. (A) Type F ascospore germination pattern of Group I, (B) Type C ascospore germination pattern ofGroup II, (C) Type B ascospore germination pattern of Group III.

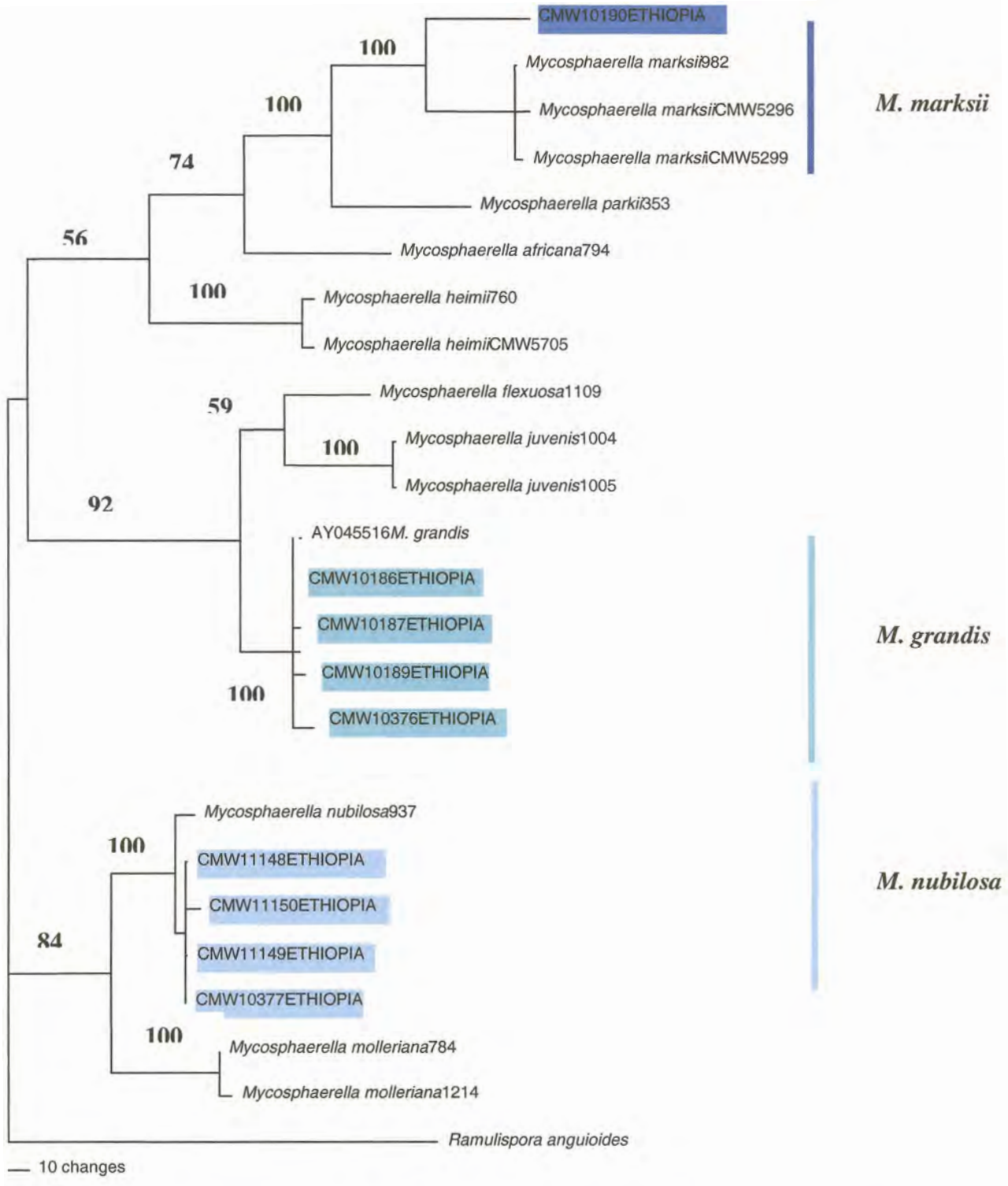



**Figure 4.** Phylogenetic tree of the ITS sequence data of *Mycosphaerella* spp. Number of characters = 705, CI = 0.691 and RI = 0.861. Bootstrap values are shown at each branch.

