Studies to consider the possible origins of three canker pathogens of *Eucalyptus* in South Africa

Submitted by

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This thesis is dedicated to my late grandfather

Livio Scribante

Wissen ist Macht wie schief gedacht

Wissen ist weinig

Können ist König

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Our Creator, God Almighty

PREFACE

A wide range of *Eucalyptus* spp. are grown commercially in plantations world-wide. Either as exotics or native, these trees are important hardwood resources used in a number of industries. Currently, approximately 19.3 million ha of commercial *Eucalyptus* plantations exist world-wide with a predicted growth of approximately 3 million ha per annum. However, it is widely recognised that *Eucalyptus* plantations are threatened by diseases. The pathogens that cause these diseases can include those native or exotic to areas where *Eucalyptus* spp. are propagated.

Chapter one of this thesis presents a review of the world-wide movement of plant pathogens, particularly in forestry. The means by which pathogens spread is discussed and the effects of the increased movement of humans and products on the spread of pathogens are considered. The impact of introduced plant diseases is illustrated using case studies of past social, economic and environmental impacts. The importance of determining the origin of pathogens and the means to accomplish this is also reviewed. The review reaches the conclusion that a holistic knowledge about pathogens is important for effective quarantine and control measures. This sets the stage for the rest of the thesis and highlights the important recent discovery of *Eucalyptus* pathogens on closely related hosts.

The experimental section of this thesis focuses on stem canker pathogens of *Eucalyptus* collected from alternative hosts belonging to the families Myrtaceae and Melastomataceae. These two families of trees reside in the Myrtales and based on molecular DNA sequence comparisons are known to be closely related. *Tibouchina* spp. reside in the Melastomataceae and are native to South America. Although they have no economic value, they are very popular as ornamentals and have been planted in a number of countries were *Eucalyptus* spp. are grown in plantations. South Africa has a number of native tree species in the Myrtaceae, including *Syzygium* spp. These species occur naturally in the same areas where *Eucalyptus* forestry is practiced commercially. Both *Tibouchina* and *Syzygium* spp. have been shown to be hosts of *Cryphonectria cubensis*, an important stem canker pathogen of *Eucalyptus* spp. A key aim of this thesis was to study canker pathogens of *Eucalyptus* spp., occurring on *Tibouchina* spp. and native *Syzygium* spp.

Cryphonectria cubensis causes a serious canker disease of many *Eucalyptus* spp. Species of *Eucalyptus, Syzygium* and *Tibouchina*, are hosts of this pathogen. Cryphonectria canker results in the formation of cankers and mortality of juvenile trees. It occurs in tropical and sub-tropical regions where high relative humidity and temperatures prevail. This disease has caused serious losses for many commercial forestry companies and has influenced the development of clonal *Eucalyptus* propagation in South Africa.

Recent studies based on morphology and DNA sequence data have indicated that *C. cubensis* represents three distinct taxonomic units and that it should possibly not reside in the genus *Cryphonectria*. The three groups encompassed in *C. cubensis* are represented by isolates from South America and central Africa, south east Asia and South Africa. Previously it was hypothesised that *C. cubensis* originated from Indonesia on *S. aromaticum*. In light of the new taxonomic data, this is, most likely not true for all three species. In the second chapter of this dissertation, I report the discovery of South African *C. cubensis* from native *Syzygium* spp. in South Africa. I discuss the importance of this finding related to the origin of South African *C. cubensis* and consider the pathogenicity of the isolates from *S. cordatum* on the native host as well as on *E. grandis*.

Chapter three of this dissertation considers the relatedness of three populations of *C. cubensis* on two exotic (*Tibouchina* and *Eucalyptus*) and one indigenous (*Syzygium*) South African hosts. The genetic structure of the three populations was determined using polymorphic DNA markers to provide some insight into the possible origin of the fungus and the kinship of the three populations. This chapter provides important information pertaining to the probable origin of South African *C. cubensis* on the exotic hosts.

Chapter four of this dissertation reports on the discovery of an undescribed *Endothiella* sp. on *Tibouchina* spp. from Australia. This fungus is characterised using DNA sequence data and morphological comparisons. The role of this fungus in causing disease was considered and the potential threat to commercial forestry and the native vegetation of Australia was discussed.

Botryosphaeria spp. are important pathogens of *Eucalyptus* spp. and various other plant genera including native plants and economically important agricultural crops. Chapter five of this dissertation reports on a survey of *Tibouchina* spp. growing in South Africa, New Zealand and Australia to determine which *Botryosphaeria* spp. occur on these trees. *Botryosphaeria* spp. infecting these trees are also compared with those infecting *Eucalyptus* spp. The comparisons are based on morphological observations and DNA sequence data. The pathogenicity of these isolates was, furthermore, tested under greenhouse conditions.

The impact and threat of exotic plant pathogens to economically important crops and to native ecosystems is increasing. Many factors are associated with this threat, not least the growing global trade, tourism and climate change. This dissertation includes studies that highlight the importance of monitoring not only commercial crops but also plants belonging to closely related genera that might act as reservoirs and alternate vectors for pathogens.

CHAPTER 1

THE MOVEMENT OF PLANT PATHOGENS ON A WORLDWIDE SCALE, THE IMPACT AND THREATS THEY POSE

INTRODUCTION

Since early times bacterial, fungal and viral pathogens have moved from their native ranges to "new" areas where they have, in many cases, become established. These pathogens are known as exotic, invasive or non-indigenous organisms. In order to discuss the movement of these pathogens, as well as the impact and threat they pose, one needs to clearly define the three above-mentioned terms.

There is no widely accepted definition for the word "non-indigenous", except that it is "the opposite of indigenous" (Flower & Flower 1969). Therefore, to define non-indigenous, one needs first to define "indigenous". Flower and Flower (1969) define "indigenous" as native or naturally belonging in an area. They further define "native" as "inborn, innate, derived from ones country or found in pure or un-combined state". Allen & Humble (2002) define a non-indigenous organism as one that occurs outside its natural ecosystem, therefore, not referring to movement between continents or countries, but between ecosystems. For this review, I will use "non-indigenous" to refer to organisms that did not originate in the specific area in which they are currently found.

The word "exotic" is generally used as a synonym for non-indigenous (Bogus *et al.* 1968). It, furthermore, describes an organism as possessing characteristics foreign to an area, with the organism not being naturalized. Bogus *et al.* (1968), however, do not define "naturalized". In a biological context, this could imply that it is not yet established, or not yet in a state of equilibrium. Flower & Flower (1969) clearly define "exotic" as "introduced from abroad". Ilson *et al.* (1984) also define exotic organisms as those from another part of the world, possessing "the charm of the unfamiliar". For this review, I use non-indigenous to refer to organisms that are introduced into an area where they did not previously occur, not necessarily from abroad or from another part of the world, but from another geographic area.

Another term often used when discussing the movement of pathogens is "invasive". This generally describes the means by which an organism has established itself and how conspicuous the result of the establishment has been. Ilson *et al.* (1984) define "invasive" as the tendency to spread or invade and use "aggression" to describe

invasiveness. Flower & Flower (1969) rather use the word "encroach" to define "invasive". "Encroach" is further defined as "intruding" (Flower & Flower 1969). In essence, "invasive" describes the intrusion of an organism from one area to another with aggression. "Intruding" implies the unwanted presence of the organism. This is also the context in which "invasive" will be used in this review.

In this review, I consider the world-wide movement of pathogens, especially those of trees. This is clearly a very broad subject that has been the topic of many previous reviews. It is impossible to treat even a small number of the diseases that have been studied and I thus make extensive use of examples. These examples are mainly those of tree pathogens, but examples from agriculture are also used in some cases. My aim is to provide a broad background to the subject of how pathogens spread and to consider prospects for reducing outbreaks of new diseases caused by non-native pathogens in the future. This review also serves as a foundation for a series of studies presented in this thesis. These studies focus to some extent on alternative hosts of some important stem canker pathogens of *Eucalyptus* spp., in areas where these trees are grown as exotics in plantations.

MEANS BY WHICH PLANT PATHOGENS SPREAD

It is reasonably well established that non-indigenous, exotic or invasive organisms are introduced from geographical areas other than those in which they are native. The means by which they spread or move deserves discussion. This is a crucial aspect of the review as it provides an understanding of not only how organisms move from one geographical area to another, but also provides an insight into preventing or restricting the movement of pathogens. Care must be taken not to confuse the natural expansion of a pathogen's range with spread or intrusion of the pathogen. Expansion is a natural process forming part of the normal dispersal of pathogens (Brown & Hovmøller 2002).

Agriculture mostly relies on a small fraction of many thousands of plant species world-wide, resulting in large areas of monoculture and low genetic diversity. Risk of global spread of plant pathogens has increased due to this limited genetic variation of crops, brought about by breeding programs and the increased use of clonal germplasm. Wheat, maize, coffee and banana immediately come to mind as examples of single

genotype crops covering vast areas in many countries. Coffee and bananas are two extreme examples where large areas are planted with single clones or cultivars (Stover & Simmonds 1987). As most of these crops and other plant material have been moved around the world, pathogens and other organisms have inadvertently moved with them (Mathys & Baker 1980).

The movement of pathogens can be divided into two main categories. These are long and short distance forms of dispersal. Long distance dispersal occurs in one step and is usually unnoticed at first (Nagarajan & Singh 1990, Agrios 1997). Examples of pathogens dispersed in this manner are the spread of the Dutch elm pathogens *Ophiostoma ulmi* (Buis.) Nannf. and *O. novo-ulmi* Brasier from Asia to Europe and the United States (Brasier 2001) and the wheat rust pathogen, *Puccinia graminis* Pers., from Mexico to Canada (Viljanen-Rollinson & Cromey 2002). Some biotrophic fungi such as rusts, powdery mildews and downy mildew pathogens are specially adapted for aerial dispersal through the production of extremely high numbers of dry spores. This adaptation is also essential for the survival of some pathogens that depend on living plant material. Long distance dispersal, therefore, enables the pathogen to move from an area with no living host to an area with abundant host tissue. Short distance spread of pathogens tends to occur more gradually than long distance spread (Brown & Hovmøller 2002). It facilitates the spread of a pathogen within an area but does not, as in the case of long distance dispersal, support the survival of certain species.

Wind dispersal

Wind dispersal of fungal pathogens takes place over both short and long distances. Long distance dispersal in the air is an important survival strategy, enabling pathogen populations to colonize new areas. Most rust pathogens, for example, are dispersed by wind (Wilkinson & Spiers 1976, McKenzie 1998, Viljanen-Rollinson & Cromey 2002). It has thus been shown that sugar cane rust was almost certainly introduced into the Dominican Republic from Cameroon in West Africa through the transport of uredinio spores by cyclonic winds in 1978 (Brown & Hovøller 2002). Another example of a rust pathogen that has been dispersed by the wind from one area to another is coffee rust (*Hemileia vastatrix* Berk. & Br.) that was spread from Angola to Brazil (Carefoot &

Sprott 1967, Bowden, Gregory & Johnson 1971), presumably by wind currents. It is also suggested that *Phytophthora infestans* (Mont.) De Bary., the causal agent of potato blight, spread from Ireland to Russia by means of cyclonic winds (Carefoot & Sprott 1967).

Spread of plant pathogens on germplasm

An increasing number of plant pathogens apparently spread both long and short distances through the movement of infected plant material (Gibson, Christensen & Munga 1964, Gilmour 1967, Edwards & Walker 1978, Walker 1987, Wingfield *et al.* 2001a). Humans have moved plant material since the early days of sea travel and global exploration. Between 1764 and 1775 Benjamin Franklin, for example, sent plant material from his post in England to America. Large numbers of agricultural crops have been moved in similar fashion between countries to be established as exotics. Between 45% and 86% of various categories of pests introduced into California and the United States in recent decades, have entered that country on horticultural imports (Annonymous 1993, Palm 2001). It has, for example, been reported that the initial introduction of potato late blight into Europe from Mexico was on infected tubers in the 1840`s (Bourke 1964, Fry *et al.* 1993, Palm 2001).

Forest pathogens have been imported into countries on plant products such as timber, seeds and nursery stock (Wingfield *et al.* 2001a). Examples of such introductions are white pine blister rust (*Cronatium ribicola* J.C. Fisch.) introduced into Canada in 1910 on infected nursery stock (Allen & Humble 2002) and *Dothistroma septospora* Hulbary. introduced into various southern hemisphere countries, presumably on infected planting stock, seeds and cones (Gibson 1979, Millar & Minter 1980, Roux 1984, Wingfield *et al.* 2001a). A number of pathogens have also been hypothesised to have been introduced into countries on *Eucalyptus* spp. imported for timber and pulp production. These, for example, are thought to include species of *Mycosphaerella* Johanson (Park & Keane 1982, Crous & Wingfield 1996, Crous 1998), *Phaeophloeospora* (Hansf.) Walker. (Heather 1967, Chimpompha 1987, Crous, Knox-Davis & Wingfield 1989, Crous, Ferreira & Sutton 1997) and *Aulographina eucalypti* (Cooke & Massee) Arx & E. Müll. (Wall & Keane 1984).

Spread of plant pathogens on seed

Many pathogens are thought to have entered countries through the trade in seeds (Noble 1979, Richardson 1979, Walker 1987). *Claviceps africana* Freder, Mantle & De Milliano was introduced from Africa and Asia into Brazil (1995) and Australia (1996) on infected seeds (Bandyopadhyay *et al.* 1998), while urediniospores of *Puccinia antirrhini* Dietel & Holway. were introduced into Australia (1952) in seed lots (Walker 1987). Rees (1981) reported that he recovered 155 fungal species, of which 27 were plant pathogens, from tropical pine seeds. Seed borne pathogens that could cause flower, cone and seed losses, have also been reported to lead to 98% infection in a given *Pinus elliottii* Engelm. seed batch (Anderson, Belcher & Miller 1984).

One of the best forestry examples of the introduction of a plant pathogen into another country on seed is that of *Diplodia pinea* (Desm.) J. Kickx. [Synonym: *Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton] (De Wet *et al.* 2003). This fungus is an endophyte in all *Pinus* spp. (Smith *et al.* 1996) and is found wherever pines grow (Gibson 1979). *Diplodia pinea* is an opportunistic pathogen and disease symptoms are expressed when the host is subjected to unfavourable growing conditions and stress (Swart, Wingfield & Knox-Davis 1987, Zwolinski, Swart & Wingfield 1990). As all pine plantations in the Southern Hemisphere are exotic, *D. pinea* is hypothesised to have been introduced from the native regions of pine species into the Southern Hemisphere in the early development of exotic pine forestry in these regions (Gibson 1979). For the establishment of pine forestry in South Africa, Australia and New Zealand, seed stock was imported from Europe, Japan and California (Brown 1883, Fieldings 1975, Poynton 1977, Eldridge & Simpson 1987, Lavery 1986, Shepherd 1990 a,b, Burgess & Wingfield 2002). Seed was also moved between South Africa, Australia and New Zealand (Poynton 1977). Because phytosanitary and certification certificates were not required for trade in seed prior to the 1950's the movement of seeds and, therefore, *D. pinea,* took place unknowingly and without control (Burgess & Wingfield 2001).

Spread of plant pathogens on alternate and alternative hosts

Economically important, as well as non-commercial alternative and alternate hosts have played a significant role in the world-wide distribution of plant pathogens. *Puccinia graminis* Pers. Pers. (wheat stem rust), introduced into America in 1660 on Barberry (Yarwood 1983) is one of the first reported cases where an alternate host has resulted in the entrance of a pathogen into a new country. Pine needle rust caused by *Coleosporium* spp. is a threat to many countries producing pine timber, and could be carried on a number of ornamentals in the *Asteraceae* family, including *Aster, Callistephus* and *Solidago* spp. (Walker 1987). The introduction of plant pathogens on economically unimportant crops is not only difficult to track due to their lack of importance, but also complicated. Due to the relative unimportance of these hosts, the control and screening of these plants would not be sufficiently thorough to prevent spread. Alternative hosts are also important sources of introductions of plant pathogens. An example of how a plant pathogen was introduced into a country on an alterantive host is *P. infestans*. It is hypothesised that *P. infestans* was introduced into Ireland on infected tomato (Fry & Goodwin 1997). The spread of plant pathogens on these hosts are, however, easier to track due to the screening of these hosts themselves.

Spread of pathogens by insects

A number of cases have been reported where insects have carried plant pathogens, not only on a small spatial scale, but also beyond country borders. For example, a large number of the Ophiostomatoid fungi are known to be vectored by insects (Upadhyay 1981, Solheim 1986, Brasier 2001, Jacobs & Wingfield 2001, Rossman 2001, Wingfield *et al.* 2001b).

The best known example of a tree pathogen that has spread by means of insects is that of the Dutch elm disease fungi (Brasier 2001). The Dutch elm disease pathogens are transmitted by bark beetles in the genus *Scolytus* (Brasier 2001) and are devastating to susceptible elm trees. Currently, there are two fungal species responsible for Dutch elm disease, namely *O. ulmi* and *O. novo-ulmi* and they have been responsible for two separate pandemics in the history of the disease. The first Dutch elm disease pandemic, caused by *O. ulmi*, began in northwest Europe around 1910 (Brasier 2001). The disease

spread eastwards forming a number of epidemic fronts across Europe and into southwest Asia (Brasier & Kirk 2001). In 1927 it was introduced into the United Kingdom and North America and by 1930 reached central Asia (Peace 1960, Brasier 1990). By the 1940's the severity of the epidemic declined after approximately 40% loss of elms was recorded in most European countries (Peace 1960).

The second Dutch elm disease pandemic was caused by *O. novo-ulmi*. Although this pandemic was only noticed in the 1970's in Britain and the neighbouring regions of Europe, it is believed that the pandemic had actually begun in the 1940's at two different locations. Each of these locations was the source of two different forms of the fungus. The first region was the Maldova-Ukraine region in eastern Europe (EAN form) and the second the southern Great Lakes area in North America (NAN form) (Brasier 1990). The EAN form moved westward across Europe reaching the Netherlands by the 1970's. The pandemic then spread to Southwest Asia (Brasier 2001). The NAN form spread across the North American continent and was present from the west to the east coast by the 1980's (Brasier 2001). The NAN form was also introduced into Britian from Canada during the 1960's (Brasier & Gibbs 1973). The second pandemic was more severe than the first. This was attributed to a more virulent form of the fungus and the late detection of the introduction due to mis-identification of the pathogen and a resulting lack in efficient quarantine (Brasier 2001). This pandemic lead to the mortality of most mature elms in Europe and approximately 30 million elms in the United Kingdom. In Northern America, the impact has been catastrophic and resulted in the death of hundreds of millions of elms (Brasier 2001).

A more recent example of the role that insects have played in the distribution of forest pathogens is from Canada. In 1998, the mortality of *Picea rubens* Sarg. (red spruce) trees was noted in the Point Pleasant Park, Halifax, Nova Scotia (Jacobs *et al.* 2003). This raised concern amongst local politicians, residents, foresters and environmentalists. Investigations into the death of the trees revealed that the causal insect was the bark beetle *Tetropium fuscum* (Fabr.) (Coleoptera: Cerambicidae). This beetle is exotic in Canada and native in northern and central Europe and Siberia (Smith & Hurley 2000). In Europe, *T. fuscum* is considered as one of the most economically important longhorn beetles on conifers (Hellrigl 1974) attacking stressed and recently dead trees (Schimitschek 1929, Hellrigl 1974). It also has a close association with *Ophiostoma*

spp., which result in blue staining of timber. After investigation of the dying trees in Canada, *Ophiostoma tertropii* Math. was commonly found in trees attacked by *T. fuscum* and was the most frequently isolated fungus from this source (Jacobs *et al.* 2003). *Ophiostoma tetropii* was, however, not isolated from *T. cinnamopterum* Kirby., the most common *Tetropium* sp. found in Canada. Jacobs *et al.* (2003) also found that the *O. tetropii* isolated from the dead trees and the galleries of *T. fuscum*, was morphologically and phylogenetically identical to the fungus associated with the same beetle in Europe. This indicated that *O. tetropii* was moved into the country from its centre of origin in Europe on the bark beetle *T. fuscum*.

Spread of plant pathogens assisted by humans

A large amount of plant material is moved around the world, but this is not nearly as great as the number of humans that commute around the world for business and pleasure. Movement of people certainly facilitates the movement of plant pathogens (Allen & Humble 2002). Humans have surpassed natural forces as the main global dispersers of vascular plants (Mack & Lonsdale 2001, Palm 2001). Although humans do not spread diseases knowingly, this happens inadvertently. This occurs through the movement of seeds, souvenirs made of plant material, the movement of ornamental plants, on baggage and even on clothes. Wellings, McIntosh & Walker (1987) reported that wheat yellow rust (*Puccinia striiformis* Westend.) was introduced into Australia from Europe on clothes. During a period of four weeks of sampling clothes and baggage at Wellington airport, it was estimated that 198 270 000 viable rust urediospores were brought into New Zealand (Sheridan 1989). It has also been shown that the *Eucalyptus* rust pathogen, *P. psidii* Winter can be isolated from clothes, camera bags and footwear of researchers working on this important forestry pathogen (Langrell *et al.* 2003). These are only two examples of the impact that humans are having on the spread of plant pathogens.