**Figure 6**. Aligned ITS sequence of *Mycosphaerella* species. (-)=Gaps, (.)= Homologous nucleotides (N)= unknown bases

```
                                  10        20        30        40        50        60        70
CMW10190Ethiopia                  TCCGTAGGTG AACC-TGCGG AGGGATCATT ACC-GAG--C GGAGGGCCC- -CGG-CCCG- -----ACCTC
Mycosphaerella_marksiiCMW9090     .......... .......G.. .......... .........- T.....TTT. .......... ..........
Mycosphaerella_marksiiCMW9091     ..G....... .........A .......... .........- T.....TTT. CG...-.... ..........
Mycosphaerella_marksiiCMW9092     .......... .......... .......... .........- T.....TTT. .......... ..........
Mycosphaerella_parkii353          .......... .......... .......... ..T......- T.....TTTC A.C..-.... ..........
Mycosphaerella_africana794        .......... .......... .......... ..T......- T......-TC A..-...... ..........
Mycosphaerella_flexuosa1109       ..G....... .......... .......... ..T......- T.....-.TC CG.-...... ..........
AY045516M.grandis                 .......... .......... .......... .........- T.......TC CG....T... ..........
CMW10186Ethiopia                  .......... .......... .......... .........- T.......TC CG....T... ..........
CMW10187Ethiopia                  .......... .......... .......... -........- T.......TC CG....T... ..........
CMW10189Ethiopia                  N......... G.AAC..... .......... .........- T.......TC CG....T... .........T
Mycosphaerella_juvenis1004        .......... .......... .......... .........- T......-TC CG.-...... ..........
Mycosphaerella_juvenis1005        .......... .......... .......... .........- T......-TC CG.-...... ..........
Mycosphaerella_heimii760          .......... .......... .......... ..T......- T......TA. .G.-.T.... ..........
Mycosphaerella_heimiiCMW5705      .......... .......... .......... ..T......- T......TA. CG.-.T.... ..........
Mycosphaerella_nubilosa937        .......... .......... .......... .........- T......--G G.A....... ..........
CMW11148Ethiopia                  .......... G.A....... .......... ..T......- T.C....--G C.A....... ..........
CMW11149Ethiopia                  NNNNNNNNNN NNNNC..... .......... ..T......- T.C....--G C.A....... .........CT
CMW11150Ethiopia                  NNNNNNNNNN N..-...... .......... ..T......- T.C....--G C.A....... ..........
CMW10377Ethiopia                  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNG C.A....... ..........
CMW10376Ethiopia                  .......... .......... .......... .........- T.......T. C...G.T... ..........
Mycosphaerella_molleriana784      .......... .......... .......... ..T......- T......--G CAA....... ..........
Mycosphaerella_molleriana1214     .......... .......... .......... ..T......- T......--G CAA....... ..........
Ramulispora_anguioides            .......... .......... .A........ .ATA...CAA T...C.T.AG .-C.C....G GAGCA.-TC.
```

```
                                  80         90         100        110        120        130        140
CMW10190Ethiopia                  ------CAA- CCCT------ -----TT--- --GT------ -------GAA ---------- -----TCA--
Mycosphaerella_marksiiCMW9090     .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_marksiiCMW9091     .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_marksiiCMW9092     .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_parkii353          .......... .......... .......... .......... .......... .......... .....C...C
Mycosphaerella_africana794        .......... .......... .......... .......... .......... .......... .....C....
Mycosphaerella_flexuosa1109       .......... .......... .......... .......... .......... ......TTCG ACCT.---..
AY045516M.grandis                 .......... ...C...... ....A..... .........A TTCC.....- .......... .....---..
CMW10186Ethiopia                  .......... ...C...... ....A..... .........A TTCC.....- .......... .....---..
CMW10187Ethiopia                  .......... ...C...... ...CA..... .........A TTCC.....- .......... .....---..
CMW10189Ethiopia                  .....C.... ...C...... ....A..... .........A TTCC.....- .......... .....---..
Mycosphaerella_juvenis1004        .......... ...C...... ....A.-... .......... .......... .......... ....TCT-.C
Mycosphaerella_juvenis1005        .......... ...C...... ....A.-... .......... .......... .......... ....TCT-.C
Mycosphaerella_heimii760          .......... .......... .......... .......... .......... .......... .....C....
Mycosphaerella_heimiiCMW5705      .......... .......... .......... .......... .......... .......... .....C....
Mycosphaerella_nubilosa937        CT....--.. ...C...... ....A.-... .......... TTTC...--- .......... .....C..CC
CMW11148Ethiopia                  C.....-..A ...C...... ....A.-... .......... TTTC...--- .......... .....C..CC
CMW11149Ethiopia                  CC....-..A ...C...... ....A.-... .........T TTTC...--- .......... .....-..CC
CMW11150BEthiopia                 C.....-..A ...C...... ....A.-... .......... TTTC...--- .......... .....C..CC
CMW10377Ethiopia                  C.....-..A ...C...... ....A.-... .......... TTTC...--- .......... .....C..CC
CMW10376Ethiopia                  C.....--.A ...C...... ....A..... .........A TT.....--- .......... .....C.G..
Mycosphaerella_molleriana784      ......-C.A ...C...... ....A.-... .......... .TTC...--- .......... ....CAA.CC
Mycosphaerella_molleriana1214     ......-C.A ...C...... ....A.-... .......... .TTC...--- .......... ....CAA.CC
Ramulispora_anguioides            TGGGGG.C.C ..TCCTCGGA GGGTT.AGAG AC..CGAGCC TCTCGGA... GCTCGGTTCA GACCT---CC
```

```
                                  150        160        170        180        190        200        210
CMW10190Ethiopia                  -AACCT---- ---------- -GTTGCTTCG G--GGGCGAC CCT-GCC--- --G-TTC-GC GGCGA-----
Mycosphaerella_marksiiCMW9090     A.C.T-.... .......... .......... .......... .......... .......... ....-C....
Mycosphaerella_marksiiCMW5296     A.C..-.... .......... .......... .......... .......... .......... ....-C....
Mycosphaerella_marksiiCMW5299     A.C.T-.... ........GT T......... .......... .......... .......... ....-C....
Mycosphaerella_parkii353          A.-.T-.... .......... T......... .......... .......... .......... ...ATC....
Mycosphaerella_africana794        ..-.T-.... ........C. T......... .......... ..C....... ...T...G.. .A.--.....
Mycosphaerella_flexuosa1109       .--.T-.... .......... ......C... .......... ..G....CTC T...---G.T .-.--C....
AY045516M.grandis                 .-C.T-.... ........CT T.....C... .......... ..G....TTC .G.GC--G-- ---TC.....
CMW10186Ethiopia                  .-C.T-.... ........CT T.....C... .......... ..G....TTC .G.GC--G-- ---TC.....
CMW10187Ethiopia                  .-C.T-.... ........CT T.....-... .......... ..G....TTC .G.GC--G-- ---TC.....
CMW10189Ethiopia                  .-C.T-.... ........CT T.....C... .......... ..G....TTC .G.GC--G-- ---TC.....
Mycosphaerella_juvenis1004        ..C.T-.... ........C. T.....C... ......T... ..G....CTC TG.G.--G-- ---CC.....
Mycosphaerella_juvenis1005        ..C.T-.... ........C. T.....C... ......T... ..G....CTC TG.G.--G-- ---CC.....
Mycosphaerella_heimii760          A.-.T-.... .......... T......... .......... .......... .G-.C.TG.G C.GT-....G
Mycosphaerella_heimiiCMW5705      A.-.T-.... .......... T......... .......... .......... .G-.C.TC.G C.GT-....G
Mycosphaerella_nubilosa937        A-C---.... .......... ......C... .......... ..G....CCC .G-.---C.- ---CC.....
CMW11148Ethiopia                  A-C---.... .......... ......C... .......... ..G....CCC .G-.---C.- ---CC.....
CMW11149Ethiopia                  A-C---.... .......... ......C... .......... ..G....CCC .G-.---C.- ---CC.....
CMW11150Ethiopia                  A-C---.... .......... ......C... .......... ..G....CCC .G-.---C.- ---CC.....
CMW10377Ethiopia                  A-C---.... .......... ......C... .......... ..G....CCC .G-.---C.- ---CC.....
CMW10376Ethiopia                  ..-....... ........CT T.....C... .......... ..G......T TC..---..G C.-TC.....
Mycosphaerella_molleriana784      A-C---.... .......... ......C... .......... ..G....... .G-.---C-- C...CC....
Mycosphaerella_molleriana1214     A-C---.... .......... ......C... .......... ..G....... .G-.---C-- C...CC....
Ramulispora_anguioides            ACC.T.GAAT AAAAAACCTT T.......T. .CA..A..C. T.GC...AGC GG-.C.TC.G C-T.TTGAG.
```

```
                                  220        230        240        250        260        270        280
CMW10190ETHIOPIA                  -GGC-GCCCC CGGGGGAAA- ----TCAAAC A-CTGCGTCA ATTTG-TGTC GGAGTA---- -CTT------
Mycosphaerella_marksiiCMW9090     ......... ......... ......... ......... ......... ......... .........
Mycosphaerella_marksiiCMW9091     ......... ......... ......... ......... ......... ......... .........
Mycosphaerella_marksiiCMW9092     ......... ......... ......... ......... ......... ......... .........
Mycosphaerella_parkii353          .-....... ...A...--T AC.T.--... C.....A... --....C... ......A... TT..A.....