Spread of plant pathogens by inconspicuous means

There are many less prominent and inconspicuous means by which pathogens move around the world. These include soil (Booth & Gibson 1972, Gibson 1979, Germishuizen 1984, Wingfield *et al.* 2001a), wooden products (Walker 1987) and even

packaging material (Campbell 2001). One forestry example is provided by *Rhizina undulata* Fr., one of the best-known soil-borne pathogens of *Pinus* spp. (Booth & Gibson 1972, Gibson 1979, Germishuizen 1984, Wingfield *et al.* 2001a). It has been hypothesised that *R. undulata* was introduced into South Africa with soil from the Boreal region (Booth & Gibson 1972, Gibson 1979, Germishuizen 1984, Wingfield *et al.* 2001a) when pine plantations were established in the country. Pathogens have also been moved around the world on unprocessed lumber, logs, wood packaging, dunnage and wood chips (Campbell 2001).

The wood wasp, *Sirex noctilio* F., is a vector for *Amylosterium areolatum* (Chaillet) Boidin and together these two organisms have the potential to cause serious damage and mortality to conifer species including *Pinus*, *Abies*, *Picea* and *Pseudotsuga* (Spradberry & Kirk 1978, 1981). In the Northern Hemisphere, where the wood wasp is native, a natural balance exists between the wasp, its symbiotic fungus and their tree hosts, to such an extent that they are considered secondary invaders (Hall 1978, Spradberry & Kirk 1978). The *S. noctilio/A. areolatum* complex was introduced into New Zealand in 1900 (Gourley 1951, Gilmour 1965, Chou 1991), Australia in 1961 (Gaurt 1970, Talbot 1977, Neumann and Minko 1981, Madden 1988) and South Africa in 1994 (Baxter, Rong & Schutte 1995, Tribe 1995, Wingfield *et al.* 2002) most likely within conifer packaging. In these Southern Hemisphere countries, the complex causes severe damage to non-indigenous *Pinus* plantations (Neuman & Marks 1990, Chou 1991, Bedding 1995) and is considered a primary problem of great economic concern (Slippers *et al.* 2000).

IMPACT OF NON-INDIGENOUS PATHOGENS

In order to appreciate the importance of the world-wide movement of plant pathogens it is necessary to consider the recorded impacts that some of these introductions have had. The impact of pathogens can be divided into three main groups, namely social, economic and environmental. In each of these categories at least one example is provided.

Economic impacts

Major losses have been recorded in plantation forestry world-wide. For example, Zwolinski *et al.* (1990) calculated the annual loss due to *D. pinea* infection of hail stressed *Pinus* spp. in South Africa at R5000-00/ha. Wright & Marks (1970) and Currie & Toes (1978) calculated the increment and potential merchantable volume loss of *Pinus* spp. for Australia and New Zealand due to *D. pinea* at 40% and 60% respectively. Millions of dollars of losses to *P. radiata* in New Zealand, Australia, Chile and Kenya (Wingfield *et al.* 2001a) are associated with crop or yield loss, decrease in quality of product and cost of control as a result of *D. septospora* infection.

Approximately 65% of losses in United States crops and forests have been attributed to introduced pathogens (Madden 2001). Infected seeds can result in the failure of germination, the death of entire seedling beds or lead to field infection after transplanting where additional losses could occur (Anderson *et al.* 1984). Yield losses due to rust diseases of cereals have been estimated at 10% of world production with losses of up to 70% for certain crops (Viljanen-Rollinson & Cromey 2002). Palm (2001) estimated the annual loss due to such introductions at approximately US$30 million. The eradication of Citrus canker in the United States, for example, cost a total of US$200 million (Madden 2001). These losses do not include the losses due to trade restrictions, embargoes (Allen & Humble 2002) or breeding programs to resolve the disease problems.

Environmental impacts

It is difficult to estimate the impact of exotic pests on the environment. It is, however, clear that these invaders harm the environment and influence the ecological balance of invaded areas (Vitousek *et al.* 1966, Walker 1987, Old & Dudzinski 1998, Palm 1999, Allen & Humble 2002). Approximately 49% of all imperilled plant species in the United States are threatened in part by alien plant pathogens (Wilcove *et al.* 1998). These alien species can change the community and forest composition of an area (Keever 1953, Mackey & Sivec 1973).

Drastic changes can combine to form debilitating chain reactions altering environmental conditions even on a micro spatial scale. Pathogens affecting the flora could have a severe impact on the fauna by altering animal habitats and food stocks (Franklin, Shugart & Harmon 1987) and in that way animal and bird diversity (Mlot 1991). Blister rust on *Pinus albicaulis* Engelm. has, for example, affected grizzly bear and nuthatch populations (Byler, Marsden & Hagle 1990, Allen & Humble 2002). These animals feed on pine nuts, therefore, a reduction in the flora population severely impacted the fauna.

Possibly the most comprehensively researched impact of invasive pathogens on indigenous vegetation and ecosystems is that asociated with *Cryphonectria parasitica* (Murr.) Barr. on the American chestnut *Castanea dentata* (Marsh.) Borkh. The American chestnut was once a dominant canopy species in the hardwood forests of the eastern United States (Anagnostakis 1987). The fruit produced by the tree was an important food source for wildlife, domestic livestock and enjoyed by humans (Anagnostakis 1987).

Chestnut blight was first noticed by Merkel in 1905 (Anagnostakis 1987) on ornamentals in the New York Zoological Gardens. *Cryphonectria parasitica* soon spread from New York and an epidemic began, which could not be stopped even though drastic chemical control was attempted (Murrill 1906). Chestnut blight progressed rapidly throughout the entire native range of chestnut trees. By 1938 the epidemic had moved abroad to Italy (Woodruf 1946) and France (Anagnostakis 1987) infecting European chestnut trees (*Castanea sativa* Mill.). Subsequent studies indicated that the fungus was spread by insects, birds, animals and possibly wind (Anagnostakis 1987). The epidemic led to the devastation of the North American chestnut tree (Anagnostakis 1987, Shearer & Tippett 1989, Castello, Leopold & Smallidge 1995, Hubbes 1999, Palm 2001). Within 50 years this, once dominant forest tree, was reduced to a multiple stemmed, low shrub (Anagnostakis & Hillman 1992). The introduction of the Japanese chestnut tree into the United States, which is resistant to infection by *C. parasitica,* has been implicated as the source of the destructive pathogen (Anagnostakis 1987, Palm 2001). The American landscape has been changed forever by chestnut blight with many possible impacts that remain unknown or poorly defined.

Social impacts

One of the best-recorded examples of the impact of a forestry pathogen on a social scale is the blight of chestnut trees caused by *C. parasitica* (Anagnostakis 1987). The wood of the chestnut tree is extremely resistant to decay due to the high levels of tannins present in the wood and bark. The high value timber was used in construction, woodwork, furniture, fencing, barrel staves and musical instruments (Gibson 1913). The fruits of the American chestnut were an important food source for wildlife, domestic livestock and humans (Anagnostakis 1987). The die-back of this native tree species due to chestnut blight led to a productivity decrease of between 15-20 % (Boyce 1961). The tannin industry, on which a number of communities largely depended, was brought to a halt (Boyce 1961). The industry managed to continue by utilizing dead trees, but the end was inevitable and thousands of people were left unemployed when the once dominant hardwood tree had disappeared from the forests.

There are many social impacts of pathogens on agriculture, including that associated with wheat stem rust caused by *Puccinia graminis* f. sp. *tritici* Eriks. & E. Henn. This pathogen has caused a scarcity of wheat in Europe and the United States. In Europe this resulted in the use of rye and in the U.S.A. corn as alternatives (Palm 2001). A more severe impact is that of ergot of rye caused by *Claviceps purpurea* (Fr.) Tulasne in France, which lead to the death of 40 000 people due to toxins produced by the fungus (Waterford & White 1982).

The most severe impact that an introduced pathogen has had on society is that associated with *P. infestans*, the cause of the Irish potato famine (Large 1940, Waterford & White 1982, Palm 2001). The first outbreak of the disease was in 1845 in Ireland (Large 1940, Carefoot & Sprott 1967, Waterford & White 1982, Palm 2001). The disease resulted in shortages of potatoes, the staple diet of the Irish people. This resulted in the death of large numbers of people and the neglect of education when children were kept out of school to forage for food (Carefoot & Sprott 1967).

The first death by starvation, as a result of the potato famine was recorded in August 1846 (Carefoot & Sprott 1867). During the period 1846 to 1866, two million people emigrated and 1 million died as a result of the famine (Large 1940, Waterford & White

1982, Palm 2001). Many more died in quarantine camps in America and England due to diseases associated with poor living conditions. *Phytophthora infestans* soon spread to European countries by means of wind (Carefoot & Sprott 1967). In 1848, hunger lead to a revolution in Europe subsequantly resulting in political changes. In 1872, blight continued to destroy millions of potato plants and tubers in England and Ireland. In 1879 crop losses amounted to 6 million pounds Sterling (Carefoot & Sprott 1967) and in 1916 late blight lead to the death of seven hundred thousand Germans (Carefoot & Sprott 1967). The disease was only brought under control in 1950 when Dr. Lindley produced a number of resistant potato varieties (Carefoot & Sprott 1967, Agrios 1997).

MEANS TO COMBAT THE SPREAD OF PLANT PATHOGENS

Quarantine measures

In order to combat the impact of invasive plant pathogens, strict control measures are needed. Since 1660, quarantine measures have been used to successfully limit the worldwide movement of pathogens. The first form of quarantine was in France when the movement of barberry, the alternate host of the wheat rust pathogen *P. graminis* (Yarwood 1983), was prevented to stop the spread of the pathogen (Palm 1999). The first plant quarantine regulations were established in Europe in 1870 after the introduction of several pests such as grapevine downy mildew [*Plasmopara viticola* (Berk. and M.A. Curtis) Berl. & De Toni.] on grapes from the United States of America into France (Palm 2001).

In 1873, Germany and the United Kingdom established a quarantine programme to prevent the introduction of Colorado potato beetle from the U.S.A. (Waterford & White 1982). The first federal plant quarantine law was established in the U.S.A. in 1912 (Waterford & White 1982). This law was initiated due to a donation of 2000 Japanese cherry trees from Tokyo to the U.S.A. These trees had to be destroyed after it was found that they were infected with an array of pests and diseases.

Since the establishment of the first Federal Plant Quarantine Act in the U.S.A. in 1912, quarantine has played a significant role in the fight against pathogen spread (Walker 1987, Palm 1999, Burgess & Wingfield 2001). This is true both in forestry and in

agriculture. The basis of quarantine is to screen imported commodities for possible pests, thereby preventing unwanted introductions. This is most strongly imposed at ports of entry into countries such as at border posts, harbours and airports.

It is well known that most introductions occur near shipping ports (Allen & Humble 2002). Screening imported commodities for plant pathogens is, however, difficult due to the quantity of entries into a country. The nature of import commodities sampled, methods and intensity of sampling, and the capacity to identify samples (e.g. insects, larvae, fruits, fungal spores and vegetative tissue) makes quarantine extremely difficult (Allen & Humble 2002).

The incubation period of non-indigenous species entering a country could be a complicating factor in the screening of non-indigenous species. Some species could enter a country on material without any screening system detecting them, due to long incubation periods of the organisms. While fungi, nematodes and bacteria can generally be isolated and identified from plant samples entering a country, some insects may need an incubation period before the adults exit the samples. This is quite frequent on, for example, dry timber such as packaging crates. Such insects could also vector pathogens (Gourley 1951, Gilmour 1965, Gaurt 1970, Talbot 1977, Neumann & Minko 1981, Walker 1987, Madden 1988, Chou 1991, Tribe 1995, Baxter *et al.* 1995, Wingfield *et al.* 2002). An example is provided where *Picea abies* (L.) Karsten bolts were used to brace large granite blocks inside shipping containers. More than 2500 adult insects representing more than 40 species of bark beetles, wood borers, and their associated parasitoids, nematodes and blue stain fungi (Allen & Humble 2002) were isolated from these bolts after an incubation period.

For quarantine to be successful it is important to know what pathogens are present in the countries of origin of the plants and which of these organisms might pose a threat to the importing country. Plant pathogen risk assessments form the basis for gathering such information. In this way, the likelihood that an organism will arrive, survive and thrive in a new habitat, can be determined. Pathogen risk assessments enable quarantine officials to focus on the most important plant pathogens (Palm 2001).

There are a number of factors that complicate crucial steps in the quarantine process. A major limiting factor in effective quarantine is the limited knowledge pertaining to fungi. Hawksworth (1991) estimated that only 5% of the worlds 1 500 000 fungi are known, with a mere 1% of these fungi held in culture collections. Campbell (2001) supported this view and estimated the percentage of named fungi to be less than 5% of the total. Walker (1987) stated that the huge number of taxonomic errors that exist, have complicated quarantine even more. This leaves a major gap in the protection system. In the past, focus has been placed on species identifications and this is an area that remains poorly studied.

In terms of identification, effective quarantine needs to delve more deeply than species level identifications. In this regard, pathogens need to be viewed as components of populations (Wingfield 2003). In pathogens capable of sexual reproduction, newly introduced genotypes crossing with existing genotypes or the hybridisation of closely related species would allow for greater gene diversity and a greater risk of pathogens overcoming tree resistance.

A number of general and more civilian measures could be taken to improve quarantine and the control of invading organisms. A cornerstone of this strategy would be the quality of staff at airports, border posts and ports (Wylie & Peters 1987). Public awareness should also be increased and could lift the burden on officials and personnel in areas where quarantine is enforced (Wylie & Peters 1987). Once an invasive organism has been noted rapid response and action is crucial. Delayed control could lead to huge financial losses and serious environmental impacts. For example, delayed control of *D. septospora* in Australia, brought about by bad weather conditions, is believed to have resulted in the subsequent spread of the disease (Eldridge & Simpson 1987).

There are approximately six examples of fungal species hybridisation (Brasier & Mehrota 1995). An excellent example is found in the case of Dutch elm disease. Limited sexual recombination between *O. ulmi* and *O. novo-ulmi* has been shown (Brasier 2001). Although it was believed that the progeny would not be able to survive in nature due to low fitness (Kile & Brasier 1990), recent research has indicated that *O. ulmi* - *O. novo-ulmi* hybrids exist in nature, but disappear when in competition with the

parent species (Brasier *et al.* 1998). Despite the low fitness of these hybrids, they could provide a "genetic bridge" allowing gene flow from one species to another possibly leading to new, more aggressive forms of the fungus (Brasier 2001). Hybridisation has also been shown between the North American (NAN) and Eurasian (EAN) races of *O. novo-ulmi* (Brasier 2001). These "new" hybridised forms of the fungus have been found to replace the pure strains in nature (Brasier 2001) and could represent a higher level of fitness.

Another example of a forest pathogen that has been considered from a population perspective is *Diplodia pinea*. This pathogen is a cosmopolitan endophyte on *Pinus* spp. and, therefore, little attention has been placed on quarantine to prevent its spread. Burgess and Wingfield (2002), however, stressed the importance of viewing the pathogen as part of a population. They reported on the impact that multiple introductions of the fungus could have on control in the sense that the introduction of multiple or new genotypes could also reduce the success of programmes aimed at breeding resistance. De Wet *et al.* (2000) also reported differences in aggressiveness in the various morphotypes of *D. pinea*, once again indicating that the presence of a fungus does not mean that quarantine is not necessary.

Molecular detection methods and diagnostic tools

In order to optimise quarantine measures, it is becoming increasingly necessary to develop detection methodologies and diagnostic tools designed to identify a broad range of organisms. Traditional diagnostic methods for identifying fungi need to be expanded and optimised using DNA-based techniques that are becoming increasingly available. These tools could provide greater diagnostic capacity with higher resolution and greater precision. Together with traditional morphology-based approaches, these precautions should provide an adequate means to identify, detect and prevent the introduction of pests and diseases into a country (Seifert, Wingfield & Wingfield 1995).

These identification tools could be grouped under the collective term "systematics". Systematics is the science of discovering, organizing and interpreting biodiversity. It increases knowledge of the biodiversity and population structure of fungi, their biology, ecology, physiology and pathology. Risk assessment and decisions pertaining to

quarantine will logically be substantially enhanced by increased knowledge of pathogens (Walker 1987, Palm 2001).

An example where systematics has promoted the control of a pathogen has been in the case of Karnal bunt caused by *Tilletia indica* Mitra. This smut fungus is difficult to distinguish from the related but non-pathogenic *T. horrida* Takah. *Tilletia indica* has posed a threat to wheat producing countries world-wide and quarantine measures were initiated to prevent the spread of Karnal bunt. Due to international quarantine regulations, the entire U.S.A. export of wheat was threatened after the discovery of Karnal bunt in that country (Yakema *et al.* 1996). However, with information obtained by means of molecular systematics it was determined that it was actually the closely related *T. horrida* that had been introduced into the USA. Trade restrictions limiting imports from the U.S.A. were thus lifted and the U.S.A. was able to maintain most of the US$5 billion wheat export market in 1996 and 1997 (Palm 1999).

Molecular systematics has been crucially important in dealing with regulations relating to many other pathogens. An example is the confusion regarding the identity of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. and *Colletorichum acutatum* J.H. Simmonds. Despite similar morphological characteristics, research has shown that taxa within the *Colletotrichum* complex differ in biological characteristics and virulence (Palm 1999). Anthracnose of lupins (*Lupinus* spp.) is attributed to *C. gloeosporioides* (Palm 1999). Because the taxon was considered to be widely distributed, the movement of infected lupin seeds was not restricted. Anthracnose of lupins, however, suddenly became a restricting factor in lupin production in in the U.S.A. (Paulitz, Atlin & Gray 1995) and Australia (Sweetingham *et al.* 1995) when above average losses ascribed to *C. gloeosporioides* were experienced. Based on rDNA sequence data, the causal agent was later identified as *C. acutatum* (Sreenivasaprasad, Mills & Brown 1994), which based on morphological characteristics is easily confused with *C. gloeosprioides*. This error in identification of *C. acutatum* was corrected based on DNA sequence data and *C. acutatum* was elevated to quarantine status. This enabled quarantine to be streamlined and increased in effectiveness. This illustrates the value and growing importance of modern diagnostic techniques to identify pathogens.

DETERMINING THE ORIGIN OF INTRODUCED PLANT PATHOGENS

A crucial link in the management of plant diseases and the enforcement of quarantine legislation is a comprehensive knowledge pertaining to the origin of pathogens. Once the origin of a pathogen is known, risk assessment is improved and centres can be identified for possible research into control measures (Linde, Zhan & McDonald 2002). Linde *et al.* (2002) stated that the centre of origin of a pathogen is the most likely area to prospect for resistance genes, because these are the locations where co-evolution is most likely to have increased the frequency of resistance alleles in natural host populations. Thus, knowledge of the area of origin of a pathogen will greatly focus quarantine measures. Numerous techniques have been considered to determine the origin of a pathogen. Some are based on historical events, others on wind patterns and more recently molecular techniques have provided more direct results with higher resolution (Taylor *et al.* 1999).

The study of biological patterns and historical records

Purdy, Krupa & Dean (1985) studied cyclonic wind patterns and were able to determine that the origin of *Puccinia melanocephala* Syd. & Syd. infection of sugarcane in the Dominican Republic, was from West Africa. They were also able to identify 1978 as the year of introduction. There are many other examples of studies involving wind patterns to determine the origin of pathogens such as *P. graminis* Pers. f. sp. *tritici* Eriks. & L. Henn. that was introduced into Canada from Mexico (Viljanen-Rollinson & Cromey 2002), *H. vastatrix* that was introduced from Angola to Brazil (Bowden *et al.* 1971) and a number of other rust species (Wilkinson & Spiers 1976, McKenzie 1998, Viljanen-Rollinson & Cromey 2002).

The use of molecular techniques

A number of hypotheses regarding the origin of pathogens and other fungi have been based on the comparison of genetic diversity and the knowledge of genetic structure of pathogen populations (Anagnostakis 1977, Anagnostakis 1982, Anagnostakis, Hau & Kranz 1986, Coates 1988, Perkins 1991, Morelet, Lipari & Powell 1992, Leslie 1993, Van Heerden *et al.* 1997, Van Zyl *et al.* 1998, Van Heerden & Wingfield 2001). Such

knowledge has provided insight into the life history of pathogens, including the evolutionary processes that shape pathogen populations in agro-ecosystems (Linde *et al.* 2002). A number of the hypotheses have been formulated to show that populations with high genotypic diversity are more likely to be native to an area than pathogen populations with a low diversity (Anagnostakis *et al.* 1986, Redlin 1991, McDonald & McDermotte 1993).

One of the most common methods that has been used to conduct population studies has been based on vegetative compatibility groups (VCG) (Anagnostakis *et al.* 1986). Using this technique, along with the study of historical events, Burgess & Wingfield (2002) were able to determine the origin and time that *D. pinea* was introduced into South Africa, New Zealand and Australia.