Mycosphaerella_africana794        ....G..... ...A..--TC A......... ......A..- -.....C... .....-.... ....AAAGTA
Mycosphaerella_flexuosa1109       ...-G..... ...C...--C ACC...-... -T....A..- -.....C... T.....TGAT AT.....GAA
AY045516M.grandis                 ...-G..... ...T...CC. A......... -T....A..- -....AC... T.....AA.T AT.-...GAA
CMW10186Ethiopia                  ...-G..... ...T...CC. A......... -T....A..- -....AC... T.....AA.T AT.-...GAA
CMW10187Ethiopia                  ...-G..... ...T...CC. A......... -T....A..- -....AC... T.....AA.T AT.-...GAA
CMW10189Ethiopia                  ...-G..... ...T...CC. A......... -T....A..- -....AC... T.....AA.T AT.-...GAA
Mycosphaerella_juvenis1004        ...-G..... ...C...CC. A..C..-... -T....A..- -.G...C... T.....AA.T AT.....GAA
Mycosphaerella_juvenis1005        ...-G..... ...C...CC. A..C..-... -T....A..- -.G...-... T.....AA.T AT.....GAA
Mycosphaerella_heimii760          C......... ...A..-CC. ATT.--.... ......A... -.-...C... ......AA.. .---..AG..
Mycosphaerella_heimiiCMW5705      C......... ...A..-CC. ATT.--.... ......A... -.-...C... ......AA.. .---..AG..
Mycosphaerella_nubilosa937        ...-G....T ..CA.A.CCC CTC.---... GG...GA..- -.G...C..- ......A.TA C---.AA...
CMW11148Ethiopia                  ...-G....T ..CA...CCC CTC.---... G.....A..- -.G...C... ......A.TA C---.AA...
CMW11149Ethiopia                  ...-G....T ..CA...CCC CTC.---... G.....A..- -.G...C... ......A.TA C---.AA...
CMW11150Ethiopia                  ...-G....T ..CA...CCC CTC.---... G.....A..- -.G...C... ......A.TA C---.AA...
CMW10377Ethiopia                  ...-G....T ..CA...CCC CTC.---... G.....A..- -.G...C... ......A.TA C---.AA...
CMW10376ETHIOPIA                  ...-G..... ...T...CC. ATC.---..A C.TCTGCATC --..TGACGT CTGAGTAAAT AT.-....GA
Mycosphaerella_molleriana784      ...-G..... ...T...CCC .TC.---... T.....A..- -.C...C... T....-..CA C---.AA...
Mycosphaerella_molleriana1214     ...-G..... ...T...CCC .TC.---... T.....A..- -.C...C... T....-..CA C---.AA...
Ramulispora_anguioides            .--TGC.TG. .A.A...CCA C...---... TCT..TT.TT .-G..A.... T......CTA TA.AATAG..
```

```
                                290        300        310        320        330        340        350

CMW10190Ethiopia                -----GTTAA -TAAA-CAAA AC-------- TTTCAACAAC GGATCTCTTG GTTCTGGCAT CGATGAAGAA
Mycosphaerella_marksiiCMW9090   .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_marksiiCMW9091   .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_marksiiCMW9092   .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_parkii353        .....-.... ...C.T-... .......... .......... .......... .......... ..........
Mycosphaerella_africana794      ..AA.-.... A----..... .......... .......... .......... .......... ..........
Mycosphaerella_flexuosa1109     TCAA.----- .----T.... .......... .......... .......... .......... ..........
AY045516M.grandis               TCAA.----- .----T.... .......... ...T...... .......... .......... ..........
CMW10186Ethiopia                TCAA.----- .----T.... .......... ...T...... .......... .......... ..........
CMW10187Ethiopia                TCAA.----- .----T.... .......... ...T...... .......... .......... ..........
CMW1089Ethiopia                 TCAA.----- .----T.... .......... ...T...... .......... .......... ..........
Mycosphaerella_juvenis1004      TCAAA----- .----T.... .......... .......... .......... .......... ..........
Mycosphaerella_juvenis1005      TCAAA----- .----T.... .......... .......... .......... .......... ..........
Mycosphaerella_heimii760        T.AAA-.... A----..... .......... .......... .......... ....CA.... ..........
Mycosphaerella_heimiiCMW5705    T.AAA-.... A----..... .......... .......... .......... ....CA.... ..........
Mycosphaerella_nubilosa937      CCAA.----- .----T...- --.TTAAAAC .......... .......... .......... ..........
CMW11148Ethiopia                CCAA.----- .----T...- --.TTAAAAC .......... .......... .......... ..........
CMW11149Ethiopia                CCAA.----- .----T...- --.TTAAAAC .......... .......... .......... ..........
CMW11150Ethiopia                CCAA.----- .----T...- --.TTAAAAC .......... .......... .......... ..........
CMW10377Ethiopia                CCAA.----- .----T...- --.TTAAAAC .......... .......... .......... ..........
CMW10376Ethiopa                 ATCAA----- .----T.... .......... ...T...... .......... .......... ..........
Mycosphaerella_molleriana784    ..AA.----- .----T...T CAATCAAAAC .......... .......... .......... ..........
Mycosphaerella_molleriana1214   ..AA.----- .----T...T CAATCAAAAC .......... .......... .......... ..........
Ramulispora_anguioides          .....----- .----.---- --........ ..AA..TTT CA.CAA.GGA TC...T.GT. .TGGC.TCG.
```

```
                                  360        370        380        390        400        410        420
CMW10190Ethiopia                  CGCAGCGAAA TGCGATAAGT AATGTGAATT GCAGAATTCA GTGAATCATC GAATCTTTGA ACGCACATTG
Mycosphaerella_marksiiCMW9090     .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_marksiiCMW9091     .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_marksiiCMW9092     .......... .......... .......... ...C...... .......... .......... ..........
Mycosphaerella_parkii353          .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_africana794        .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_flexuosa1109       .......... .......... .......... .......... .......... .......... ..........
AY045516M.grandis                 .......... .......... ....-..... .......... .......... .......... ..........
CMW10186Ethiopia                  .......... .......... .......... .......... .......... .......... ..........
CMW10187Ethiopia                  .......... .......... .......... .......... .......... .......... ..........
CMW10189Ethiopia                  .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_juvenis1004        .......... .......... .........C .......... .......... .......... ..........
Mycosphaerella_juvenis1005        .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_heimii760          .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_heimiiCMW5705      .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_nubilosa937        .......... .......... .......... .......... .......... .......... ..........
CMW11148Ethiopia                  .......... .......... .......... .......... .......... .......... ..........
CMW11149Ethiopia                  .......... .......... .......... .......... .......... .......... ..........
CMW11150Ethiopia                  .......... .......... .......... .......... .......... .......... ..........
CMW10377Ethiopia                  .......... .......... .......... .......... .......... .......... ..........
CMW10376Ethiopia                  .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_molleriana784      .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_molleriana1214     .......... .......... .......... .......... .......... .......... ..........
Ramulispora_anguioides            T.A..AACGC A....A.T.C G..AA.T.A. .TGA.T.G.. .AATTCAG.G A.TCA.CGA. T.TTTG.AC.
```

```
                                430       440       450       460       470       480       490

CMW10190Ethiopia                CGCCCCGTGG TATTCCGCGG GGCATGCCTG TTCGAGCGTC ATTTCA-CCA CTCGAG--TC TGACTCGGTA
Mycosphaerella_marksiiCMW9090   .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_marksiiCMW9091   .......... .......... ..T....... .......... .......... .......... ..........
Mycosphaerella_marksiiCMW9092   .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_parkii353        .......... .......... .......... .......... .......... .......... ..........
Mycosphaerella_africana794      .......... .......... .......... .......... .......... ...A....C. .AG..T....
Mycosphaerella_flexuosa1109     ......T... .......G.. .......... .......... .......... ...A....C. ..G..T....
AY045516M.grandis               ......TC.. .......A.. .......... .......... .......... ...A....C. ..G..T....
CMW10186Ethiopia                ......TC.. .......A.. .......... .......... .......... ...A....C. ..G..T....
CMW10187Ethiopia                ......T... .......A.. .......... .......... .......... ...A....C. ..G..T....
CMW10189Ethiopia                ......TC.. .......A.. .......... .......... .......... ...A....C. ..G..T....
Mycosphaerella_juvenis1004      ......T... .......G.. .......... .......... .......... ...A....C. ..G..T....
Mycosphaerella_juvenis1005      ......T... .......G.. .......... .......... .......... ...A....C. ..G..T....
Mycosphaerella_heimii760        .....TC... .......G.. .......... .......... .......... ...A....C. ..G..T....
Mycosphaerella_heimiiCMW5705    .....TC... .......G.. .......... .......... .......... ...A....C. ..G..T....
Mycosphaerella_nubilosa937      .....TC... .......GA. .......... .......... .......... ...C....C. CCG..T....
CMW11148Ethiopia                .....TC... .......GA. .......... .......... .......... ...C....C. CCG..G...C