With the growing availability of DNA-based methods, a number of techniques based on genotypic rather than phenotypic characteristics have been developed to study populations. These techniques not only makes it possible to calculate genotypic diversity in a fungal population, but also provide an insight into the genetic structure of populations. This is achieved by determining gene flow (Slatkin 1987, Slatkin & Barton 1989, Boeger, Chen & McDonald 1993), mode of reproduction, gametic disequilibrium and population differentiation (Agapow & Austin 2001). To examine fungal populations at such a fine spatial scale requires the use of genetic markers that can discriminate among individuals in a population, which are usually morphologically identical (McDonald & Martinez 1990). A number of methods based on these principles have been developed and used and these will impact positively on quarantine.

The first DNA markers to be used for fungal evolutionary studies were restriction fragment length polymorphisms (RFLPs). This technique has been used with great success in numerous studies (Jeffereys, Wilson & Thein 1985a, b, Taylor 1986, Perkins & Turner 1988, McDonald *et al.* 1989, Leuchtman & Clay 1989, Gilbert *et al.* 1990, McDonald & Martinez 1990, Smith *et al.* 1990) and continues to be widely used in population genetic studies (Taylor *et al.* 1999). This approach is advantageous as it is highly reproducible and co-dominant (Hantula, Dusabenyagasani & Hamelin 1996).

The analysis of randomly amplified polymorphic DNA (RAPD) is based on random priming of DNA with short primers (Welsh & McClelland 1990, Williams *et al.* 1990). This technique is similar to RFLP analysis in that it assays DNA sequence variation in short regions. Instead of analysing restriction endonuclease recognition sequences it focuses on polymerase chain reaction (PCR) priming regions (Williams *et al.* 1990) and has been particularly popular for the study of fungal population genetics (Hantula *et al.* 1996). The disadvantage of RAPD markers is that they are not co-dominant.

Amplified fragment polymorphism (AFLP) analysis is a PCR based assay that selectively amplifies DNA fragments (Maughan *et al.* 1996, Rosendahl & Taylor 1997). DNA is digested with two endonucleases and site-specific adapters are then ligated to the DNA fragments. Primers complementary to the adapters and to the restriction sites are designed with selective nucleotides added to the 3′ ends of the primers (Steinger *et al.* 2002). Thus, only DNA fragments with nucleotides flanking the restriction sites that match the selective nucleotides of the primers are amplified during PCR (Maughan *et al.* 1996, Mueller, Lipari & Milgroom 1996). Resolution of the resulting DNA fragments on standard sequencing gels allows for the detection of AFLP`s. Similar to RAPD analysis, AFLP assay requires no prior sequencing knowledge, but can detect a much greater number of loci than those detected by RAPD analysis. Therefore, one could screen thousands of samples or independent genetic loci (Young *et al.* 1988, Maughan *et al.* 1996, Mueller *et al.* 1996).

The amplification of microsatellites using flanking primers, followed by analysis of length polymorphisms within each locus is a more efficient and accurate technique in determining genetic structure of a population (Hantula *et al.* 1996). This technique has been used to reflect intra-specific relationships in fungal pathogen populations and genetic diagnostics as reported by Moon, Tapper & Scott (1999), Queller, Stassmann & Hughes (1993), Rafalski & Tingey (1993), Longato & Bonfante (1997), Neu *et al.* (1999), Lynn & Heun (1993) and Barnes *et al.* (2001). These markers enable the detection and characterisation of multiple alleles at a specific locus. A number of studies have used microsatellite markers to study population structure, genetic diversity and related questions (Queller *et al.* 1993). In recent years this approach has also been used successfully to study a number of tree pathogens (O'Donnell, Cigelnik & Nirenberg, 1998, Barnes *et al.* 2001, Carbone & Kohn 2001)

POSSIBLE THREAT OF PATHOGENS FROM NATIVE HOSTS TO NON-INDIGENOUS PLANTATION SPECIES

There are numerous examples where pathogens have moved from hosts native to a country onto exotic forestry species, impacting negatively on the non-indigenous plantation species. One of most interesting examples is that of *Eucalyptus* rust caused by *P. psidii*. This is the only rust able to infect the Myrtaceae and it is not known in the native range of *Eucalyptus* spp. (Coutinho *et al.* 1998). The pathogen was first reported from *Psidium pomiferum* L. in 1884 from its native habitat, Brazil (Winter 1884). In 1973, a serious outbreak of *P. psidii* on *Eucalyptus* was reported from Brazil where it led to excessive losses in nurseries and young plantations (Ferreira 1981). This fungus poses a huge threat to *Eucalyptus* forestry world-wide and also to species in the Myrtaceae in countries such as Australia where this family of plants shows a great diversity. The occurrence of the pathogen on *Callistemon speciosus* (Sims) CD. in South America (Ferreira 1989), another Australian native, has highlighted the threat *P. psidii* could pose to a number of native myrtaceous species in Australia. The cross infection of *P. psidii* from a native host to an introduced species is a good example of the unfortunate consequence of exposing a non-indigenous plant species to a native pathogen.

The canker pathogen *Cryphonectria cubensis* (Bruner) Hodges. was first reported on *Eucalyptus* in Cuba (Bruner 1917). This was followed by reports on a wide range of *Eucalyptus* spp. world-wide, including those from Northern Australia (Davison & Coates 1991), Asia (Florence, Sharma & Mohanan 1986), South and Central America (Hodges, Geary & Cordell 1979, Hodges *et al.* 1986) and Africa (Gibson 1980, Wingfield, Swart & Abear 1989). *Cryphonectria cubensis* has also been reported as an opportunistic pathogen causing die-back of *Syzygium aromaticum* (L.) Merr. & Perry (clove) in Brazil and Indonesia (Hodges *et al.* 1986). The pathogen was found to be more aggressive on *Eucalyptus* than on *S. aromaticum* (Hodges *et al.* 1986). Hodges *et al.* (1986) stated that this *S. aromaticum/Eucalyptus* connection was somewhat analogous to the situation with the chestnut blight fungus that posed no threat to its native host in its centre of origin (Asia) but had a devastating effect on the European and American chestnuts. This hypothesis led the researchers to believe that the origin

of *C. cubensis* is Indonesia, with *S. aromaticum* its native host and that it had cross-infected to *Eucalyptus*.

Until 2001, *C. cubensis* was known only on genera in the Myrtaceae. However, in 2001 the fungus was reported on native *Tibouchina urvilleana* (CD.) Long. and *T. lepidota* Baill. from Colombia, where the pathogen also occurs on *Eucalyptus* spp. (Wingfield *et al.* 2001b). These two species reside in the Melastomataceae, a family recently shown to be closely related to the Myrtaceae. Both families reside in the Myrtales (Conti *et al.* 1997). In the study by Wingfield *et al.* (2001b) sufficient evidence could, however, not be gathered to conclusively state whether the fungus had moved from the native *Tibouchina* spp. to the non-indigenous *Eucalyptus* spp.

In 2002, after the first report of *C. cubensis* on *Tibouchina* spp. from South Africa (Myburg *et al.* 2002a), Myburg *et al.* (2002b) reported that the South African fungus is distinct from the fungus with the same name, occurring in the rest of the world, based on ITS and β-tubulin and Histone *H3* gene sequences. This raises the question as to the origin of the South African fungus and the possibility of it having a native host in Africa. A number of native Myrtaceae occur in South Africa including *Syzygium cordatum* Hachst. and other *Syzygium* spp., *Eugenia* spp. and *Metrosideros angustifolius* (L.) J.E.Sm. These trees grow in close proxomity to *Eucalyptus* plantations and any of these native African Myrtales could represent the indigenous hosts of South African *C. cubensis*

CONCLUSIONS

The huge threat and potential impact of invasive plant pathogens, make it clear that all possible means should be exercised to combat new introductions. With changes in global trade patterns new introductions of pathogens are likely to continue to occur and most probably to increase in number. If increased efforts are not made to curb these introductions, the world stands to experience new and devastating disease problems equivalent in scale to late blight of potato in Ireland, the Chestnut blight in the United States and others.

In order to achieve reduced movement of pathogens, substantially increased knowledge of pathogens will become increasingly crucial. Means of spread, techniques for identification and detection, and areas of origin of high-risk pathogens must be determined. In this regard, new technologies are providing improved methods that are likely to reduce risks. However, these increased opportunities are counterbalanced by increased trade and movement of people and products, which clearly increase risks.

In developing strategies to reduce the movement of alien pathogens, it is important to consider trends and experiences of the past and to learn from previous oversights. Similarly, knowledge of successes can be used to improve current control strategies. As the problem of invasive species is cosmopolitan and relevant globally, it is clear that co-operative ventures between countries will be essential. Invasive species do not recognise political borders and, therefore, humans should adopt the same view. Only through co-operation between all parties involved, together with research, training and education will it be possible to combat the spread and reduce the threat imposed by invasive species.

In the future, I predict that we will increasingly have to deal not only with pathogens introduced into new environments, but also with pathogens moving from native to exotic hosts. These "new" pathogens will also threaten trees in their areas of origin. In the research chapters of this thesis, I deal with alternative hosts of some important canker pathogens of *Eucalyptus* spp. Some of these pathogens clearly threaten *Eucalyptus* spp. and perhaps other related trees, in their areas of origin. Hopefully these studies will contribute, at least in part, to reducing threats that have perhaps not previously been recognised.

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CHAPTER 2

DISCOVERY OF *CRYPHONECTRIA CUBENSIS* ON NATIVE *SYZYGIUM* SPECIES IN SOUTH AFRICA

ABSTRACT

Cryphonectria cubensis is a destructive pathogen on *Eucalyptus* species in South and Central America, South and Central Africa and South-east Asia. It also causes cankers on *Syzygium aromaticum* (clove) in South America, Central Africa and South-east Asia and on *Tibouchina* spp. (glory flower) in South America and South Africa. It has previously been suggested that *C. cubensis* was introduced into South Africa, possibly from South America. During disease surveys in indigenous forests of South Africa, fruiting structures resembling those of *C. cubensis* were found on native *S. cordatum* (water berry) and *S. guineense* (water pear). The fungus from these *Syzygium* spp. was identified based on morphological characteristics and β-tubulin gene sequences. Fruiting structures were identical to those of *C. cubensis* from *Eucalyptus* spp. in South Africa. Analysis of β-tubulin gene sequences of *C. cubensis* isolates confirmed this identification. Pathogenicity trials showed that the fungus is more virulent on exotic *Eucalyptus* spp. than on native *S. cordatum*. Results of this study strongly suggest that the fungus known as *C. cubensis* in South Africa is a native pathogen. This adds convincing evidence to the view that *C. cubensis* in South Africa is a species different to that occurring elsewhere in the world.

INTRODUCTION

Cryphonectria cubensis (Bruner) Hodges is one of the most threatening canker pathogens to *Eucalyptus* plantations in tropical and sub-tropical regions, globally (Boerboom & Maas 1970, Hodges, Geary & Gordell 1979, Sharma, Mohanan & Florance 1985a,b, Wingfield, Swart & Abear 1989). The disease, commonly known as Cryphonectria canker, favours relatively high temperatures, rainfall and humidity (Hodges *et al.* 1979, Sharma *et al.* 1985a,b). Currently, losses due to Cryphonectria canker are minimised by means of breeding and propagation of disease tolerant *Eucalyptus* clones and hybrids (Alfenas, Hodges & Jeng 1984, Conradie, Swart & Wingfield 1992).

Cryphonectria cubensis has been reported on three host genera in two families, residing in the order Myrtales. The fungus was first reported on *Eucalyptus* in Cuba (Bruner 1917). This was followed by reports on a wide range of *Eucalyptus* spp. world-wide, including those from Australia (Davison & Coates 1991), Asia (Sharma *et al.* 1985b, Florence, Sharma & Mohanan 1986), South and Central America (Hodges *et al.* 1979, Hodges *et al.* 1986) and Africa (Gibson 1980, Wingfield *et al.* 1989). *Cryphonectria cubensis* has also been reported as an opportunistic pathogen causing die-back of *Syzygium aromaticum* (L.) Merr. & Perry (clove) in Brazil and Indonesia (Hodges *et al.* 1986).

Until recently, *C. cubensis* was known to occur only on genera in the Myrtaceae. However, in 2001 the fungus was reported on native *Tibouchina urvilleana* (DC.) Logn. and *T. lepidota* Baill. from Colombia, where it causes girdling cankers and die-back of stems and branches of these ornamental trees residing in the Melastomataceae (Wingfield *et al.* 2001). Both these families, however, reside in the Myrtales and have been shown, based on molecular phylogenies, to be closely related (Conti *et al.* 1997). More recently, Cryphonectria canker has also been found on *T. granulosa* Cogn. in northern KwaZulu/Natal, South Africa (Myburg *et al.* 2002a). The discovery of *C. cubensis* on native Melastomataceae in South America has led Wingfield *et al.* (2001) to question the hypothesis that the fungus originated on cloves in Indonesia (Hodges *et al.* 1986). Based on sequence data for the ITS region of the ribosmal DNA operon, Myburg, Wingfield & Wingfield (1999) showed that isolates of *C. cubensis* from South-

east Asia and South America reside in discrete clades, with South African isolates grouping with those from South America. Biological differences between *C. cubensis* isolates from South Africa and the rest of the world (Myburg *et al.* 2002b) prompted subsequent studies using multiple gene trees including the ITS and β–tubulin genes. These studies showed that the South African fungus is distinct from the fungus with the same name occurring in the rest of the world. They also suggested that it probably represents a distinct species, possibly native to South Africa (Myburg *et al.* 2002b).

The aims of this study were to investigate the possible occurrence of *C. cubensis* on native Myrtaceae in South Africa and to compare isolates from native hosts with those from *Eucalyptus* and *Tibouchina* spp. in other parts of the world. This was done based on morphological characteristics, comparison of β–tubulin gene sequences and pathogenicity studies.

MATERIALS AND METHODS

Disease symptoms and collection of samples

Syzygium cordatum Hachst. and *S. guineense* (CD.) Willd. trees showing signs of die-back and the formation of cracks and cankers, were identified in many parts of the KwaZulu-Natal, Tzaneen (Northern province), Hazyview and Sabie (Mpumalanga province) areas of South Africa. Plant material bearing fruiting bodies resembling those of *C. cubensis,* was collected for further analysis. Material was incubated in moist chambers for one day to induce the production of spores. Single conidial and ascopore isolations were made on 2% Malt Extract Agar (MEA) (20g Biolab Malt Extract, 15g Biolab Agar, 1 litre water) and incubated at 25°C. Isolates are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

Morphological comparisons

Bark specimens with fruiting structures collected from cankers on *Syzygium* spp. were compared with *C. cubensis* specimens from *Eucalyptus* spp., used in previous studies

(Wingfield *et al.* 1989, Myburg *et al.* 2002a,b) (Table 1). For these comparisons, fruiting structures were embedded in Leica embedding medium and sectioned using a Leica CM1100 cryostat (Setpoint Technologies, Johannesburg, South Africa). Specimens were sectioned at –20 °C to a thickness of 12µm. The sectioned structures were subsequently studied using standard light microscopy. A size range was obtained for the conidiomatal and ascomatal stromata from two of each of these structures. Ten measurements were taken of conidiophores, conidia, asci and ascospores, for each collection. Colour notations used were those described by Rayner (1970). Bark specimens from the *Syzygium* spp. have been deposited in the herbarium of the National Collection of Fungi, Pretoria, South Africa (PREM) (Table 1).

DNA isolation and amplification

Isolates (Table 2) were inoculated into 1.5 ml micro centrifuge tubes containing 750 µl, 30 % Malt Extract Broth and incubated at 25 °C for four days. DNA was isolated as described by Van der Merwe *et al.* (2001). The β-tubulin gene region was amplified using primer pairs Bt1a/Bt1b and Bt2a/Bt2b (Glass & Donaldson 1995). Amplification of the β-tubulin gene regions was done according to Myburg *et al.* (2002b). Amplification reactions were performed on a Perkin Elmer GeneAmp PCR System 9700 thermocycler (Perkin-Elmer Applied Biosystems, Inc. Foster City, California). PCR products were visualised on 2% agarose-ethidium bromide stained gels using ultra violet light.

DNA sequencing

PCR products were purified using a High Pure PCR Product Purification Kit (Roche Diagnostic GmbH, Mannheim, Germany). DNA fragments were sequenced with the same primer pairs used in the PCR amplification reactions. An ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS (Perkin-Elmer, Warrington, United Kingdom) was used for the sequencing, using an ABI PRISM 3100™ automated sequencer. Sequences were aligned using ClustalX (Thompson *et al.* 1997) and manually adjusted using Sequence Navigator version 1.0.1

(Perkin-Elmer Applied BioSystems Inc., Foster City, California). All sequences obtained in this study have been deposited in Genbank (Table 2).

Data analyses were performed using PAUP* (Phylogenetic Analysis Using Parsimony) version 4.0b (Swofford 1998). Analyses were done using the heuristic search option with TBR (tree-bisection-reconnection) branch swapping. Gaps inserted during sequence alignment, were treated as fifth base (NEWSTATE). A bootstrap analysis (50% majority rule, 1000 replications) was done to determine the confidence levels of the tree branching points (Felsenstein 1995). Previously published sequences for *C. parasitica* (Murr.) Barr (Myburg *et al.* 1999) and *C. eucalypti* (Venter *et al.* 2001, Venter *et al.* 2002) were used for comparative purposes. *Diaporthe ambigua* Nits. was used as an outgroup taxon to root the phylogenetic tree. The stringency of the branch nodes was tested using Markov Chain Monte Carlo Algorithms (MCMC) (Larget & Simon 1999) in Bayesian Analysis (Lutzoni, Pagel & Reeb 2001). Random trees were obtained through 100 000 generations, with every 10th tree sampled. The first 1500 trees were discarded as the burnin period. A general time reversal model was used and four MCMC chains were run simultaneously in the analysis. The sampled trees were summarised in a consensus tree showing posterior probabilities of the branches.

Pathogenicity

In order to determine whether the fungus isolated from the *Syzygium* spp. was the causal agent of the cankers and die-back observed on the trees, and to consider the possible pathogenicity of the fungus on *Eucalyptus* spp., infection studies were performed. The *S. cordatum* and *E. grandis* trees for inoculation had stem diameters ranging from 10 to 23 mm and were arranged in a complete randomised design. All trees were maintained under greenhouse conditions for a two-week acclimatisation period, prior to inoculation. The greenhouse was subjected to natural day/night conditions and a temperature of approximately 25 °C was maintained.

In order to assess the reciprocal pathogenicity of isolates on *S. cordatum* and *Eucalyptus*, the fastest growing isolate from *S. cordatum* (CMW 9364) and an isolate (CMW 2113) from *E. grandis*, previously shown to represent a higher level of

pathogenicity in a population of *C. cubensis* isolates (Van Zyl & Wingfield 1999), were each inoculated into the stems of 20 *S. cordatum* and 20 *E. grandis* (clone ZG14) trees. Ten trees per species were inoculated with sterile MEA plugs to serve as controls. Wounds were made on the stems of trees approximately 150 mm above the soil level, using a 10 mm diameter cork borer. Mycelial plugs of a similar size were taken from the actively growing edges of 7-day-old cultures and placed in the wounds with the mycelium towards the cambium. Wounds were sealed with laboratory film (Parafilm "M", American National Can™ Chicago, IL.) to protect the inoculated fungus and cambium from desiccation.

Lesion lengths associated with the inoculations, were measured after six weeks. To determine the variance between isolates and between trees, inoculation data were subjected to analysis of variance using the General Linear Model procedure of SAS (SAS/STAT Users guide, Version 6, 1989).

RESULTS

Disease symptoms and collection of samples

A number of trees showing branch die-back and cankers, were found in all the areas surveyed. Disease symptoms included branch die-back associated with cracking of the bark, and the development of stem cankers. Fruiting bodies of a fungus, resembling the anamorph of *C. cubensis,* were found between the cracks and on dead areas of the stems, in all the areas surveyed. Teleomorph structures, resembling *C. cubensis,* were also common on trees in the Northern Province (30 % of isolates) and Zululand area (~25% of isolates). No teleomorph structures were found on trees in the Mpumalanga province.

Morphological comparisons

The fungus found on native South African *Syzygium* spp. had a morphology similar to that of *C. cubensis* from South Africa (Wingfield *et al.* 1989, Myburg *et al.* 2002a, b). *Conidiomata* were superficial to slightly immersed, pyriform to clavate (Fig. 1a, 1b), uni- to multilocular (Fig. 1b), blackened with an umber (13m) interior, 300-420(-450)

µm wide, base (280-)330-500 µm long, base above the bark surface (60-)80-320(-370) µm long (Fig. 1b). *Necks* connected to single or separate locules occurred per structure, and these were up to 570 µm long and (110-)120-160(-170) µm wide. *Conidiophores* were hyaline, cylindrical or flask shaped with attenuated apices, frequently septate, with or without branching underneath the septum, (7.5-) 9.5-15.5 µm long, 1-1.5 µm wide (Fig. 1c). *Conidiogenous cells* were enteroblastic, phialidic, determinate. *Conidia* were exuded as bright luteous (19) spore tendrils or droplets, hyaline, non-septate, oblong to oval, 3-3.5 µm long, 1.5 µm wide (Fig. 1d).

Ascomata were characterised by long perithecial necks emerging from the bark with weakly developed, predominantly prosenchymatous, cinnamon (13'') to orange (15) coloured stromatic tissue at the bases of the necks (Figs. 1e, f). *Perithecia* were semi-immersed in the bark, black, base globose, (220-) 230-400 (-490) µm in length and (250-) 270-350 (-380) µm wide (Fig. 1f). *Perithecial necks* were periphysate, up to 1220 µm long as they emerge through the bark, covered in umber (13m) tissue as it extends beyond the stroma giving them a black appearance (Fig. 1f). Extended parts of the perithecial necks were (100-) 106-150 (-155) µm wide, width of the actual perithecial neck (65-) 74-111 (-113) µm. *Asci* contained eight ascospores, were fusoid to ellipsoid, non-stipitate, with non-amyloid refractive rings, (26-) 27-31.5 (-32) µm long, 6-7 (-7.5) µm wide (Fig. 1g). *Ascospores* were hyaline, septate, fusoid to oval, with rounded apices, (5-) 5.5-6.5 (-7) µm long, (1.5-) 2-2.5 (-3) µm wide (Fig. 1g).

DNA sequencing

PCR amplification with the two primer pairs resulted in fragments of 537 bp (Bt1a/Bt1b) and 495 bp (Bt2a/Bt2b), respectively. The fragments were sequenced and resulted in sequences of 464 bp (Bt1a/Bt1b) and 425 bp (Bt2a/Bt2b), before alignment. Aligned sequences of the combined data resulted in a data set of 1016 characters, consisting of 599 constant characters, 317 parsimony-informative characters and 100 variable characters that were parsimony- uninformative. The heuristic search produced three most parsimonious trees with most variation in the clade including the South African strains (Fig. 2). A strict bootstrap consensus tree (length of tree = 684 steps, CI = 0.918, RI = 0.951, RC = 0.806 and HI = 0.139) was generated from the 100 variable characters and most branches were well supported with high bootstrap values (Fig. 2).

Posterior probability values calculated for the branch nodes supported the bootstrap values.

Results of the DNA sequence analysis support the outcome of the morphological comparison, with isolates from *Syzygium* spp. grouping with *Tibouchina* and *Eucalyptus* spp. from South Africa. This confirms the identification of the fungus as the same as the South African strain of *C. cubensis* (Fig. 2). The *Syzygium* isolates grouped within the South African *C. cubensis* clade, and separate from isolates in the South-east Asian and South American clades (bootstrap support 99%, posterior probability 100%), consistent with geographical origins of the isolates. The larger *C. cubensis* clade grouped separately from other *Cryphonectria* spp. (bootstrap support 100%, posterior probability 100%). The bootstrap support for the branch node separating the South American and south-east Asian groups (bootstrap support 51%), with no support using MCMC analysis, suggest that these two groups are closely related.

Pathogenicity

Inoculation of *S. cordatum* and the *E. grandis* clone (ZG14) with *C. cubensis* resulted in the formation of lesions on both hosts, within six weeks (Table 3). Some of the *Eucalyptus* plants showed signs of decline and produced epicormic shoots on the stems within this time period. Epicormic shoots were present on some *S. cordatum* trees, but no signs of die-back were visible. The control inoculations produced no lesions on either host. Isolates from both *Syzygium* and *Eucalyptus* were both more pathogenic on the *E. grandis* than on the *S. cordatum* trees ($P > 0.0001$) (Fig. 3, Table 3).

DISCUSSION

Cryphonectria cubensis has been known in South Africa since 1989, where it caused a serious disease of *Eucalyptus* spp. (Wingfield *et al.* 1989). Because of the common occurrence of this fungus on *Eucalyptus* spp. elsewhere in the world, it has been assumed that *C. cubensis* was accidentally introduced into South Africa (Van Heerden & Wingfield 2001). The discovery of the fungus on native South African trees in this study is intriguing and alters our view on the origin of the fungus. The fungus was found to be common on *S. cordatum* and *S. guineense* in several distantly located areas

of South Africa, discounting the possibility of a chance infection. Analysis of DNA sequence data also confirmed that the fungus on the *Syzygium* spp., is the same as that found on *Eucalyptus* and *Tibouchina* spp. in South Africa.

The survey resulting in the discovery of *C. cubensis* on native *Syzygium* spp. was planned to broadly cover the natural distribution range of these trees in South Africa. *Cryphonectria cubensis* was collected from all three major geographical areas surveyed. The occurrence of the fungus on *Syzygium* spp. in KwaMbonambi on the Zululand coast, a major *Eucalyptus* forestry region where Cryphonectria canker resulted in serious losses during the 1990`s (Van Heerden *et al.* 1997), might have suggested an origin of the fungus on *Eucalyptus*. However, the other regions surveyed on the Zululand coast (Amanzimgwenia, Kosi bay and Kosi mouth) are isolated from *Eucalyptus* plantings. Furthermore, the discovery of the fungus in the northern and eastern regions of the country where Cryphonectria canker is not known on *Eucalyptus,* even though susceptible trees are planted, was surprising. The climatic conditions in the latter areas are generally considered to be unsuitable for the disease. These findings provide strong evidence that the fungus that we have known as *C. cubensis* on *Eucalyptus* and *Tibouchina* in South Africa, represents a native fungus, originating on *Syzygium* spp.

The teleomorph of *C. cubensis* was found frequently on samples collected from native *Syzygium* spp. This is particularly interesting, because teleomorph structures are generally absent on *Eucalyptus* and *Tibouchina* spp. in South Africa. For example, Van Heerden & Wingfield (2001) conducted an extensive survey of Cryphonectria canker in *Eucalyptus* plantations and failed to detect the sexual structures of the causal agent. The teleomorph of the South African fungus has not been found frequently. It has been recorded once on *Eucalyptus*, where it occurred on the roots of *E. grandis* (Wingfield *et al.* 1989) and a limited number of times on *Tibouchina* spp. (Myburg *et al.*, 2002a). If it is true that the fungus is native on *Syzygium* spp., and has adapted to infect *Eucalyptus* spp., recombination may be occurring on native hosts and resulting in new genotypes of the fungus able to infect *Eucalyptus* spp.

Based on sequence data for the ITS region of the ribosomal DNA operon, Myburg *et al.* (1999) showed that isolates of *C. cubensis* from South-east Asia and South America

reside in discrete clades. In that study, South African isolates grouped with those from South America. A subsequent study using multiple gene trees, initiated due to the biological differences of *C. cubensis* in South America and South Africa, showed that the South African fungus is distinct (Myburg *et al.* 2002b). Results of the present study, showing that the fungus occurs on native trees, adds substantial evidence to the view that the South African fungus known as *C. cubensis,* represents a distinct taxon that is in all likelihood, native to Southern Africa.

Pathogenicity trials in this study showed that *S. cordatum* was more tolerant to infection by *C. cubensis* than the *Eucalyptus* clone tested. This result is not surprising, as a native host would be expected to be more resistant to infection than an exotic host of known susceptibility (Leppik 1970, Newhouse 1990). *Syzygium cordatum* trees inoculated in this study were all raised from seed and each plant represented a distinct genotype. In contrast, the *Eucalyptus* trees were all of a single genotype known to be susceptible to *C. cubensis*. Nevertheless, a highly pathogenic isolate from *Eucalyptus* did minimal damage on inoculated *S. cordatum* trees, providing strong evidence for a relatively high level of resistance in the native South African tree. The data also support the view that the fungus is probably native on *S. cordatum*.

Results of this study provide good evidence to suggest that the fungus known as *C. cubensis* in South Africa is native to this country. They also support the previous studies comparing species of *Cryphonectria* and related fungi based on DNA sequence data (Myburg *et al.* 2002a) that have shown that the fungus known as *C. cubensis* should probably reside in a discrete genus. Assigning a name to the South African fungus, prior to completion of phylogenetic studies at the family and generic levels, would be premature. However, we do expect that the fungus will represent a new species, residing in a new genus together with *C. cubensis sensu stricto.*

Discovery of a native host for the fungus known as *C. cubensis* in South Africa, and thus showing that the fungus on *Eucalyptus* probably originated on *Syzygium,* has important implications for forestry internationally. Previous studies have shown that the South African fungus is considerably more pathogenic than *C. cubensis* from elsewhere in the world (Roux *et al.* 1999). In pathogenicity tests, the South African fungus rapidly colonises the cambium and girdles trees (Van Zyl & Wingfield, 1999). This is different

to *C. cubensis* in South-east Asia and South America, which tends to cause stem cankers that develop less rapidly (Van Zyl & Wingfield, 1999). Clearly, the South African fungus is a threat to *Eucalyptus* forestry elsewhere in the world. Furthermore, it is probably not present in Australia and, if it were to enter that country, it could have a devastating effect on native Australian Myrtaceae. Every effort should thus be made to restrict its spread from South Africa.

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Table 1. Specimens used in the morphological comparisons.

Herbarium no.[a]	Identity	Host	Origin	Date	Collector
PREM 49379	*C. cubensis*	*E. grandis*	South Africa	1988	M.J. Wingfield
PREM 49377	*C. cubensis*	*E. grandis*	South Africa	1986	M.J. Wingfield
PREM 49378	*C. cubensis*	*E. grandis*	South Africa	1987	M.J. Wingfield
PREM 57293	*C. cubensis*	*E. grandis*	South Africa	2001	M. Gryzenhout
PREM 57357	*C. cubensis*	*T. granulosa*	South Africa	1999	J. Roux
PREM 57358	*C. cubensis*	*T. granulosa*	South Africa	1999	J. Roux
PREM 57294	*C. cubensis*	*E. grandis*	Colombia	2000	M.J. Wingfield
PREM 57297	*C. cubensis*	*Eucalyptus* sp.	Indonesia	2001	M.J. Wingfield
PREM 57474	*C. cubensis*	*S. cordatum*	South Africa	2001	R.N. Heath
PREM 57475	*C. cubensis*	*S. guineense*	South Africa	2001	M. Gryzenhout
PREM 57476	*C. cubensis*	*S. cordatum*	South Africa	2001	R.N. Heath
PREM 57477	*C. cubensis*	*S. cordatum*	South Africa	2002	R.N. Heath
PREM 57478	*C. cubensis*	*S. cordatum*	South Africa	2002	R.N. Heath
PREM 57479	*C. cubensis*	*S. cordatum*	South Africa	2002	R.N. Heath
PREM 57480	*C. cubensis*	*S. cordatum*	South Africa	2002	R.N. Heath

[a] PREM, National Collection of Fungi, Pretoria, South Africa.

Table 2. Isolates used for molecular comparison and pathogenicity trials.

Culture no.[a]	Isolate identity	Host	Origin	Genbank accession number
CMW 9364	*C. cubensis*	*S. cordatum*	South Africa	AY149284 [c]
CMW 9366	*C. cubensis*	*S. cordatum*	South Africa	AY149285 [c]
CMW 10036	*C. cubensis*	*S. cordatum*	South Africa	AY149286 [c]
CMW 10046	*C. cubensis*	*S. cordatum*	South Africa	AY149287 [c]
CMW 10076	*C. cubensis*	*S. cordatum*	South Africa	AY149288 [c]
CMW 10086	*C. cubensis*	*S. cordatum*	South Africa	AY149289 [c]
CMW 10092	*C. cubensis*	*S. guineense*	South Africa	AY149290 [c]
CMW 62	*C. cubensis*	*Eucalyptus* sp.	South Africa	AF273063 [b]; AF273458 [b]
CMW 2113	*C. cubensis*	*Eucalyptus* sp.	South Africa	AF273067 [b]; AF273462 [b]
CMW 8755	*C. cubensis*	*Eucalyptus* sp.	South Africa	AF273064 [b]; AF273459 [b]
CMW 9327	*C. cubensis*	*Tibouchina* sp.	South Africa	AF273060 [b]; AF 273455 [b]
CMW 9328	*C. cubensis*	*Tibouchina* sp.	South Africa	AF273061 [b]; AF 273456 [b]
CMW 9932	*C. cubensis*	*Tibouchina* sp.	South Africa	AF273062 [b]; AF 273457 [b]
CMW 8758	*C. cubensis*	*Tibouchina* sp.	South America	AF273068 [b]; AF273463 [b]
CMW 1853	*C. cubensis*	*S. aromaticum*	South America	AF273070 [b]; AF273465 [b]
CMW 9927	*C. cubensis*	*Tibouchina* sp.	South America	AF292034 [b]; AF292037 [b]
CMW 9929	*C. cubensis*	*Tibouchina* sp.	South America	AF292035 [b]; AF292038 [b]
CMW 9928	*C. cubensis*	*Tibouchina* sp.	South America	AF292036 [b]; AF292038 [b]
CMW 8756	*C. cubensis*	*Eucalyptus* sp.	South east Asia	AF273077 [b]; AF375606 [b]
CMW 9906	*C. cubensis*	*Eucalyptus* sp.	South east Asia	AF273069 [b]; AF 273464 [b]
CMW9903	*C. cubensis*	*Eucalyptus* sp.	South east Asia	AF273070 [b]; AF273465 [b]
CMW 7036	*C. eucalypti*	*Eucalyptus* sp.	South Africa	AF368341 [d]; AF368340 [d]
CMW 7037	*C. eucalypti*	*Eucalyptus* sp.	Australia	AF368343 [d]; AF368342 [d]
CMW 7038	*C. eucalypti*	*Eucalyptus* sp.	Australia	AF368345 [d]; AF368344 [d]
CMW 7047	*C. parasitica*	*Q. virginiana*	America	AF273469 [d]; AF273073 [d]
CMW 7048	*C. parasitica*	*Q. virginiana*	America	AF273470 [d]; AF273076 [d]
CMW 1651	*C. parasitica*	*C. dentata*	America	AF273074 [d]; AF273467 [d]
CMW 1652	*C. parasitica*	*C. dentata*	America	AF273468 [d]; AF27075 [d]
CMW 5288	*D. ambigua*	*Malus* sp.	South Africa	AF543819;AF543821 [e]

[a] Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa.
[b] β-tubulin 1 and 2 sequence data obtained from Myburg *et al.* (2002a, b).
[c] β-tubulin 1 and 2 sequence data generated in this study.
[d] β-tubulin 1 and 2 sequence data obtained from Venter *et al.* (2002).
[e] β-tubulin 1 and 2 sequence data obtained from Genbank.

Table 3. Lesion lengths on one-year-old *Eucalyptus grandis* (ZG 14 clones) and *Syzygium cordatum* six weeks after inoculation in the greenhouse.

ISOLATE	[a] Lesion length (mm)	
	Syzygium cordatum	*Eucalyptus grandis* (ZG 14)
CMW 2113	49.9	146.5
CMW 9364	108.9	133.0
Control	10	10

[a] Each value is the average of 20 measurements for each isolate

$P > 0.0001$

CV = 43.24

R-Square = 0.81

F = 7.45

Figure 1. Light micrographs of the fruiting structures of *Cryphonectria cubensis* from *Syzygium* spp. in South Africa. (a). Conidioma on bark. (b). Longitudinal section through conidioma. (c). Conidiophores. (d). Conidia. (e). Ascoma on bark. (f). Longitudinal section through ascoma showing limited tissue (arrow). (g). Asci. and ascospores. Bars: a-b, e-f = 100 µm; c-d, g = 10 µm.

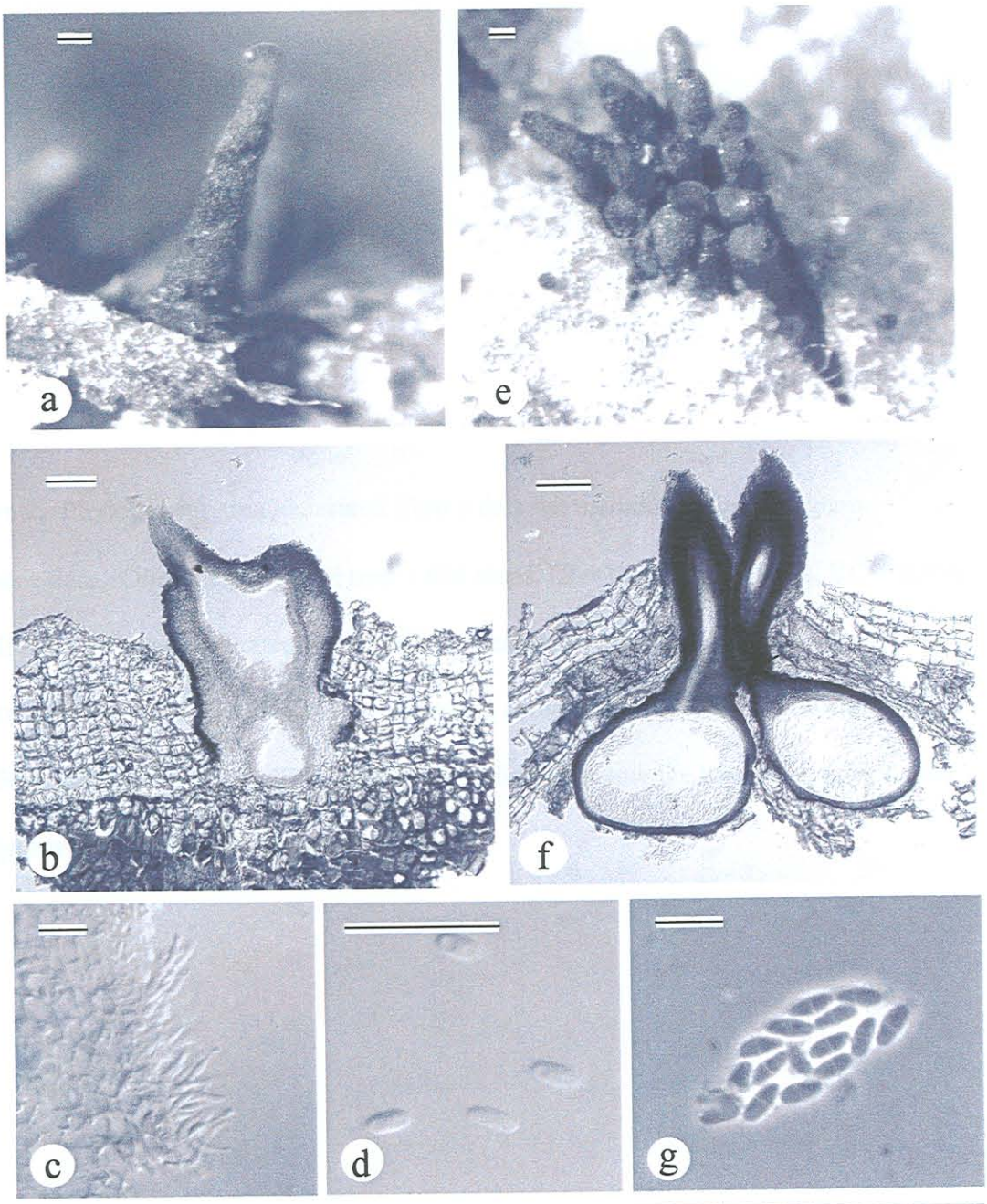

a
e
b
f
c
d
g

Figure 2. Phylogenetic tree generated from a data set including β-tubulin gene sequence data. One tree (length of tree = 684 steps, CI = 0.918, RI = 0.951, RC = 0.806 and HI = 0.139) was generated from heuristic searches performed on the data set. Bootstrap values (1000 replicates) are indicated above the branches with those lower than 50% are not shown. MCMC values are indicated below the branches. *Diaporthe ambigua* was used to root the tree.