CMW11149Ethiopia                .....TC... .......GA. .......... .......... .......... ...C....C. CCG..G...C
CMW11150Ethiopia                .....TC... .......GA. .......... .......... .......... ...C....C. CCG..G...C
CMW10377Ethiopia                .....TC... .......GA. .......... .......... .......... ...C....C. CCG..G...C
CMW10376Ethiopia                ......TC.. .......A.. .......... .......... .......... ...A....C. ..G..T....
Mycosphaerella_molleriana784    .....TC... .......GA. .......... .......... ...A...... ...CG.C.CT C-G..G....
Mycosphaerella_molleriana1214   .....TC... .......GA. .......... .......... ...A...... ...CG.C.CT C-G..G....
Ramulispora_anguioides          .A.ATT.C.C CC.CTG.TAT TC.GG.GGGC A.GCCTGT.. GAGCGTCATT A.AACCAC.. AAG...TC-G
```

```
                                  500        510        520        530        540        550        560

CMW10190Ethiopia                  TTGGGCGCCG CGTT-T--CG -ACGCGCGCC -------TTA AAGTTT-CCG GCTG-GACCG TCCGTCTCCG
Mycosphaerella_marksiiCMW99090    .......T.. .......... ..T....... .......... .......... .......... ..........
Mycosphaerella_marksiiCMW9091     .......T.. .......... ..T....... .......... .......... .......... ..........
Mycosphaerella_marksiiCMW9092     .......T.. .......... ..T....... .......... .......... .......... ..........
Mycosphaerella_parkii353          .......T.. ..GCT..C.. .C........ ........C. ....C..... ......G.A. C.........
Mycosphaerella_africana794        .......T.. ..G-T..C.. .-...--... .......... ....C..... .....AG.A. .T......TA
Mycosphaerella_flexuosa1109       .......AG. ..GCT..C.. GC...C...- ........C. ....C..... -......... A.......TA
AY045516M.grandis                 .......... ..G.T.GC.. .-...--... ........C. ....C..... .....AG..A A.T.....TA
CMW10186Ethiopia                  .......... ..G.T.GC.. .-...--... ........C. ....C..... .....AG..A A.T.....TA
CMW10187Ethiopia                  .......... ..G.T.GC.. .-...--... ........C. ....C..... .....AG..A A.T.....TA
CMW10189Ethiopia                  .......... ..G.T.GC.. .-...--... ........C. ....C..... .....AG..A A.T.....TA
Mycosphaerella_juvenis1004        .......AG. ..GCT...-- .-....-... GCCCGCC.C. ....C..... ........G. AT......TA
Mycosphaerella_juvenis1005        .......AG. ..GCT...-- .-....-... GCCCGCC.C. ....C..... ........G. AT......TA
Mycosphaerella_heimii760          .......T.. ..GCT-.C.. .-...--... .......... ....C.T... .....AG.T. ........TA
Mycosphaerella_heimiiCMW5705      .......T.. ..GCT-.C.. .-......C-- .......... ....C.T... .....AG.T. ........TA
Mycosphaerella_nubilosa937        .......... ..GCC..C.. .-...-GC.- ........C. .T..C..... ..C..AG... A.......TC
CMW11148Ethiopia                  .......... ..GCC..C.. .-...-GC.- ........C. .T..C..... ..C..AG... A.......TC
CMW11149Ethiopia                  .......... ..GCC..C.. .-...-GC.- ........C. .T..C..... ..C..AG... A.......TC
CMW11150Ethiopia                  .......... ..GCC..C.. .-...-GC.- ........C. .T..C..... ..C..AG... A.......TC
CMW10377Ethiopia                  .......... ..GCC..C.. .-...-GC.- ........C. .T..C..... ..C..AG... A.......TC
CMW10376Ethiopia                  .......... ..G.T.GC.. .-......C-- ........C. ....C..... .....AG..A A.T.....TA
Mycosphaerella_molleriana784      .......... ..GC.-...- TC....---- ...CGCC.CG ....C..... ..C..AG... A........A
Mycosphaerella_molleriana1214     .......... T.GC.-...- TC....---- ...CGCC.CG ....C..... ..C..AG... A...G....A
Ramulispora_anguioides            C.T..TATT. G.G.TCGCG. TTT-..---- ..CGGCC--- ---.C.AAAC T.A.T.G.G. .G.C.G..G.
```

```
                                570        580        590        600        610        620        630
CMW10190Ethiopia                AGCGTTGTGG CATCTGTC-- ---------- TC-GCT---A GG--GAGT-C GCGGAGGGCG -TT---GGCC
Mycosphaerella_marksiiCMW9090   .......... .......... .......... .......... .......C.. .......... ..........
Mycosphaerella_marksiiCMW9091   .......... .......... .......... .......... .......C.. .......... ..........
Mycosphaerella_marksiiCMW9092   .......... .......... .......... .......... .......C.. .......... ..........
Mycosphaerella_parkii353        .......... ....----.. ACA.GTTC.. .......... .......... .....C.... ..C.......
Mycosphaerella_africana794      .......... ..-------.T ATA..TT... .......G.. --AA....T. .-...C...- ...TT.....
Mycosphaerella_flexuosa1109     .........A .--------.T AAA..TTGGA -.C...TGT- --......AT .-...C.T.- .C...C....
AY045516M.grandis               .......... -------TTT AATCAT.... -.C...TGT- --.....AT. .---.A.... A--..C....