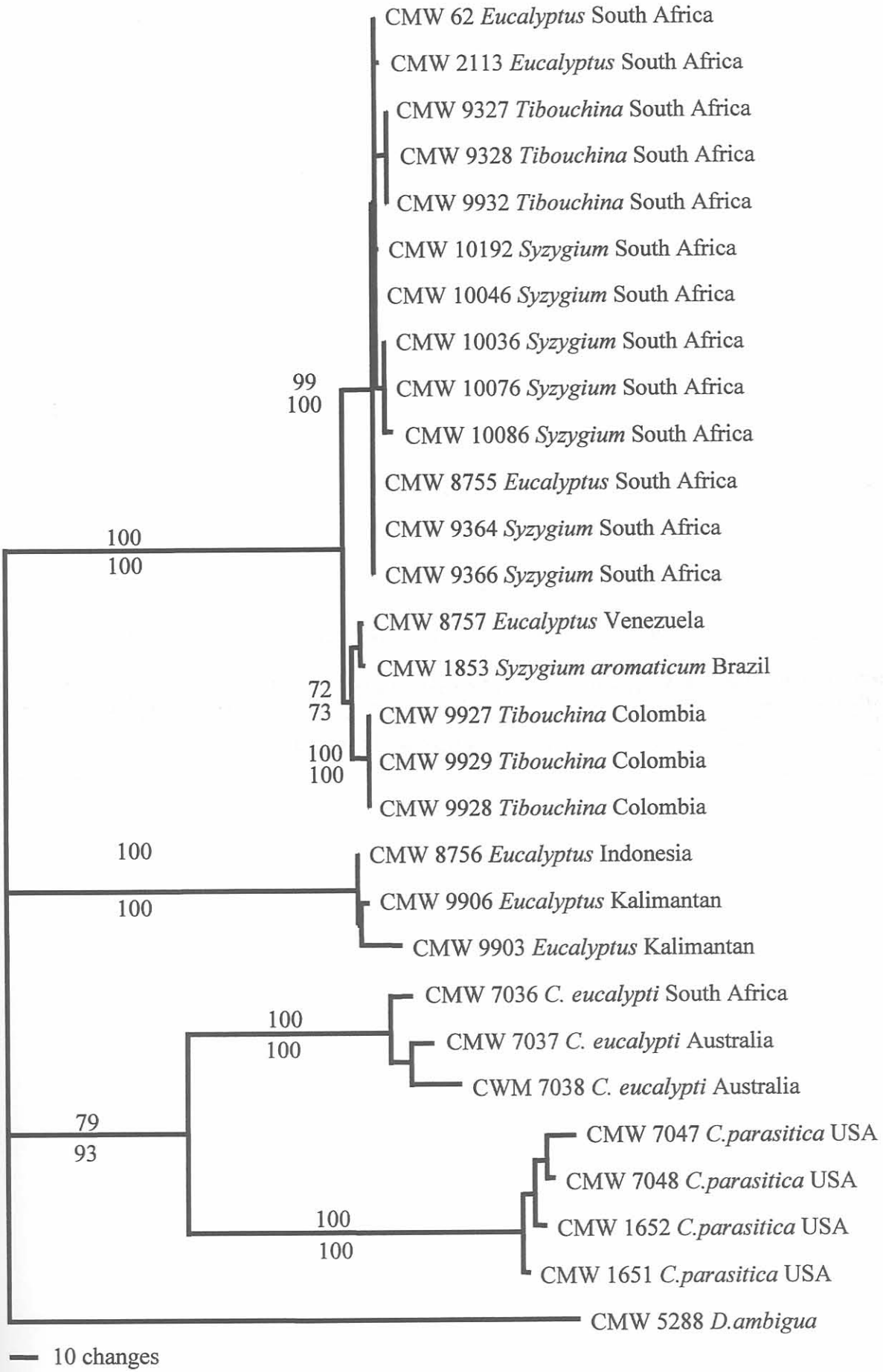
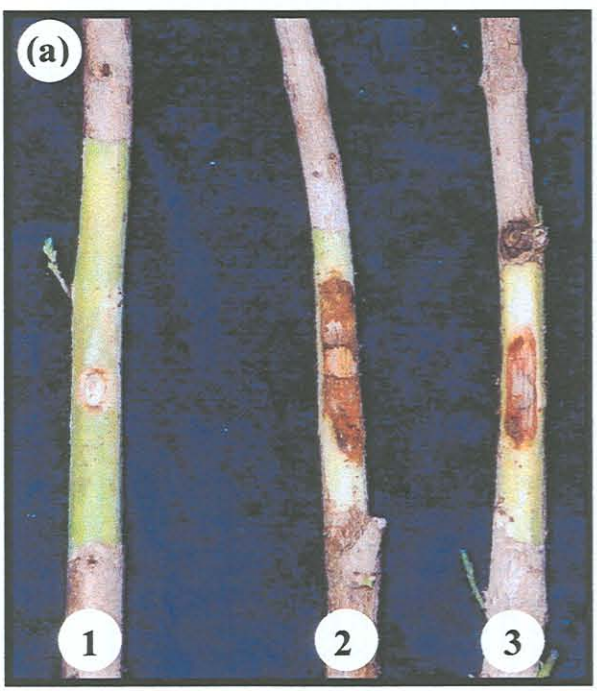
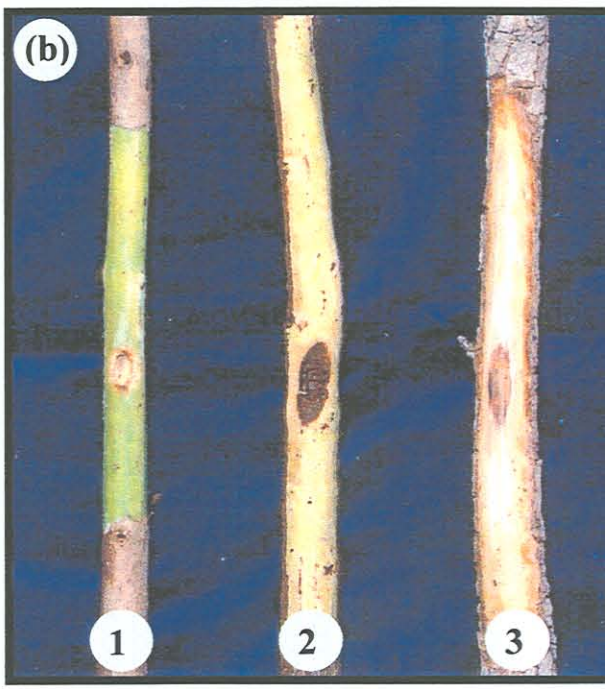


Figure. 3. Lesions resulting from pathogenicity trials. (a) Lesions formed on *Eucalyptus grandis* clones (a2=CMW 2113, a3=CMW 9364) with control inoculation producing no lesions (a1). (b) Lesions formed on *Syzygium cordatum* trees (b2=CMW 2113, b3=CMW 9364) with control inoculation producing no lesions (b1).





APPENDIX 1

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                                        10        20        30        40        50        60        70
CMW 62 Eucalyptus South Africa          TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 2113 Eucalyptus South Africa        TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACGGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 8755 Eucalyptus South Africa        TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 9327 Tibouchina South Africa        TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 9328 Tibouchina South Africa        TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 9932 Tibouchina South Africa        TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 9364 Syzygium South Africa          TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 9366 Syzygium South Africa          TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 10192 Syzygium South Africa         TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCTAGTTG ACCCAGCAGA TGTTCGACCC
CMW 10046 Syzygium South Africa         TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 10036 Syzygium South Africa         TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 10076 Syzygium South Africa         TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 10086 Syzygium South Africa         TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 8757 Eucalyptus Venezuela           TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 1853 Syzygium aromaticum            TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 9927 Tibouchina Colombia            TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 9929 Tibouchina Colombia            TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 9928 Tibouchina Colombia            TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 8756 Eucalyptus Indonesia           TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 9906 Eucalyptus Kalimantan          TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 9903 Eucalyptus Kalimantan          TGACCAGCCG TGGCGCCCAC TCCTTCCGGG CTGTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 7036 C. eucalypti South Africa      TGACCAGCCG TGGCGCCCAC TCTTTCCGCG CCCTCACCGT GCCCGAGTTG ACCCAGCAAA TGTTCGACCC
CMW 7037 C. eucalypti Australia         TGACCAGCCG TGGCGCCCAC TCTTTCCGCG CCCTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CWM 7038 C. eucalypti Australia         TGACCAGCCG TGGCGCCCAC TCTTTCCGCG CCCTCACCGT GCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 7047 C. parasitica USA              TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CCCTCACCGT CCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 7048 C. parasitica USA              TGACCAGCCG TGGCGCCCAC TCTTTCCGCG CCCTCACCGT CCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 1651 C. parasitica USA              TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CCCTCACCGT CCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 1652 C. parasitica USA              TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CCCTAACCGT CCCCGAGTTG ACCCAGCAGA TGTTCGACCC
CMW 5288 D. ambigua                     TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CCGTCACCGT TCCTGAGCTC ACCCAGCAAA TGTTCGACCC
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                                          80         90         100        110        120        130        140
CMW 62 Eucalyptus South Africa            CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC
CMW 2113 Eucalyptus South Africa          CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC
CMW 8755 Eucalyptus South Africa          CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC
CMW 9327 Tibouchina South Africa          CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC
CMW 9328 Tibouchina South Africa          CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC
CMW 9932 Tibouchina South Africa          CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC
CMW 9364 Syzygium South Africa            CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC
CMW 9366 Syzygium South Africa            CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC
CMW 10192 Syzygium South Africa           CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC
CMW 10046 Syzygium South Africa           CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC
CMW 10036 Syzygium South Africa           CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC
CMW 10076 Syzygium South Africa           CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC
CMW 10086 Syzygium South Africa           CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC
CMW 8757 Eucalyptus Venezuela             CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC
CMW 1853 Syzygium aromaticum              CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC
CMW 9927 Tibouchina Colombia              CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC
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CMW 9928 Tibouchina Colombia              CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC
CMW 8756 Eucalyptus Indonesia             CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC
CMW 9906 Eucalyptus Kalimantan            CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC
CMW 9903 Eucalyptus Kalimantan            CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCCGCCAT CTTGTAAGTC
CMW 7036 C. eucalypti South Africa        CAAGAACATG ATGGCTGCCT CGGACTTCCG CAACGGCCGT TACCTGACGT GCTCTGCCAT CTTGTACGTT
CMW 7037 C. eucalypti Australia           CAAGAACATG ATGGCTGCCT CGGACTTCCG CAACGGCCGT TACCTGACGT GCTCTGCCAT CTTGTACGTT
CWM 7038 C. eucalypti Australia           CAAGAACATG ATGGCTGCCT CGGACTTCCG CAACGGCCGT TACCTGACGT GCTCTGCCAT CTTGTACGTT
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CMW 7048 C. parasitica USA                CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACAT GCTCTGCCAT CTTGTAAGTT
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CMW 1652 C. parasitica USA                CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACAT GCTCTGCCAT CTTGTAAGTT
CMW 5288 D. ambigua                       CAAGAACATG ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT GCTCTGCCAT CTTGTAAGTC
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                                        150        160        170        180        190        200        210
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CMW 8755 Eucalyptus South Africa        CCCC-G---- CCCCTCGCGC CTCGGGGCGC CTCGGCCGAA GC-------- TCGTC--TGC TAA-------
CMW 9327 Tibouchina South Africa        CCCC-G---- CCCCTCGCGC CTCGGGGCGC CTCGGCCGAA GC-------- TCGTC--TGC TAA-------
CMW 9328 Tibouchina South Africa        CCCC-G---- CCCCTCGCGC CTCGGGGCGC CTCGGCCGAA GC-------- TCGTC--TGC TAA-------
CMW 9932 Tibouchina South Africa        CCCC-G---- CCCCTCGCGC CTCGGGGCGC CTCGGCCGAA GC-------- TCGTC--TGC TAA-------
CMW 9364 Syzygium South Africa          CCCC-G---- CCCCTCGCGC CTCGGGGCGC CTCGGCCGAA GC-------- TCGTC--TGC TAA-------
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CMW 10192 Syzygium South Africa         CCCC-G---- CCCCTCGCGC CTCGGGGCGC CTCGGCCGAA GC-------- TCGTC--TGC TAA-------
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CMW 10076 Syzygium South Africa         CCCC-G---- CCCCTCGCGC CTCGGGGCGC CTCGGCCGAA GC-------- TCGTC--TGC TAA-------
CMW 10086 Syzygium South Africa         CCCC-G---- CCCCTCGCGC CTCGGGGCGC CTCGGCCGAA GC-------- TCGTC--TGC TAA-------
CMW 8757 Eucalyptus Venezuela           TCCC-G---- CCCCTCGCGC CTCGGAGAGC ATCGGCCGAA GC-------- TTGTC--TGC TAA-------
CMW 1853 Syzygium aromaticum            TCCC-G---- CCCCTCGCGC CTCGAAGAGC ATCGGCCGAA GC-------- TTGTC--TGC TAA-------
CMW 9927 Tibouchina Colombia            CCCC-G---- CCCCTCGCGC CTCGGGGAGC ATCGGCCGAG GC-------- TTGTC--TGC TAA-------
CMW 9929 Tibouchina Colombia            CCCC-G---- CCCCTCGCGC CTCGGGGAGC ATCGGCCGAG GC-------- TTGTC--TGC TAA-------
CMW 9928 Tibouchina Colombia            CCCC-G---- CCCCTCGCGC CTCGGGGAGC ATCGGCCGAG GC-------- TTGTC--TGC TAA-------
CMW 8756 Eucalyptus Indonesia           CCCC-G---- CCCCTCGCGC CTCGGGGAGC ATCGGCCGAA GC-------- TTGTC--TGC TAA-------
CMW 9906 Eucalyptus Kalimantan          CCCC-G---- CCCCTCGCGC CTCGGGGAGC ATCGGCCGAA GC-------- TTGTC--TGC TAA-------
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CMW 7037 C. eucalypti Australia         TTC-TGCTTC TTTCTCT-AT CTCACAAATC -TCGGATCCA CCTCTCGGGC TTGTTTTTGC TAACCCTG-C
CWM 7038 C. eucalypti Australia         TT-------- -------TGT CTCACAAATC -TCGGATCCA CCTCTCGGGC TTGTTTTTGC TAACCCTG-C
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CMW 1652 C. parasitica USA              TTCTTGTCTT TTCCTCGCAA GTCTCGACGA ACGTCTTGGG CTGTTTG-GC TAA------- ---CCCTGTC
CMW 5288 D. ambigua                     CCCTAAGTCC CCTCGCACAA ATAAAA--TG GTCGCGCGCT GACACTGTGC ---------- ----------
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CMW 2113 Eucalyptus South Africa    ------CCCT CATCG---TC CAGCCGTGGC AAGGTCTCCA TGAAGGAGGT CGAGGACCAG ATGCGCAACG
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CMW 9327 Tibouchina South Africa    ------CCCT CATCG---TC CAGCCGTGGC AAGGTCTCCA TGAAGGAGGT CGAGGACCAG ATGCGCAACG
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CMW 9932 Tibouchina South Africa    ------CCCT CATCG---TC CAGCCGTGGC AAGGTCTCCA TGAAGGAGGT CGAGGACCAG ATGCGCAACG
CMW 9364 Syzygium South Africa      ------CCCT CATCG---TC CAGCCGTGGC AAGGTCTCCA TGAAGGAGGT CGAGGACCAG ATGCGCAACG
CMW 9366 Syzygium South Africa      ------CCCT CATCG---TC CAGCCGTGGC AAGGTCTCCA TGAAGGAGGT CGAGGACCAG ATGCGCAACG
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CMW 10046 Syzygium South Africa     ------CCCT CATCG---TC CAGCCGTGGC AAGGTCTCCA TGAAGGAGGT CGAGGACCAG ATGCGCAACG
CMW 10036 Syzygium South Africa     ------CCCT CATCG---TC CAGCCGTGGC AAGGTCTCCA TGAAGGAGGT CGAGGACCAG ATGCGCAACG
CMW 10076 Syzygium South Africa     ------CCCT CATCG---TC CAGCCGTGGC AAGGTCTCCA TGAAGGAGGT CGAGGACCAG ATGCGCAACG
CMW 10086 Syzygium South Africa     ------CCCT CATCG---TC CAGCCGTGGC AAGGTCTCCA TGAAGGAGGT CGAGGACCAG ATGCGCAACG
CMW 8757 Eucalyptus Venezuela       ------CTCT TATCG---TC CAGCCGTGGC AAGGTCTCCA TGAAGGAGGT TGAGGACCAG ATGCGCAATG
CMW 1853 Syzygium aromaticum        ------CTCT TATCG---TC CAGC-GTGGC AAGGTCTCCA TGAAGGAGGT TGAGGACCAG ATGCGCAATG
CMW 9927 Tibouchina Colombia        ------CTCT TATCG---TC CAGCCGTGGC AAGGTCTCCA TGAAGGAGGT CGAGGACCAG ATGCGCAATG
CMW 9929 Tibouchina Colombia        ------CTCT TATCG---TC CAGCCGTGGC AAGGTCTCCA TGAAGGAGGT CGAGGACCAG ATGCGCAATG
CMW 9928 Tibouchina Colombia        ------CTCT TATCG---TC CAGCCGTGGC AAGGTCTCCA TGAAGGAGGT CGAGGACCAG ATGCGCAATG
CMW 8756 Eucalyptus Indonesia       ------CTCT TATCG---TC CAGCCGTGGC AAGGTCTCCA TGAAGGAGGT TGAGGACCAG ATGCGCAACG
CMW 9906 Eucalyptus Kalimantan      ------CTCT TATCG---TC CAGCCGTGGC AAGGTCTCCA TGAAGGAGGT TGAGGACCAG ATGCGCAACG
CMW 9903 Eucalyptus Kalimantan      ------CTCT TATCG---TC CAGCCGTGGC AAGGTCTCCA TGAAGGAGGT TGAGGACCAG ATGCGCAACG
CMW 7036 C. eucalypti South Africa  TTTCCTCTCT -CCCC---TA CAGCCGTGGC AAGGTCTCCA TGAAGGAGGT CGAGGACCAG ATGCGCAACG
CMW 7037 C. eucalypti Australia     TTTCCTCTCT -CCCC---TA CAGCCGTGGC AAGGTCTCCA TGAAGGAGGT CGAGGACCAG ATGCGCAACG
CWM 7038 C. eucalypti Australia     TTTCCTCTCT -CCCC---TA CAGCCGGGGC AAGGTCTCCA TGAAGGAGGT CGAGGACCAG ATGCGCAACG
CMW 7047 C. parasitica USA          TTT-CTCTCT TCCCCTTCTC TAGCCGGGGT AAGGTCTCCA TGAAGGAAGT CGAGGACCAG ATGCGCAACG
CMW 7048 C. parasitica USA          TTT-CTCTCT TCCCCTTCTT TAGCCGTGGT AAGGTCTCCA TGAAGGAAGT CGAGGACCAG ATGCGCAACG
CMW 1651 C. parasitica USA          TTT-CTCTCT TCCCCTTCTC AAGCCGTGGT AAGGTCTCCA TGAAGGAAGT CGAGGACCAG ATGCGCAACG
CMW 1652 C. parasitica USA          TTT-CTCTCT TCCCCTTCTC TAGCCGTGGT AAGGTCTCCA TGAAGGAAGT CGAGGACCAG ATGCGCAACG
CMW 5288 D. ambigua                 ---------- --------TC TAGCCGTGGA AAGGTCTCCA TGAAGGAGGT CGAGGACCAG ATGCGCAACG
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	290	300	310	320	330	340	350
CMW 62 *Eucalyptus* South Africa	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 2113 *Eucalyptus* South Africa	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 8755 *Eucalyptus* South Africa	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 9327 *Tibouchina* South Africa	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 9328 *Tibouchina* South Africa	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TTTGCTCCAT
CMW 9932 *Tibouchina* South Africa	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 9364 *Syzygium* South Africa	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 9366 *Syzygium* South Africa	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 10192 *Syzygium* South Africa	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 10046 *Syzygium* South Africa	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 10036 *Syzygium* South Africa	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 10076 *Syzygium* South Africa	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 10086 *Syzygium* South Africa	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 8757 *Eucalyptus* Venezuela	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 1853 *Syzygium aromaticum*	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 9927 *Tibouchina* Colombia	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 9929 *Tibouchina* Colombia	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 9928 *Tibouchina* Colombia	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 8756 *Eucalyptus* Indonesia	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 9906 *Eucalyptus* Kalimantan	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 9903 *Eucalyptus* Kalimantan	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TTTGCTCCAT
CMW 7036 *C. eucalypti* South Africa	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 7037 *C. eucalypti* Australia	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CWM 7038 *C. eucalypti* Australia	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 7047 *C. parasitica* USA	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATTCC	CAACAACGTC	CAGACCGCCC	TTTGCTCCAT
CMW 7048 *C. parasitica* USA	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATTCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 1651 *C. parasitica* USA	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATTCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 1652 *C. parasitica* USA	TCCAGAGCAA	GAACTCGTCC	TACTTCGTCG	AGTGGATTCC	CAACAACGTC	CAGACCGCCC	TCTGCTCCAT
CMW 5288 *D. ambigua*	TCCAGAGCAA	GAACTCATCC	TACTTCGTCG	AGTGGATCCC	CAACAACGTC	CAGACCGCCC	TCTGCTCGAT

	360	370	380	390	400	410	420
CMW 62 *Eucalyptus* South Africa	CCCCCCCAAG	GGTCTCAAGA	TGTCCTCCAC	CTTTGTTGGC	AACTCCACTG	CCATCCAGGA	GCTCTTCAAG
CMW 2113 *Eucalyptus* South Africa	CCCCCCCAAG	GGTCTCAAGA	TGTCCTCCAC	CTTTGTTGGC	AACTCCACTG	CCATCCAGGA	GCTCTTCAAG
CMW 8755 *Eucalyptus* South Africa	CCCCCCCAAG	GGTCTCAAGA	TGTCCTCCAC	CTTTGTTGGC	AACTCCACTG	CCATCCAGGA	GCTCTTCAAG
CMW 9327 *Tibouchina* South Africa	CCCCCCCAAG	GGTCTCAAGA	TGTCCTCCAC	CTTTGTTGGC	AACTCCACTG	CCATCCAGGA	GCTCTTCAAG
CMW 9328 *Tibouchina* South Africa	CCCCCCCAAG	GGTCTCAAGA	TGTCCTCCAC	CTTTGTTGGC	AACTCCACTG	CCATCCAGGA	GCTCTTCAAG
CMW 9932 *Tibouchina* South Africa	CCCCCCCAAG	GGTCTCAAGA	TGTCCTCCAC	CTTTGTTGGC	AACTCCACTG	CCATCCAGGA	GCTCTTCAAG
CMW 9364 *Syzygium* South Africa	CCCCCCCAAG	GGTCTCAAGA	TGTCCTCCAC	CTTTGTTGGC	AACTCCACTG	CCATCCAGGA	GCTCTTCAAG
CMW 9366 *Syzygium* South Africa	CCCCCCCAAG	GGTCTCAAGA	TGTCCTCCAC	CTTTGTTGGC	AACTCCACTG	CCATCCAGGA	GCTCTTCAAG
CMW 10192 *Syzygium* South Africa	CCCCCCCAAG	GGTCTCAAGA	TGTCCTCCAC	CTTTGTTGGC	AACTCCACTG	CCATCCAGGA	GCTCTTCAAG
CMW 10046 *Syzygium* South Africa	CCCCCCCAAG	GGTCTCAAGA	TGTCCTCCAC	CTTTGTTGGC	AACTCCACTG	CCATCCAGGA	GCTCTTCAAG
CMW 10036 *Syzygium* South Africa	CCCCCCCAAG	GGTCTCAAGA	TGTCCTCCAC	CTTTGTTGGC	AACTCCACTG	CCATCCAGGA	GCTCTTCAAG
CMW 10076 *Syzygium* South Africa	CCCCCCCAAG	GGTCTCAAGA	TGTCCTCCAC	CTTTGTTGGC	AACTCCACTG	CCATCCAGGA	GCTCTTCAAG
CMW 10086 *Syzygium* South Africa	CCCCCCCAAG	GGTCTCAAGA	TGTCCTCCAC	CTTTGTTGGC	AACTCCACTG	CCATCCAGGA	GCTCTTCAAG
CMW 8757 *Eucalyptus* Venezuela	CCCCCCCAAG	GGTCTCAAGA	TGTCCTCCAC	CTTTGTTGGC	AACTCCACTG	CCATCCAGGA	GCTCTTCAAG
CMW 1853 *Syzygium aromaticum*	CCCCCCCAAG	GGTCTCAAGA	TGTCCTCCAC	CTTTGTTGGC	AACTCCACTG	CCATCCAGGA	GCTCTTCAAG
CMW 9927 *Tibouchina* Colombia	CCCCCCCAAG	GGTCTCAAGA	TGTCCTCCAC	CTTTGTTGGC	AACTCCACTG	CCATCCAGGA	GCTCTTCAAG
CMW 9929 *Tibouchina* Colombia	CCCCCCCAAG	GGTCTCAAGA	TGTCCTCCAC	CTTTGTTGGC	AACTCCACTG	CCATCCAGGA	GCTCTTCAAG
CMW 9928 *Tibouchina* Colombia	CCCCCCCAAG	GGTCTCAAGA	TGTCCTCCAC	CTTTGTTGGC	AACTCCACTG	CCATCCAGGA	GCTCTTCAAG
CMW 8756 *Eucalyptus* Indonesia	CCCCCCCAAG	GGTCTCAAGA	TGTCCTCCAC	CTTTGTTGGC	AACTCCACTG	CCATCCAGGA	GCTCTTCAAG
CMW 9906 *Eucalyptus* Kalimantan	CCCCCCCAAG	GGTCTCAAGA	TGTCCTCCAC	CTTTGTTGGC	AACTCCACTG	CCATCCAGGA	GCTCTTCAAG
CMW 9903 *Eucalyptus* Kalimantan	CCCCCCCAAG	GGTCTCAAGA	TGTCCTCCAC	CTTTGTTGGC	AACTCCACCG	CCATCCAGGA	GCTCTTCAAG
CMW 7036 *C. eucalypti* South Africa	CCCCCCCAAG	GGCCTCAAGA	TGTCCTCCAC	CTTTGTCGGC	AACTCCACCG	CCATCCAGGA	GCTCTTCAAG
CMW 7037 *C. eucalypti* Australia	CCCCCCCAAG	GGCCTCAAGA	TGTCCTCCAC	CTTTGTCGGC	AACTCCACCG	CCATCCAGGA	GCTCTTCAAG
CWM 7038 *C. eucalypti* Australia	CCCCCCCAAG	GGCCTCAAGA	TGTCCTCCAC	CTTTGTCGGC	AACTCCACCG	CCATCCAGGA	GCTCTTCAAG
CMW 7047 *C. parasitica* USA	TCCCCCCAGG	GGCCTAAAGA	TGTCCTCGAC	CTTTGTCGGC	AACTCCACCG	CCATCCAGGA	GCTCTTAAAG
CMW 7048 *C. parasitica* USA	TCCCCCCAGG	GGCCTCAAGA	TGTCCTCGAC	CTTTGTCGGC	AACTCCACCG	CCATCCAGGA	GCTCTTCAAG
CMW 1651 *C. parasitica* USA	TCCCCCCAAG	GGCCTCAAGA	TGTCCTCGAC	CTTTGTCGGC	AACTCCACCG	CCATCCAGGA	GCTCTTCAAG
CMW 1652 *C. parasitica* USA	TCCCCCCAAG	GGCCTCAAGA	TGTCCTCGAC	CTTTGTCGGC	AACTCCACCG	CCATCCAGGA	GCTCTTCAAG
CMW 5288 *D. ambigua*	CCCTCCCAAG	GGTCTCAAGA	TGTCCTCTAC	CTTCGTGGGT	AACTCGACTG	CTATCCAGGA	GCTGTTCAAG