CMW10186Ethiopia                .......... -------TTT AATCAT.... -.C...TGT- --.....AT. .---.A.... A--..C....
CMW10187Ethiopia                .......... -------TTT AATCAT.... -.C...TGT- --.....AT. .---.A.... A--..C....
CMW10189Ethiopia                .......... -------TTT AATCAT.... -.C...TGT- --.....AT. .---.A.... A--..C....
Mycosphaerella_juvenis1004      .........A .T..G---.. .....TTGGA -.C...TGC- --......A. .-...---.. ..CCTC....
Mycosphaerella_juvenis1005      .........A .T..G---.. .....TTGGA -.C...TGC- --......A. .-...---.. ..CCTC....
Mycosphaerella_heimii760        .......... .--------.. AACTAT.... -TC...T.C- ..AG.--... .-..T..C.. .--..C....
Mycosphaerella_heimiiCMW5705    .......... .--------.. AACTAT.... -TC...T.C- ..AG.--... .-..T..C.. .--..C....
Mycosphaerella_nubilosa937      .......... ..------.. ..CTACTGTT ..GCT-.G.. C..G.GAC.. .-.TCT.... .--.CGC...
CMW11148Ethiopia                .......... ..------.. ..CTACTGTT ..GCT-.G.. C..G.GAC.. .-.TCT.... .--GCGC...
CMW11149Ethiopia                .......... ..------.. ..CTACTGTT ..GCT-.G.. C..G.GAC.. .-.TCT.... .--GCGC...
CMW11150Ethiopia                .......... ..------.. ..CTACTGTT ..GCT-.G.. C..G.GAC.. .-.TCT.... .--GCGC...
CMW10377Ethiopia                .......... ..------.. ..CTACTGTT ..GCT-.G.. C..G.GAC.. .-.TCT.... .--GCGC...
CMW10376ETHIOPA                 .......... -------TTT AATCAT.... -.C...TGT- -.AG-.-... .---.A.... A--..C....
Mycosphaerella_molleriana784    .......... ..CAAC.GTT T.CG...CTT ...--GGG.. C-..----.. .-.TCT.... .--GCGC...
Mycosphaerella_molleriana1214   .......... ..CAAC.GTT T.CG...CTT ...--GGG.. C-..----.. .-.TCT.... .--GCGC...
Ramulispora_anguioides          CT.TAC.C.T AG.AATA-CT CC.....TCG -.GAT....- --..----.G A-.TCC..TA .--GGTTTA.
```

```
                                    640        650        660        670        680        690      697

CMW101901Ethiopia                    --------GT TAAAC----- ---------A CCCCATCA-A AGGTTGACCT CGGATCAGGT AGGGATA
Mycosphaerella_marksiiCMW9090       .......... .......... .......... .......... .......... .......... .......
Mycosphaerella_marksiiCMW9091       .......... .......... .......... .......... .......... .......... .......
Mycosphaerella_marksiiCMW9092       .......... .......... .......... .......... .......... .......... .......
Mycosphaerella_parkii353            .......... ....T..... .......... .......... .......... .......... .......
Mycosphaerella_africana794          .......... ....TC.... TTTC...TT. --------.. .......... .......... .......
Mycosphaerella_flexuosa1109         .......... .......... ..TTATTAC. .-------.. .......... .......... .......
AY045516M.grandis                   .......... .......... ..TTATT.C. --------.. .......... .......... .......
CMW10186Ethiopia                    .......... .......... ..TTATT.C. --------.. .......... .......... .......
CMW10187Ethiopia                    .......... .......... ..TTATT.C. --------.. .......... .......... .......
CMW10189Ethiopia                    .......... .......... ..TTATT.C. --------.. .......... .......... .......
Mycosphaerella_juvenis1004          .......... .....CC... TTTTAT..C. --------.. .......... .......... .......
Mycosphaerella_juvenis1005          .......... .....CC... TTTTAT..C. --------.. .......... .......... .......
Mycosphaerella_heimii760            .......... ....TC.... TTT.....C. .-------.. .......... .......... .......
Mycosphaerella_heimiiCMW5705        .......... ....TC.... TTT.....C. .-------.. .......... .......... .......
Mycosphaerella_nubilosa937          .......... .....CC... TTT.....C. ..------A. .......... .......... .......
CMW11148Ethiopia                    .......... .....CC... TTT.....C. ..------A. .......... .......... .......
CMW11149Ethiopia                    .......... .....CC... TTT.....C. ..------A. .......... .........- .......
CMW11150Ethiopia                    .......... .....CC... TTT.....C. ..------A. .......... .......... .......
CMW10377Ethiopia                    .......... .....CC... TTT.....C. ..------A. .......... .......... .......
CMW10376Ethiopa                     .......... ....-C.... ..TTATT.C. --------.. .......... .......... .......
Mycosphaerella_molleriana784        .......... .....CC... TT...T..C. .-------A. .......... .......... .......
Mycosphaerella_molleriana1214       .......... .....CC... TT...T..C. .-------A. .......... .......... .......
Ramulispora_anguioides              TTGCCAAC-- -..C.CCCAA TTTTTT.AC- --------.- .......... .......... .......
```