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                                        430        440        450        460        470        480        490
CMW 62 Eucalyptus South Africa      CGTATCGGCG AGCAGTTCAC -----TG-CT ATGTT-CCGT CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 2113 Eucalyptus South Africa    CGTATCGGCG AGCAGTTCAC -----TG-CT ATGTT-CCGT CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 8755 Eucalyptus South Africa    CGTATCGGCG AGCAGTTCAC -----TG-CT ATGTT-CCGT CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 9327 Tibouchina South Africa    CGTATCGGCG AGCAGTTCAC -----TG-CT ATGTT-CCGT CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 9328 Tibouchina South Africa    CGTATCGGCG AGCAGTTCAC -----TG-CT ATGTT-CCGT CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 9932 Tibouchina South Africa    CGTATCGGCG AGCAGTTCAC -----TG-CT ATGTT-CCGT CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 9364 Syzygium South Africa      CGTATCGGCG AGCAGTTCAC -----TG-CT ATGTT-CCGT CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 9366 Syzygium South Africa      CGTATCGGCG AGCAGTTCAC -----TG-CT ATGTT-CCGT CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 10192 Syzygium South Africa     CGTATCGGCG AGCAGTTCAC -----TG-CT ATGTT-CCGT CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 10046 Syzygium South Africa     CGTATCGGCG AGCAGTTCAC -----TG-CT ATGTT-CCGT CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 10036 Syzygium South Africa     CGTATCGGCG AGCAGTTCAC -----TG-CT ATGTT-CCGT CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 10076 Syzygium South Africa     CGTATCGGCG AGCAGTTCAC -----TG-CT ATGTT-CCGT CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 10086 Syzygium South Africa     CGTATCGGCG AGCAGTTCAT -----TG-TT ATGTT-CCGT CGCAAGGCTT TCTTGCATTT GTACACTGGG
CMW 8757 Eucalyptus Venezuela       CGTATCGGCG AGCAGTTCAC -----TG-CT ATGTT-CCGT CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 1853 Syzygium aromaticum        CGTATCGGCG AGCAGTTCAC -----TG-CT ATGTT-CCGT CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 9927 Tibouchina Colombia        CGTATCGGCG AGCAGTTCAC -----TG-CT ATGTT-CCGT CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 9929 Tibouchina Colombia        CGTATCGGCG AGCAGTTCAC -----TG-CT ATGTT-CCGT CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 9928 Tibouchina Colombia        CGTATCGGCG AGCAGTTCAC -----TG-CT ATGTT-CCGT CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 8756 Eucalyptus Indonesia       CGTATCGGCG AGCAGTTCAC -----TG-CT ATGTT-CCGT CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 9906 Eucalyptus Kalimantan      CGTATCGGCG AGCAGTTCAC -----TG-CT ATGTT-CCGT CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 9903 Eucalyptus Kalimantan      CGTATCGGCG AGCAGTTCAC -----TG-CT ATGTT-CCGT CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 7036 C. eucalypti South Africa  CGTGTTGGCG AGCAGTTCAC CGCCATGTCT ATGTT-CCGG CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 7037 C. eucalypti Australia     CGTGTTGGCG AGCAGTTCAC CGCCATGTCT ATGTT-CCGG CGCAAGGCTT TCTTGCATTG GTACACTGGG
CWM 7038 C. eucalypti Australia     CGTGTTGGCG AGCAGTTCAC CGCCATG-CT ATGTTTCCGG CGCAAGGCTT TCTTGCATTG GTACACTGGG
CMW 7047 C. parasitica USA          CGTGTCGGCG AGCAGTTCAC CGCCA----- -TGTT-CCGG CGCAAGGCTT TCTTGCATTG GTACACTGGT
CMW 7048 C. parasitica USA          CGTGTCGGCG AGCAGTTTAC CGCCA----- -TGTT-CCGG CGCAAGGCTT TCTTGCATTG GTACACTGGT
CMW 1651 C. parasitica USA          CGTGTCGGCG AGCAGTTTAC CGCCA----- -TGTT-CCGG CGCAAGGCTT TCTTGCATTG GTACACTGGT
CMW 1652 C. parasitica USA          CGTGTCGGCG AGCAGTTTAC CGCCA----- -TGTT-CCGG CGCAAGGCTT TCTTGCATTG GTACACTGGT
CMW 5288 D. ambigua                 CGTGTGGGCG AGCAGTTCAC TGCCA----- -TGTT-CCGG CGCAAGGCTT TCTTGCATTG GTACACTGGC
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                                        500        510        520        530        540        550        560
CMW 62 Eucalyptus South Africa          ACGCGACACG GC--GGTCT- CGAGACCGCG ATGGTAGTGG TGGC-----T TCAGTACTGA CCGCG-ACCG
CMW 2113 Eucalyptus South Africa        ACGCGACACG GC--GGTCT- CGAGACCGCG ATGGTAGTGG TGGC-----T TCAGTACTGA CCGCG-ACCG
CMW 8755 Eucalyptus South Africa        ACGCGACACG GC--GGTCT- CGAGACCGTG ATGGTAGTGG TGGC-----T TCAGTACTGA CCGCG-ACCG
CMW 9327 Tibouchina South Africa        ACGCGACACG GC--GGTCTG CGAGACCGAG ATGGTAGTGG TGGC-----T TCAGTACTGA CCGCGGACCG
CMW 9328 Tibouchina South Africa        ACGCGACACG GC--GGTCTG CGAGACCGAG ATGGTAGTGG TGGC-----T TCAGTACTGA CCGCGGACCG
CMW 9932 Tibouchina South Africa        ACGCGACACG GC--GGTCTG CGAGACCGAG ATGGTAGTGG TGGC-----T TCAGTACTGA CCGCGGACCG
CMW 9364 Syzygium South Africa          ACGCGACACG GC--GGTCT- CGAGACCGTG ATGGTAGTGG TGGC-----T TCAGTACTGA CCGCG-ACCG
CMW 9366 Syzygium South Africa          ACGCGACACG GC--GGTCT- CGAGACCGTG ATGGTAGTGG TGGC-----T TCAGTACTGA CCGCG-ACCG
CMW 10192 Syzygium South Africa         ACGCGACACG GC--GGTCT- CGAGACCGCG ATGGTAGTGG TGGC-----T TCAGTACTGA CCGCG-ACCG
CMW 10046 Syzygium South Africa         ACGCGACACG GC--GGTCT- CGAGACCGCG ATGGTAGTGG TGGC-----T TCAGTACTGA CCGCG-ACCG
CMW 10036 Syzygium South Africa         ACGGGACAGG AC--GGTCT- CGAGACCGCG ATGGTAGTGG TGGC-----T TCAGTACTGA CCGCG-ACCG
CMW 10076 Syzygium South Africa         ACGGGACAGG AC--GGTCT- CGAGACCGCG ATGGTAGTGG TGGC-----T TCAGTACTGA CCGCG-ACCG
CMW 10086 Syzygium South Africa         ACGGGACAGG AC--GGTCT- CGAGACCGCG ATGGTAGTGG TGGC-----T TCAGTACTGA CCGCG-ACCG
CMW 8757 Eucalyptus Venezuela           ACGCGACACG GC--GGTCT- CGAGACCGCG ATGGTGGTGG TGGC-----T TCAGTACTGA CCGCG-ACCG
CMW 1853 Syzygium aromaticum            ACGCGACACG GC--GGTCT- CGAGACCGTG ATGGTGGTGG TGGC-----T TCAGTACTGA CCGCG-ACCG
CMW 9927 Tibouchina Colombia            ACGCGACACG GC--GGTCT- CGAGACCGTG ATGGTGGTGG TGGC-----T TTAGTACTGA CCGCG-ACCC
CMW 9929 Tibouchina Colombia            ACGCGACACG GC--GGTCT- CGAGACCGTG ATGGTGGTGG TGGC-----T TTAGTACTGA CCGCG-ACCC
CMW 9928 Tibouchina Colombia            ACGCGACACG GC--GGTCT- CGAGACCGTG ATGGTGGTGG TGGC-----T TTAGTACTGA CCGCG-ACCC
CMW 8756 Eucalyptus Indonesia           ACGCGACACG GC--GGTCT- CGAGACCGTG ATGGTGGTGG TGGC-----T TCAGTACTGA CCGCG-ACCG
CMW 9906 Eucalyptus Kalimantan          ACGCGACACG GC--GGTCT- CGAGACCGCG ATGGTAGTGG TGGG-----T TCAGTACTGA CCGCG-ACCG
CMW 9903 Eucalyptus Kalimantan          ACGCGACACG GC--GGTCT- CGAGACCGTG ATGGTGGTGG TGGC-----T TCAGTACTGA CCGCG-ACCG
CMW 7036 C. eucalypti South Africa      ACACGATACG GC--GATGT- --CGACC--- -TCGTGCAGC AGAC-----T TGGGTGCTGA CCTCG-ACCG
CMW 7037 C. eucalypti Australia         ACCCGATACG GC--GATGT- --CGACC--- -TCGTGCAGC AGAC-----T TGGGTGCTGA CCTCG-ACGC
CWM 7038 C. eucalypti Australia         ACCCGATACG GC--GATGT- --CGACC--- -TCGTGCAGC AGAC-----T TGGGTGCTGA CCTCG-ACCG
CMW 7047 C. parasitica USA              TTTCGACACG AT--ACAG-- CTACATCGAC ATCGTGCAGC AGAC-----T TGGATGCTGA CCTCG-ACAA
CMW 7048 C. parasitica USA              TTTCGACACG AT--ACAG-- CTACATCGAC ATCGTGCAGC AGAC-----T TGGATGCTGA CCTCG-ACAA
CMW 1651 C. parasitica USA              TTTCGACACG AT--ACAG-- CTACATCGAC ATCGTGCAGC AGAC-----T TGGATGCTGA CCTCG-ACAA
CMW 1652 C. parasitica USA              TTTCGACACG AT--ACAG-- CTACATCGAC ATCGTGCAGC AGAC-----T TGGATGCTGA CCTCG-ACAA
CMW 5288 D. ambigua                     -----ACCGC CGCGAC-G-- CTCGACACGC GACAATACGA CCTCGAAGCA TCGTTGCTGA CCTCG-ACAT
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                                    570        580        590        600        610        620        630
CMW 62 Eucalyptus South Africa      C-AGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTCCT GTTGCACCAG
CMW 2113 Eucalyptus South Africa    C-AGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTCCT GTTGCACCAG
CMW 8755 Eucalyptus South Africa    C-AGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTCCT GTTGCACCAG
CMW 9327 Tibouchina South Africa    C-AGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTCCT GTTGCACCAG
CMW 9328 Tibouchina South Africa    C-AGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTCCT GTTGCACCAG
CMW 9932 Tibouchina South Africa    C-AGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTCCT GTTGCACCAG
CMW 9364 Syzygium South Africa      C-AGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTCCT GTTGCACCAG
CMW 9366 Syzygium South Africa      C-AGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTCCT GTTGCACCAG
CMW 10192 Syzygium South Africa     C-AGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTCCT GTTGCACCAG
CMW 10046 Syzygium South Africa     C-AGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTCCT GTTGCACCAG
CMW 10036 Syzygium South Africa     C-AGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTCCT GTTGCACCAG
CMW 10076 Syzygium South Africa     C-AGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTCCT GTTGCACCAG
CMW 10086 Syzygium South Africa     C-AGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTCCT GTTGCACCAG
CMW 8757 Eucalyptus Venezuela       C-AGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTCCT GCTGCACCAG
CMW 1853 Syzygium aromaticum        C-AGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTCCT GCTGCACCAG
CMW 9927 Tibouchina Colombia        C-AGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTCCT GCTGCACCAG
CMW 9929 Tibouchina Colombia        C-AGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTCCT GCTGCACCAG
CMW 9928 Tibouchina Colombia        C-AGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTCCT GCTGCACCAG
CMW 8756 Eucalyptus Indonesia       C-AGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTCCT GCTGCACCAG
CMW 9906 Eucalyptus Kalimantan      C-AGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTCCT GCTGCACCAG
CMW 9903 Eucalyptus Kalimantan      C-AGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTCCT GCTGCACCAG
CMW 7036 C. eucalypti South Africa  C-AGGCAAAC CATCTCGGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAT GTACC---AC ACCATA-CCC
CMW 7037 C. eucalypti Australia     C-AGGCAAAC CATTTCGGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAT GTACC---AC ACCATA-CCC
CWM 7038 C. eucalypti Australia     C-AGGCAAAC CATTTCGGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAT GTACC---AC ACCATA-CCC
CMW 7047 C. parasitica USA          T-AGGCAAAC CATCTCCGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTATC TCGGCTTCCC
CMW 7048 C. parasitica USA          T-AGGCAAAC CATCTCCGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTATC TCGGCTTCCC
CMW 1651 C. parasitica USA          T-AGGCAAAC CATCTCCGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTATC TCGGCTTCCC
CMW 1652 C. parasitica USA          T-AGGCAAAC CATCTCCGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAC GTACCCTATC TCGGCTTCCC
CMW 5288 D. ambigua                 TTAGGCAAAC CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGCGTGTAT GCACC----- TCACATTCCC
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                                        640        650        660        670        680        690        700
CMW 62 Eucalyptus South Africa          GC---GGCGC GCC------- ---------- -TCGAGCTTC CC-GCTGACC A-CTGCACAG CTACAACGGC
CMW 2113 Eucalyptus South Africa        GC---GGCGC GCC------- ---------- -TCGAGCTTC CC-GCTGACC A-CTGCACAG CTACAACGGC
CMW 8755 Eucalyptus South Africa        GC---GGCGC GCC------- ---------- -TCGAGCTTC CC-GCTGACC A-CTGCACAG CTACAACGGC
CMW 9327 Tibouchina South Africa        GC---GGCGC GCC------- ---------- -TCGAGCTTC CC-GCTGACC A-CTGCACAG CTACAACGGC
CMW 9328 Tibouchina South Africa        GC---GGCGC GCC------- ---------- -TCGAGCTTC CC-GCTGACC A-CTGCACAG CTACAACGGC
CMW 9932 Tibouchina South Africa        GC---GGCGC GCC------- ---------- -TCGAGCTTC CC-GCTGACC A-CTGCACAG CTACAACGGC
CMW 9364 Syzygium South Africa          GC---GGCGC GCC------- ---------- -TCGAGCTTC CC-GCTGACC A-CTGCACAG CTACAACGGC
CMW 9366 Syzygium South Africa          GC---GGCGC GCC------- ---------- -TCGAGCTTC CC-GCTGACC A-CTGCACAG CTACAACGGC
CMW 10192 Syzygium South Africa         GC---GGCGC GCC------- ---------- -TCGAGCTTC CC-GCTGACC A-CTGCACAG CTACAACGGC
CMW 10046 Syzygium South Africa         GC---GGCGC GCC------- ---------- -TCGAGCTTC CC-GCTGACC A-CTGCACAG CTACAACGGC
CMW 10036 Syzygium South Africa         GC---GGCGC GCC------- ---------- -TCGAGCTTC CC-GCTGACC A-CTGCACAG CTACAACGGC
CMW 10076 Syzygium South Africa         GC---GGCGC GCC------- ---------- -TCGAGCTTC CC-GCTGACC A-CTGCACAG CTACAACGGC
CMW 10086 Syzygium South Africa         GC---GGCGC GCC------- ---------- -TCGAGCTTC CC-GCTGACC A-CTGCACAG CTACAACGGC
CMW 8757 Eucalyptus Venezuela           GC---GGCGC GCC------- ---------- -TCGAGCTTC CC-GCTGACC A-CTGCACAG CTACAACGGC
CMW 1853 Syzygium aromaticum            GC---GGCGC GCC------- ---------- -TCGAGCTTC CC-GCTGACC A-CTGCACAG CTACAACGGC
CMW 9927 Tibouchina Colombia            AC---GGCGC GCC------- ---------- -TCGAGCTTC CC-GCTGACC A-CTGCACAG CTACAACGGC
CMW 9929 Tibouchina Colombia            AC---GGCGC GCC------- ---------- -TCGAGCTTC CC-GCTGACC A-CTGCACAG CTACAACGGC
CMW 9928 Tibouchina Colombia            AC---GGCGC GCC------- ---------- -TCGAGCTTC CC-GCTGACC A-CTGCACAG CTACAACGGC
CMW 8756 Eucalyptus Indonesia           GC---GGCGC GCC------- ---------- -TCGAGCTTC CC-GCTGACC A-CCGCACAG CTACAACGGC
CMW 9906 Eucalyptus Kalimantan          GC---GGCGC GCC------- ---------- -TCGAGCTTC CC-GCTGACC A-CCGCACAG CTACAACGGC
CMW 9903 Eucalyptus Kalimantan          GC---GGCGC GCC------- ---------- -TCGAGCTTC CC-GCTGACC A-CCGCACAG CTACAACGGC
CMW 7036 C. eucalypti South Africa      TACACGGCGG CCCACGCAAG ATGGACGCGG CTCGGGCTTT CCTGCTAACC ACCCGCGTAG CTACAACGGC
CMW 7037 C. eucalypti Australia         TACACGGCGG CCCACGCAAG ATGGACGCGG CTCGGGCTCT CCTGCTGACC ACCCGCATAG CTACAACGGC
CWM 7038 C. eucalypti Australia         TACACGGCGG CCCACGCAAG ATGGACGCGG CCCGGGCTCT CCTGCTGACC ACCCGCATAG CTACAACGGC
CMW 7047 C. parasitica USA              AAG------- ------CAAG ACAGACGCGA CTTGAGCTTT CCTGCTGACC A-CCACATAG CTACAACGGC
CMW 7048 C. parasitica USA              AAG------- ------CAAG ACAGACGCGA CTTGAGCTTT CCTGCTGACC A-CCACATAG CTACAACGGC
CMW 1651 C. parasitica USA              AAG------- ------CAAG ACAGACGCGA CTTGAGCTTT CCTGCTGACC A-CCACATAG CTACAACGGC
CMW 1652 C. parasitica USA              AAG------- ------CAAG ACAGACGCGA CTTGAGCTTT CCTGCTGACC A-CCACATAG CTACAACGGC
CMW 5288 D. ambigua                     -TGCC----- --CACTGATC TTGGCCTCT- CTTCCGGCTT GGCACTGACA ATCGCA-TAG TTACAACGGC
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                                        710        720        730        740        750        760        770
CMW 62 Eucalyptus South Africa          ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCT-GTCGG- -GAC-CAGG-
CMW 2113 Eucalyptus South Africa        ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCT-GTCGG- -GAC-CAGG-
CMW 8755 Eucalyptus South Africa        ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCT-GTCGG- -GAC-CAGG-
CMW 9327 Tibouchina South Africa        ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCT-GTCGG- -GAC-CAGG-
CMW 9328 Tibouchina South Africa        ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCT-GTCGG- -GAC-CAGG-
CMW 9932 Tibouchina South Africa        ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCT-GTCGG- -GAC-CAGG-
CMW 9364 Syzygium South Africa          ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCT-GTCGG- -GAC-CAGG-
CMW 9366 Syzygium South Africa          ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCT-GTCGG- -GAC-CAGG-
CMW 10192 Syzygium South Africa         ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCT-GTCGG- -GAC-CAGG-
CMW 10046 Syzygium South Africa         ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCT-GTCGG- -GAC-CAGG-
CMW 10036 Syzygium South Africa         ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCT-GTCGG- -GAC-CAGG-
CMW 10076 Syzygium South Africa         ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCT-GTCGG- -GAC-CAGG-
CMW 10086 Syzygium South Africa         ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCT-GTCGG- -GAC-CAGG-
CMW 8757 Eucalyptus Venezuela           ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCT-GTCGG- -GAC-CAGG-
CMW 1853 Syzygium aromaticum            ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCT-GTCGG- -GAC-CAGG-
CMW 9927 Tibouchina Colombia            ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCT-GTCGG- -GAC-CAGG-
CMW 9929 Tibouchina Colombia            ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCT-GTCGG- -GAC-CAGG-
CMW 9928 Tibouchina Colombia            ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCT-GTCGG- -GAC-CAGG-
CMW 8756 Eucalyptus Indonesia           ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCT-GTCGG- -GAC-CAGT-
CMW 9906 Eucalyptus Kalimantan          ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCT-GTCGG- -GAC-CAGT-
CMW 9903 Eucalyptus Kalimantan          ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCT-GTCGG- -GAC-CAGT-
CMW 7036 C. eucalypti South Africa      ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCTTGTCGGC TGAC-CAGGC
CMW 7037 C. eucalypti Australia         ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCTTATCGGG TGAC-CAGGC
CWM 7038 C. eucalypti Australia         ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTATG TCTTATCGGG TGAC-CAGGC
CMW 7047 C. parasitica USA              ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTTA ACGAGGTATG TCTTATCGGG TGAT-CAAG-
CMW 7048 C. parasitica USA              ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTTA ACGAGGTATG TCTTATCGGG TGAT-CAAG-
CMW 1651 C. parasitica USA              ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTTA ACGAGGTATG TCTTATCGGG TGATACAAG-
CMW 1652 C. parasitica USA              ACCTCCGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTTA ACGAGGTATG TCTTATCGGG TGAT-CAAG-
CMW 5288 D. ambigua                     ACTTCTGAGC TCCAGCTCGA GCGCATGAAC GTCTACTTCA ACGAGGTAAG TCAACAACTG CACATCATCC
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                                    780        790        800        810        820        830        840
CMW 62 Eucalyptus South Africa      CTGGGGC-GT CATCCCGCCC GCGAACCCCC TGTGCGTGAC CGAGCTCCCG C--------- ----------
CMW 2113 Eucalyptus South Africa    CTGGCGC-GT CATCCCGCCC GCGAACCCCC TGTGCGTGAC CGAGCTCCCG C--------- ----------
CMW 8755 Eucalyptus South Africa    CTGGGGC-GT CATCCCGCCC GCGAACCCCC TGTGCGTGAC CGAGCTCCCG C--------- ----------
CMW 9327 Tibouchina South Africa    CTGGGGC-GT CATCCCGCCC GCGAACCCCC TGTGCGTGAC CGAGCTCCCG C--------- ----------
CMW 9328 Tibouchina South Africa    CTGGGGC-GT CATCCCGCCC GCGAACCCCC TGTGCGTGAC CGAGCTCCCG C--------- ----------
CMW 9932 Tibouchina South Africa    CTGGGGC-GT CATCCCGCCC GCGAACCCCC TGTGCGTGAC CGAGCTCCCG C--------- ----------
CMW 9364 Syzygium South Africa      CTGGGGC-GT CATCCCGCCC GCGAACCCCC TGTGCGTGAC CGAGCTCCCG C--------- ----------
CMW 9366 Syzygium South Africa      CTGGGGC-GT CATCCCGCCC GCGAACCCCC TGTGCGTGAC CGAGCTCCCG C--------- ----------
CMW 10192 Syzygium South Africa     CTGGGGC-GT CATCCCGCCC GCGAACCCCC TGTGCGTGAC CGAGCTCCCG C--------- ----------
CMW 10046 Syzygium South Africa     CTGGGGC-GT CATCCCGCCC GCGAACCCCC TGTGCGTGAC CGAGCTCCCG C--------- ----------
CMW 10036 Syzygium South Africa     CTGGGGC-GT CATCCCGCCC GCGAACCCCC TGTGCGTGAC CGAGCTCCCG C--------- ----------
CMW 10076 Syzygium South Africa     CTGGGGC-GT CATCCCGCCC GCGAACCCCC TGTGCGTGAC CGAGCTCCCG C--------- ----------
CMW 10086 Syzygium South Africa     CTGGGGC-GT CATCCCGCCC GCGAACCCCC TGTGCGTGAC CGAGCTCCCG C--------- ----------
CMW 8757 Eucalyptus Venezuela       CTGGGGC-GT CATCCCGCCC GCGAACCCCC TGTGCGTGAC CGAGCTCCCG C--------- ----------
CMW 1853 Syzygium aromaticum        CTGGGGC-GT CATCCCGCCC GCGAACCCCC TGTGCGTGAC CGAGCTCCCG C--------- ----------
CMW 9927 Tibouchina Colombia        CTGGGGC-GT CATCCCGCCC GCGAACCCCC TGTGCGTGAC CGAGCTCCCG C--------- ----------
CMW 9929 Tibouchina Colombia        CTGGGGC-GT CATCCCGCCC GCGAACCCCC TGTGCGTGAC CGAGCTCCCG C--------- ----------
CMW 9928 Tibouchina Colombia        CTGGGGC-GT CATCCCGCCC GCGAACCCCC TGTGCGTGAC CGAGCTCCCG C--------- ----------
CMW 8756 Eucalyptus Indonesia       CTGGGGC-GT CATCCCGCCC GCGAACCCCC TGTGCGTGAC CGAGCTCCCG C--------- ----------
CMW 9906 Eucalyptus Kalimantan      CTGGGGC-GT CATCCCGTCC GCGAACCCCC TGTGCGTGAC CGAGCTCCCG C--------- ----------
CMW 9903 Eucalyptus Kalimantan      CTGGGGC-GT CATCCCGTCC GCGAACCCCC TGTGCGTGAC CGAGCTCCCG C--------- ----------
CMW 7036 C. eucalypti South Africa  CTCCAGCCAT CATCCTGCCT CCTGCCTCCT CCTTCCA-TC GGGACTTCTG TGGCCTG-AC C---------
CMW 7037 C. eucalypti Australia     CTCGAGCCAT CATCCTGCCT CCTCCCTCCT CATCCCC-TC GGGGCTTTTG TGGCCTG-AC C---------
CWM 7038 C. eucalypti Australia     CTCGAGCCAG CATCCTGCCT CCTGCCTCCT CATCCCC-TC GGGGCTTTTG TGGCCTG-AC C---------
CMW 7047 C. parasitica USA          CTCAAGCTTC CA-CCTCGGC CACCCCCCCC CCCCC-TTTC CGGGG-CCCT C-----G-A- ----------
CMW 7048 C. parasitica USA          CTCAAGCTTC CA-CCTCGGC CAACCCCCCC CCCCCCTTTC CGGGG-CCCT C-----G-A- ----------
CMW 1651 C. parasitica USA          CTCAAGCTTC CA-CCTGGGC CAACCCCCCC CCCCC-TTTC CGGGG-CCTT C----TG-A- ----------
CMW 1652 C. parasitica USA          CTCAAGCTTC -A-CCTCGGC -AACCCCCCC CCCCC-TTTC CGGGG-CCTT ------G-A- ----------
CMW 5288 D. ambigua                 ATCCGACCAT ---CTC--C- AACACGG--- -----TTTAC TG----CC-G TCGCCCGGAC CTCG------
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                                        850        860        870        880        890        900        910
CMW 62 Eucalyptus South Africa          ---------- ---------- ---------T GACGCGCTCC T-GTCACA-G GCCTCCGGCA ACAAGTATGT
CMW 2113 Eucalyptus South Africa        ---------- ---------- ---------T GACGCGCTCC T-GTCACA-G GCCTCCGGCA ACAAGTATGT
CMW 8755 Eucalyptus South Africa        ---------- ---------- ---------T GACGCGCTCC T-GTCACA-G GCCTCCGGCA ACAAGTATGT
CMW 9327 Tibouchina South Africa        ---------- ---------- ---------T GACGCGCTCC T-GTCACA-G GCCTCCGGCA ACAAGTATGT
CMW 9328 Tibouchina South Africa        ---------- ---------- ---------T GACGCGCTCC T-GTCACA-G GCCTCCGGCA ACAAGTATGT
CMW 9932 Tibouchina South Africa        ---------- ---------- ---------T GACGCGCTCC T-GTCACA-G GCCTCCGGCA ACAAGTATGT
CMW 9364 Syzygium South Africa          ---------- ---------- ---------T GACGCGCTCC T-GTCACA-G GCCTCCGGCA ACAAGTATGT
CMW 9366 Syzygium South Africa          ---------- ---------- ---------T GACGCGCTCC T-GTCACA-G GCCTCCGGCA ACAAGTATGT
CMW 10192 Syzygium South Africa         ---------- ---------- ---------T GACGCGCTCC T-GTCACA-G GCCTCCGGCA ACAAGTATGT
CMW 10046 Syzygium South Africa         ---------- ---------- ---------T GACGCGCTCC T-GTCACA-G GCCTCCGGCA ACAAGTATGT
CMW 10036 Syzygium South Africa         ---------- ---------- ---------T GACGCGCTCC T-GTCACA-G GCCTCCGGCA ACAAGTATGT
CMW 10076 Syzygium South Africa         ---------- ---------- ---------T GACGCGCTCC T-GTCACA-G GCCTCCGGCA ACAAGTATGT
CMW 10086 Syzygium South Africa         ---------- ---------- ---------T GACGCGCTCC T-GTCACA-G GCCTCCGGCA ACAAGTATGT
CMW 8757 Eucalyptus Venezuela           ---------- ---------- ---------T GACGCGCTCC T-GTCACA-G GCCTCCGGCA ACAAGTATGT
CMW 1853 Syzygium aromaticum            ---------- ---------- ---------T GACGCGCTCC T-GTCACA-G GCCTCCGGCA ACAAGTATGT
CMW 9927 Tibouchina Colombia            ---------- ---------- ---------T GACGCGCTCC T-GTCACA-G GCCTCCGGCA ACAAGTATGT
CMW 9929 Tibouchina Colombia            ---------- ---------- ---------T GACGCGCTCC T-GTCACA-G GCCTCCGGCA ACAAGTATGT
CMW 9928 Tibouchina Colombia            ---------- ---------- ---------T GACGCGCTCC T-GTCACA-G GCCTCCGGCA ACAAGTATGT
CMW 8756 Eucalyptus Indonesia           ---------- ---------- ---------T GACGCGCTCC T-GTCACA-G GCCTCCGGCA ACAAGTATGT
CMW 9906 Eucalyptus Kalimantan          ---------- ---------- ---------T GACGCGCTCC T-GTCACA-G GCCTCCGGCA ACAAGTATGT
CMW 9903 Eucalyptus Kalimantan          ---------- ---------- ---------T GACGCGCTTC TTGTCACAAG GCTTCGGGCA ACAAGTATGT
CMW 7036 C. eucalypti South Africa      ---------- ------GAGC TTGCCCTTCT GACGCGTTTC TCGTCCA--G GCCTCCGGCA ACAAGTATGT
CMW 7037 C. eucalypti Australia         ---------- ------GAGC TTGCCCTTCT GACGCGTTTC TCGTCCA--G GCCTCCGGCA ACAAGTATGT
CWM 7038 C. eucalypti Australia         ---------- ------GAGC CTGCCCTTCT GACGCGTTTC TCGTCCA--G GCCTCCGGCA ACAAGTATGT
CMW 7047 C. parasitica USA              --CTTCTGGT ATAGGCGAGC TTCCTCTTCT GACGCGCTTC TTGTCCA--G GCCTCCGGCA ACAAGTATGT
CMW 7048 C. parasitica USA              --CTTCTGGT ATAGGCGAGC TTCCTCTTCT GACGCGCTTC TTGTCCA--G GCCTCCGGCA ACAAGTATGT
CMW 1651 C. parasitica USA              --CTTCTGGT ATAGGCGAGC ATCCTCTTCT GACGCGCTTC TTGTCCA--G GCCTCCGGCA ACAAGTATGT
CMW 1652 C. parasitica USA              --CTTCTGGT ATAGGCGAGC TTCCTCTTCT GACGCGCTTC TTGTCCA--G GCCTCCGGCA ACAAGTATGT
CMW 5288 D. ambigua                     ---------- ---------- --------CT AACGTG-TTA TCGCCCA--G GCCTCCGGCA ACAAGTATGT
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                                        920        930        940        950        960        970        980
CMW 62 Eucalyptus South Africa          CCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGCACCATG GACGCCGTCC GTGCCGGCCC CTTCGGCCAG
CMW 2113 Eucalyptus South Africa        CCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGCACCATG GACGCCGTCC GTGCCGGCCC CTTCGGCCAG
CMW 8755 Eucalyptus South Africa        CCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGCACCATG GACGCCGTCC GTGCCGGCCC CTTCGGCCAG
CMW 9327 Tibouchina South Africa        CCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGCACCATG GACGCCGTCC GTGCCGGCCC CTTCGGCCAG
CMW 9328 Tibouchina South Africa        CCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGCACCATG GACGCCGTCC GTGCCGGCCC CTTCGGCCAG
CMW 9932 Tibouchina South Africa        CCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGCACCATG GACGCCGTCC GTGCCGGCCC CTTCGGCCAG
CMW 9364 Syzygium South Africa          CCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGCACCATG GACGCCGTCC GTGCCGGCCC CTTCGGCCAG
CMW 9366 Syzygium South Africa          CCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGCACCATG GACGCCGTCC GTGCCGGCCC CTTCGGCCAG
CMW 10192 Syzygium South Africa         CCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGCACCATG GACGCCGTCC GTGCCGGCCC CTTCGGCCAG
CMW 10046 Syzygium South Africa         CCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGCACCATG GACGCCGTCC GTGCCGGCCC CTTCGGCCAG
CMW 10036 Syzygium South Africa         CCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGCACCATG GACGCCGTCC GTGCCGGCCC CTTCGGCCAG
CMW 10076 Syzygium South Africa         CCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGCACCATG GACGCCGTCC GTGCCGGCCC CTTCGGCCAG
CMW 10086 Syzygium South Africa         CCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGCACCATG GACGCCGTCC GTGCCGGCCC CTTCGGCCAG
CMW 8757 Eucalyptus Venezuela           CCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGCACCATG GACGCCGTCC GTGCCGGCCC CTTCGGCCAG
CMW 1853 Syzygium aromaticum            CCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGCACCATG GACGCCGTCC GTGCCGGCCC CTTCGGCCAG
CMW 9927 Tibouchina Colombia            CCCCCGCGCC GTCCTCGTCG ACCTCGAGCC CGGCACCATG GACGCCGTCC GTGCCGGCCC CTTCGGCCAA
CMW 9929 Tibouchina Colombia            CCCCCGCGCC GTCCTCGTCG ACCTCGAGCC CGGCACCATG GACGCCGTCC GTGCCGGCCC CTTCGGCCAA
CMW 9928 Tibouchina Colombia            CCCCCGCGCC GTCCTCGTCG ACCTCGAGCC CGGCACCATG GACGCCGTCC GTGCCGGCCC CTTCGGCCAA
CMW 8756 Eucalyptus Indonesia           TCCCCGCGCC GTCCTCGTCG ATCTCGAGCC TGGCACCATG GACGCCGTCC GTGCCGGCCC CTTCGGCCAG
CMW 9906 Eucalyptus Kalimantan          TCCCCGCGCC GTCCTCGTCG ATCTCGAGCC TGGCACCATG GACGCCGTCC GTGCCGGCCC CTTCGGCCAG
CMW 9903 Eucalyptus Kalimantan          TCCCCGCGCC GTCCTTGTTG ATCTCGAACC TGGCACCATG GACGCCGTTC GTGCCGGCCC CTTCGGCCAG
CMW 7036 C. eucalypti South Africa      TCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGTACCATG GATGCCGTCC GCGCCGGCCC CTTCGGCCAG
CMW 7037 C. eucalypti Australia         TCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGTACCATG GATGCCGTCC GCGCCGGCCC CTTCGGCCAG
CWM 7038 C. eucalypti Australia         TCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGTACCATG GATGCCGTCC GCGCCGGCCC CTTCGGCCAG
CMW 7047 C. parasitica USA              TCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGTACCATG GATGCCGTCC GCGCTGGCCC CTTTGGTCAG
CMW 7048 C. parasitica USA              TCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGTACCATG GATGCCGTCC GCGCTGGCCC CTTTGGTCAG
CMW 1651 C. parasitica USA              TCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGTACCATG GATGCCGTCC GCGCTGGCCC CTTTGGTCAG
CMW 1652 C. parasitica USA              TCCCCGCGCC GTCCTCGTCG ATCTCGAGCC CGGTACCATG GATGCCGTCC GCGCTGGCCC CTTTGGTCAG
CMW 5288 D. ambigua                     TCCTCGCGCC GTCCTCGTCG ATCTCGAGCC CGGTACCATG GACGCCGTCC GTGCCGGTCC CTTTGGCCAG
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                                          990        1000       1010