## SUMMARY

In Ethiopia, the planting of exotic species commenced with the introduction of *Eucalyptus globulus* approximately 110 years ago. Today several different *Eucalyptus*, *Pinus*, *Cupressus* and Australian *Acacia* species are planted to provide wood for fuel/energy and raw material for furniture and construction. In many areas, people are dependent solely on wood to provide for their basic fuel and construction needs. Despite this, little attention has been given to improve the silvicultural and management practices of plantations in Ethiopia. In particular, disease surveillance and management has never received due attention. The aim of the studies that make up this thesis have been to address the issue of diseases of plantation trees in Ethiopia. Studies have thus focused on the prevalence, identity and importance of major diseases of especially *Eucalyptus* and *Pinus* spp.

As a background to this thesis, available information on diseases of exotic tree species in Africa has been reviewed and this is presented in the first chapter. In the review, diseases of the major exotic plantation species including *Eucalyptus*, *Pinus*, *Cupressus* and *Acacia* species have been considered. A section was also devoted to highlight tree diseases reported from Ethiopia. The review shows clearly that there is a great lack of information on diseases of exotic plantation species in most African countries, with the exception of South Africa. This suggests the need for more pathology studies in African plantations. The review also highlights the importance of diseases in plantation forests.

In Ethiopia, little information is available on tree diseases in plantation forests. To partially address this problem, disease surveys were conducted in 2000 and 2001 in *Eucalyptus* and *Pinus* plantations in South and South Western Ethiopia. The results of this survey showed that a number of pathogens, known from other countries, including Armillaria root rot, stem canker and foliage diseases are found in plantations of Ethiopia. The major diseases discovered during the survey are discussed in Chapter two of this thesis and an indication is given of their impact and distribution.

During the disease survey, Armillaria root rot was found to be associated with both exotic and native tree species. Morphological and molecular identification techniques revealed that the *Armillaria* sp. collected in this study is *A. fuscipes*. This is discussed in chapter three, where I also provide preliminary data regarding the host range and distribution of Armillaria root rot in Ethiopia. Prior to this study it was suggested that *A. mellea* is responsible for Armillaria root rot of

hard woods in Ethiopia. The current study, however, showed that at least two *Armillaria* spp., *A. mellea* and *A. fusicpes* are causing Armillaria root rot in the country. Of significance is the fact that *A. fuscipes* was isolated from two indigenous tree species, *A. abyssinica* and *J. excelsa.*

Chapter four of this thesis deals with the identity of the fungus causing stem canker on *Eucalyptus camaldulensis.* Disease symptoms identical to those caused by *Coniothyrium zuluense* were commonly found on *E. camaldulensis* in restricted areas in Western Ethiopia. The causative agent was determined based on DNA sequence analysis of the ITS 1, ITS 2 and 5.8S gene region and β-tubulin genes. According to the phylogenetic tree generated for these sequence data, the Ethiopian *Coniothyrium* isolates seem to be closely related to *C. zuluense,* however, the Ethiopian isolates formed a separate group. This may suggest that *C. zuluense* represents a species complex, but this needs further investigation. Coniothyrium canker is considered to be one of the most serious diseases of *Eucalyptus* spp. especially to the sawn timber and construction industry as it weakens and flaws the timber. It occurrence in Ethiopia is, therefore, of great importance.

Disease symptoms similar to those of Botryosphaeria canker on *Eucalyptus* were commonly observed in all the areas where surveys were conducted. *Botryosphaeria* spp. are known as opportunistic stress related and endophytic pathogens on a wide range of woody plants, worldwide. In Ethiopia, symptoms similar to those associated with *Botryosphaeria* infection elsewhere, were found in almost all plantations surveyed. The disease was found on several *Eucalyptus* spp. including *E. globulus*, *E. saligna*, *E. grandis* and *E. citriodora.* Both morphological and molecular identification techniques were used to determine the identity of the fungus and the results are presented in chapter five. It was shown that *B. parva* is responsible for Botryosphaeria stem canker of *Eucalyptus* spp. in Ethiopia and the pathogenicity of Ethiopian isolates was also tested. This pathogen can have a serious effect on *Eucalyptus* in Ethiopia, as growing conditions in the country are often harsh and many people rely on coppicing to reproduce their stands. All these factors are conducive to stress and thus to *Botryosphaeria* infection.

*Diplodia pinea* is a fungus that commonly resides in the cones of *Pinus* spp. and it tends to move from these sites to infect stems, when trees are under stress. Therefore, isolations were made from *Pinus patula* cones to determine whether *D. pinea* was present in these structures in Ethiopia. Chapter 6 of the thesis provides results of this study. It was expected that *D. pinea* would be the most common inhabitant of the cones. Contrary to this, a *Fusicoccum* sp. was found more

frequently than *D. pinea*. The results presented in this chapter show clearly that the A morphotype of *D. pinea* is found in cones of *P. patula* in Ethiopia. The *Fusicoccum* sp. found associated with *P. patula* cones is most closely related to *B. parva*. Results of greenhouse inoculation studies showed that both these fungi are pathogenic to *Pinus tadea*, with *D. pinea* being the more pathogenic.

Serious leaf spot and shoot die-back symptoms were observed on leaves of *E. globulus* at several localities. The leaf blotch symptoms closely resemble those caused by *Mycosphaerella* spp. Even though 30 different *Mycosphaerella* spp. are known to be associated with *Eucalyptus* species world-wide, the cause of Mycosphaerella leaf blotch on *E. globulus* in Ethiopia is not known. Morphological and DNA based comparisons were used to determine the identity of the species found in Ethiopia and the results are provided in chapter seven. I was thus able to show that three *Mycosphaerella* spp. namely, *M. marksii, M. grandis* and *M. nubilosa* are involved in causing Mycosphaerella leaf disease of *E. globulus* in Ethiopia. This is the first report of these species from Ethiopia and the first report of *M. grandis* from a country other than Australia.

The results presented in the various chapters making up this thesis provide the first detailed studies on diseases of plantation trees in Ethiopia. Most tree diseases discussed in the thesis are first reports for the country. The thesis provides information on the identity of the pathogens and their significance in plantation development in Ethiopia. It also highlights the need for adequate management and silvicultural practices, as well as the need for selecting disease tolerant provenances and/or individuals. The information presented in the thesis also expands the host range and geographic distribution of all the pathogens included in the study, giving the study international significance.