CMW 62 Eucalyptus South Africa            CTGTTCCGCC CCGACAACTT CGTCTTCGGC CAGTCC
CMW 2113 Eucalyptus South Africa          CTGTTCCGCC CCGACAACTT CGTCTTCGGC CAGTCC
CMW 8755 Eucalyptus South Africa          CTGTTCCGCC CCGACAACTT CGTCTTCGGC CAGTCC
CMW 9327 Tibouchina South Africa          CTGTTCCGCC CCGACAACTT TGTCTTCGGC CAGTCC
CMW 9328 Tibouchina South Africa          CTGTTCCGCC CCGACAACTT TGTCTTCGGC CAGTCC
CMW 9932 Tibouchina South Africa          CTGTTCCGCC CCGACAACTT TGTCTTCGGC CAGTCC
CMW 9364 Syzygium South Africa            CTGTTCCGCC CCGACAACTT CGTCTTCGGC CAGTCC
CMW 9366 Syzygium South Africa            CTGTTCCGCC CCGACAACTT CGTCTTCGGC CAGTCC
CMW 10192 Syzygium South Africa           CTGTTCCGCC CCGACAACTT CGTCTTCGGC CAGTCC
CMW 10046 Syzygium South Africa           CTGTTCCGCC CCGACAACTT CGTCTTCGGC CAGTCC
CMW 10036 Syzygium South Africa           CTGTTCCGCC CCGACAACTT CGTCTTCGGC CAGTCC
CMW 10076 Syzygium South Africa           CTGTTCCGCC CCGACAACTT CGTCTTCGGC CAGTCC
CMW 10086 Syzygium South Africa           CTGTTCCGCC CCGACAACTT CGTCTTCGGC CAGTCC
CMW 8757 Eucalyptus Venezuela             CTGTTCCGCC CCGACAACTT CGTCTTCGGC CAGTCC
CMW 1853 Syzygium aromaticum              CTGTTCCGCC CCGACAACTT CGTCTTCGGC CAGTCC
CMW 9927 Tibouchina Colombia              CTGTTCCGCC CCGACAACTT CGTCTTCGGC CAGTCC
CMW 9929 Tibouchina Colombia              CTGTTCCGCC CCGACAACTT CGTCTTCGGC CAGTCC
CMW 9928 Tibouchina Colombia              CTGTTCCGCC CCGACAACTT CGTCTTCGGC CAGTCC
CMW 8756 Eucalyptus Indonesia             CTGTTCCGCC CCGACAACTT CGTCTTCGGC CAGTCC
CMW 9906 Eucalyptus Kalimantan            CTGTTCCGCC CCGACAACTT CGTCTTCGGC CAGTCC
CMW 9903 Eucalyptus Kalimantan            CTGTTCCGGC CCGACAACTT CGTCTTCGGC CAGTCC
CMW 7036 C. eucalypti South Africa        CTGTTCCGTC CCGACAACTT CGTCTTCGGC CAGTCC
CMW 7037 C. eucalypti Australia           CTGTTCCGTC CCGACAACTT CGTCTTCGGC CAGTCC
CWM 7038 C. eucalypti Australia           CTGTTCCGTC CCGACAACTT CGTCTTCGGC CAGTCC
CMW 7047 C. parasitica USA                CTGTTCCGTC CCGACAACTT CGTCTTTGGC CAGTCC
CMW 7048 C. parasitica USA                CTGTTCCGTC CCGACAACTT CGTCTTTGGC CAGTCC
CMW 1651 C. parasitica USA                CTGTTCCGTC CCGACAACTT CGTCTTTGGC CAGTCC
CMW 1652 C. parasitica USA                CTGTTCCGTC CCGACAACTT CGTCTTTGGC CAGTCC
CMW 5288 D. ambigua                       CTCTTCCGCC CCGACAACTT CGTCTTCGGC CAGTCC
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CHAPTER 3

GENETIC COMPARISON OF *CRYPHONECTRIA CUBENSIS* ISOLATES FROM NATIVE AND EXOTIC HOSTS IN SOUTH AFRICA

ABSTRACT

Cryphonectria cubensis is one of the most important fungal canker pathogens of *Eucalyptus* trees in tropical and sub-tropical regions of the world. Although it is best known on *Eucalyptus*, this pathogen has been recorded on three genera of trees in two families residing in the Myrtales. The fungus was first reported in South Africa in 1989, and has hindered the development of clonal propagation of *Eucalyptus* spp.in the country. Recent studies based on morphological and DNA sequence comparisons have shown that *C. cubensis* in South Africa is distinct from the fungus of the same name, occurring in other parts of the world. This has led to speculation that the South African form of *C. cubensis* represents a distinct taxon, native to this country. The recent discovery of Cryphonectria canker on native *Syzygium* spp. in South Africa supports this view. The purpose of this study was to compare the genetic structure of South African isolates of *C. cubensis* from native *Syzygium* spp. and those from exotic *Eucalyptus* and *Tibouchina* spp., using vegetative compatibility tests and polymorphic DNA (SSR) markers. Unique alleles were found for several DNA markers in each of the three groups of isolates examined. This suggests that the isolates on the different hosts could have originated from a yet unknown host. There were more unique genotypes amongst isolates from *Eucalyptus* and *Tibouchina,* than from the native *Syzygium* spp. This indicated that *C. cubensis* on *Tibouchina* and *Eucalyptus* could have originated from *Syzygium* spp. and that *C. cubensis* has been on this native host for longer than it has been on the two exotic hosts. This study provides the first clear evidence that *C. cubensis* in South Africa is native to this country or at least to the African continent.

INTRODUCTION

Cryphonectria cubensis (Bruner) Hodges is the causal agent of Cryphonectria canker of *Eucalyptus* trees in plantations grown in the tropics and sub -tropics (Boerboom & Maas 1970, Hodges, Geary & Cordell 1979, Sharma, Mohanan & Florence 1985a, b, Wingfield, Swart & Abear 1989), causing tree death through stem girdling. Cryphonectria canker is most common in regions with relatively high temperatures, rainfall and humidity (Hodges *et al.* 1979, Sharma *et al.* 1985a,b). In many of these areas, it is regarded as an important constraint to exotic plantation forestry (Bruner 1917, Boerboom & Maas 1970, Hodges & Reis 1974, Gibson 1980, Wingfield *et al.* 1989).

C. cubensis is best known on *Eucalyptus* spp, but has also been reported from three tree genera in the Myrtaceae and Melastomataceae. While morphologically very different, species in these two tree families are closely related, as reflected in comparisons of DNA sequence data and both reside in the Myrtales (Conti *et al.* 1997). Hosts of *C. cubensis* residing in the Myrtaceae include many *Eucalyptus* spp. (Bruner 1917, Hodges *et al.* 1979, Gibson 1980, Sharma *et al.* 1985a,b, Wingfield *et al.* 1989, Davison & Coates 1991) and *Syzygium* spp. (Hodges, Alfenas & Ferreira 1986, Chapter 2, *This dissertation*). In the Melastomataceae, hosts include species of *Tibouchina* (Wingfield *et al.* 2001, Myburg *et al.* 2002a).

Recent studies have shown differences between South African strains of *C. cubensis* and those occurring in other parts of the world. Based on comparisons of sequence data for the ITS regions of the ribosomal RNA operon, Myburg, Wingfield & Wingfield (1999) showed that isolates of *C. cubensis* from South-east Asia and South America reside in discrete clades. The South African isolates grouped together with those from South America. Because symptoms associated with the fungus on *Eucalyptus* spp. in South Africa differ from those in South America, Myburg *et al.* (1999) initiated more detailed comparisons using multiple gene trees. Results showed that the South African fungus is distinct from the fungus of the same name occurring in other parts of the world and that it probably represents a distinct species (Myburg *et al.* 2002b). It has also been suggested that *C. cubensis* would more appropriately be

accommodated in a genus other than *Cryphonectria* (Venter *et al.* 2001, Gryzenhout *et al.* 2002, Myburg *et al.* 2003).

Population genetic studies on fungal pathogens have been used to gain insight into the origin and relatedness of species (O'Donnell, Cigelnik & Nirenberg, 1998, Carbone & Kohn 2001). Various techniques have been used to determine the population structure of fungi, including the determination of phenotypic characters such as vegetative compatibility groups (VCGs) (Anagnostakis & Kranz 1987, Correll, Gordon & McCain 1992, Leslie 1993). In tree pathogens, VCGs have for example been used to study populations of *C. cubensis* (Van Heerden *et al.* 1997) and the closely related chestnut blight pathogen, *C. parasitica* (Murr.) Barr. (Anagnostakis, Hau & Kranz, 1987). Numerous molecular markers have also been used to study the population biology of plant pathogens (McDonald & McDermott 1993). In recent years, co-dominant polymorphic markers have become the preferred tools to study populations of pathogens (Taylor *et al.* 2000). These markers have the advantage of allowing the detection and characterisation of multiple alleles at a specific locus. Of these markers, microsatellites are useful because they are abundant in eukaryotic genomes (Toth, Gaspari & Jurka 2000), highly polymorphic, are easy to score and are thought to be selectively neutral (Tautz 1989). A recent study has thus used polymorphic markers to study mode of reproduction, gene flow and population differentiation in *C. cubensis* (Van der Merwe 2002).

Cryphonectria canker has recently been discovered on native *Syzygium* spp. in South Africa (Heath *et al.* 2003). This discovery and the fact that the fungus in South Africa appears to be unique, has led to the view that it might be native to this country. In this study, we consider the genetic diversity of *C. cubensis* isolates from three different hosts in South Africa.

MATERIALS AND METHODS

Isolates

South African *C. cubensis* isolates used in this study originated from three tree genera (Table 1). Isolates from *Eucalyptus* were obtained from the culture collection (CMW)

of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa and were the same as those used in previous studies by Van Heerden *et al.* (1997). Isolates from the native South African trees *Syzygium cordatum* Hachst. and *S. guineense* (CD.) Willd. were the same as those used in Chapter 2 (*this dissertation*). *Tibouchina* isolates collected by Myburg *et al.* (2002) were used and supplemented with isolates collected specifically for this study from the KwaZulu-Natal Province. Techniques used to make these isolations were the same as those presented previously (Chapter 2, *This dissertation*). All isolates were obtained from single conidia, each from a different tree. All isolates are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

Vegetative compatibility tests

VCGs were used to estimate the genotypic diversities for the isolates from exotic *Eucalyptus*, *Tibouchina* and native *Syzygium* spp. The VCGs were assessed on oatmeal agar (Van Heerden & Wingfield 2001) (Fig. 1) and a medium described by Powell (1995) using the pH indicator, bromocresol green. Only one isolate per tree was used, since previous studies have shown that *Eucalyptus* trees are consistently infected by a single VCG (Van Heerden *et al.* 1997). Each set of isolates was initially assessed individually. VCGs previously determined for the *Eucalyptus* isolates by Van Heerden & Wingfield (2001) were used for comparative purposes. VCG's were determined for the *Syzygium* and *Tibouchina* populations using the technique described by Anagnostakis (1977). Subsequently, one representative isolate for each VCG from each of the three collections was used to compare the VCG's present on the different trees.

After the incubation period, isolates forming barrage reactions, or that had not merged, were scored as incompatible genotypes (Fig. 1). The vegetative compatible isolates merged at the point of mycelial interaction and formed confluent mycelial mats (Fig. 1). To assess the diversity of the VCGs, the number of VCGs and the frequency of isolates belonging to each VCG was determined for each of the three sets of isolates. Two different statistical parameters were used to determine the degree of diversity for these isolates. The first parameter was Stoddart & Taylor's

(1988) genotypic diversity (G). To compare diversity levels between populations, the genotypic diversity (G) was divided by the sample size (N) to obtain the maximum percentage of genotypic diversity ($\hat{G}$) (Stoddart & Taylor 1988, McDonald *et al.* 1994).

The second parameter used to consider the VCG data was the Shannon Index (SI) (Bowman *et al.* 1971, Groth & Roelfs 1986). This takes into account the frequency and evenness of the distribution of a particular phenotype. The SI was normalised to obtain H_s (normalised Shanon index) before populations of different sizes could be compared (Sheldon 1969). The H_s value was subsequently treated as an indication of phenotypic diversity based on VCGs.

Analyses using microsatellite markers

DNA isolation and amplification

Mycelium from actively growing cultures (Table 1) was inoculated into 1.5 ml microcentrifuge tubes containing 750 µl, 30% (w/v) Malt Extract Broth (Merck laboratory Supplies, Germany). DNA was isolated using a modified version (Van der Merwe 2002) of the technique of Murray & Thompson (1980).

Polymorphic loci were amplified from genomic DNA from 34 isolates from *Eucalyptus* spp., 37 isolates from *Tibouchina* spp. and 62 isolates from *Syzygium* spp., using six specific primer pairs developed by Van der Merwe, Wingfield &Wingfield (2003) (Table 2). Polymerase chain reactions (PCR) consisted of 25 ng genomic DNA, 1 mM of each dNTP (Promega, Madison, Wisconsin, U.S.A.), 0.2 µM of each locus-specific primer, 0.5 units SuperTherm *Taq* Polymerase (Southern Cross Biotechnology, South Africa), 10 × Reaction Buffer (Southern Cross Biotechnology, South Africa), 2.5 mM $MgCl_2$ (Southern Cross Biotechnology, South Africa) and 0.32 M 2-pyrrolidinone (Aldrich Chemical Company Inc., U.S.A). Sterile water was used to adjust the final volume to 12.5 µl. Amplification reactions were performed on a Perkin Elmer GeneAmp PCR System 9700 thermocycler (Perkin-Elmer Applied Biosystems, Inc. Foster City, California).

Conditions for the PCR consisted of an initial denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 1 min, primer annealing for 1 min at 50 °C (*COL3* and *SA1* loci) or 54 °C (*SA2*, *SA6*, *SA9* and *SA10* loci), and extension at 72 °C for 1 min. The reaction was terminated with a final extension at 68 °C for 30 min to ensure completion of the reactions. PCR products were visualised on a 2 % agarose-ethidium bromide gel using ultraviolet light. Product sizes were estimated by comparison with a 100 bp standard size marker (Promega, Madison, Wisconsin, U.S.A.).

Genescan analysis

PCR products (0.2 µl) were added to 0.4 µl loading dye (Perkin Elmer Corporation, California, USA), 0.4 µl formamide and 0.2 µl of the internal size standard GENESCAN-500 TAMRA (Perkin Elmer Corporation, California, USA). Samples were denatured at 95 °C for five min and immediately transferred to ice, followed by size fractionation using a GeneScan® PAGE gel on an ABI Prism 377 Automatic DNA Sequencer. Allele sizes were determined using GeneScan® 2.1 analysis software (Perkin Elmer Corporation) and Genotyper® 3.0 (Perkin Elmer Corporation).

Statistical analyses

Statistical analysis was performed using Multilocus 1.2 (Agapow & Burt 2001). Several analyses were performed to test for partitions and to calculate genotypic diversity, linkage disequilibrium, population differentiation and gene flow for the three sets of isolates.

In order to test for the presence of partitions within the total data set, Nei's (1973) generalised measure of G_{st} was calculated. When G_{st} approximates zero, most of the gene diversity is found within subdivisions (populations) and, therefore, population differentiation is low. Conversely, when G_{st} approaches 1, each sub-division is homogeneous and most of the variation exists between subdivisions. The G_{st} value was calculated over all isolates sampled, as well as for all combinations of subdivisions based on host origin.

Genotypic diversity, as defined by Stoddart & Taylor (1988) was modelled against the number of loci (1000 re-sampling repetitions) to produce a sigmoidal graph indicating

when the diversity present in the natural population had been adequately sampled. When the graph reaches a plateau, it indicates that sampling was sufficient. Genotypic diversity (G) defines the probability that two individuals considered at random have different genotypes (Nei 1973). In addition to genotypic diversity, the gene diversity (Nei 1973) was calculated for each locus to consider the probability of sampling two different alleles at the same locus, within the population. This statistic is equivalent to the heterozygosity value for diploid organisms. For each population, the mean of H ($\overline{H}$) was calculated to facilitate comparisons between populations.

Gametic (linkage) disequilibrium ($\bar{r}_s$) (Agapow & Burt 2001) was calculated to determine whether two individuals that are the same at a certain locus, are more likely to be the same at another locus and provides an indication of mode of reproduction. This analysis is similar to the Index of Association (I_A) and linkage disequilibrium ($\bar{r}_d$) (Agapow & Burt 2001) but is adapted to be applied to ordered alleles and can test whether alleles tending in the same direction are positively or negatively associated. Alleles are considered as integers and all are summated for each individual, after which the variance can be calculated. This value can be compared to the variance expected in the absence of gametic disequilibrium, which is the sum of the variance for each locus. Thus, the position of the observed value relative to a modelled randomly mating population, is an indication of the level of clonality or outcrossing within the population. The difference between $\bar{r}_s$, I_A and $\bar{r}_d$ is that $\bar{r}_s$ analysis the allelic values directly and the sums are over all individuals, whereas I_A and $\bar{r}_d$ computes a distance matrix and calculates the sums over all pairs of individuals.

Population differentiation (θ) is based on the number of characters shared, and different between populations. It, therefore, allows for the determination of differentiation between populations in either the allelic or genotypic distribution. It is calculated using a randomised data set with 1000 randomisations using Weir's (1996) formulation of Wright's F_{ST} for haploids. Gene flow is defined as $\frac{1}{\theta}$, the inverse of population differentiation, and is expressed as a relative value. Therefore, a low $\frac{1}{\theta}$-

value indicates low levels of gene flow, while a high value indicates high levels of gene flow. These values are supported by a probability value. Due to gene flow's inverse relationship to population differentiation (θ), a higher population differentiation value will result in a low gene flow value and *vice versa*.

RESULTS

Isolates

A total of 100 *Eucalyptus* isolates obtained from previous collections by Van Heerden & Wingfield (2001) were used for VCG analysis. These isolates originated from *E. grandis* in the KwaZulu-Natal Province (Fig. 2). Other isolates collected were those from *S. cordatum* (61) in the Northern Province, KwaZulu-Natal Province and the Mpumalanga Province and from *S. guineense* (1) in the Mpumalanga Province. A total of 37 isolates were collected from *T. granulosa* in the KwaZulu-Natal Province.

Vegetative compatibility tests

In the study conducted by Van Heerden & Wingfield (2001), 23 VCGs were identified from a collection of *Eucalyptus* isolates. The genotypic diversity for that population was 9.6 with a maximum percentage of genotypic diversity ($\hat{G}$) of 0.4 % (Table 3). The phenotypic diversity of this population, as measured with the normalised Shannon Index (H_s), was 55 %.

The *Syzygium* population resulted in 32 VCGs (Table 3) with an average of two isolates per VCG. Of the 62 isolates in this population, 21 belonged to a different VCG, with the largest VC group consisting of 10 isolates (Table 3). The VCG of the isolate from *S. guineense* was not represented among those from *S. cordatum*. The $\hat{G}$ value for this population was 26 % (Table 3), while H_s was 36 % (Table 3).

The *Tibouchina* population consisted of 10 VCGs with an average of 3.8 isolates per VCG (Table 3). Only two of the isolates resided in unique VCGs with six isolates representing the VCG-group including the largest number of isolates. This population had a $\hat{G}$ of 22 % (Table 3), and H_s of 24 %.

The test conducted to compare the three different populations showed that there were five VCGs common to both *Syzygium* and *Eucalyptus*. There was one VCG common to both *Syzygium* and *Tibouchina* and no VCGs were shared between the *Eucalyptus* and *Tibouchina* populations.

Analyses using microsatellite markers

Genescan analysis

A total of seven loci were screened using the six primer pairs available. The alleles for these loci ranged in size from 169 to 286 bp (Table 4). Primers designed for locus *SA9* targeted two independent polymorphic loci in the genome. All but one of these loci were polymorphic. Locus *SA1* was monomorphic (285 bp) for the isolates from *Syzygium*, while loci *COL3* and *SA2* could not be amplified in isolates from *Tibouchina*. The data points for *COL3* and *SA2* were thus treated as missing data in all subsequent analyses.

Statistical analyses

Based on G_{st} (Nei 1973), the total data set could be divided into partitions that correlate with the host from which isolates originated (G_{st} = 0.67). G_{st} between subdivisions based on host origin could also be calculated (Fig. 3). The G_{st} was indirectly related to the number of shared VCGs. The highest G_{st} (0.5) value was between the *Eucalyptus* and *Tibouchina* populations. These two populations also did not share VCGs.

The maximum percentage of genotypic diversity for the *Eucalyptus*, *Tibouchina* and *Syzygium* populations was 45 %, 33 % and 5 %, respectively. The corresponding gene diversity ($\overline{H}_s$) values were 0.43, 0.57 and 0.17. When genetic variation (G_{ST}) (Stoddart & Taylor 1988) was re-sampled per locus for each population, only the population from *Syzygium* did not reach a plateau, even though this population was comprised of the highest number of isolates (Fig. 4). The graphs obtained for both the *Eucalyptus* and *Tibouchina* populations reached a plateau, indicating that the maximum amount of genetic diversity had been sampled for those hosts (Fig. 4).

The observed genetic disequilibrium value ($\bar{r}_s$) for the *Syzygium* and *Eucalyptus* populations was within the ranges of the distribution for the randomised data set, with values of 0.12 (P=0.412) and 0.001 (P=0.928) respectively (Fig. 5A-B). The non-significant P-values indicate that the null hypothesis of random mating cannot be rejected. The observed $\bar{r}_s$-value for the *Tibouchina* population was not within the range of the distribution of the randomised data set but outside the set at 0.39, supported by a probability value of P<0.002 (Fig. 5C). Due to the high level of significance of this P-value, the null hypothesis can be rejected, indicating that the alleles are in gametic disequilibrium. This suggests that the primary mode of reproduction for this population is clonal.

Population differentiation was tested between all populations in all possible combinations. Of these combinations, only the test between the *Syzygium* and *Eucalyptus* populations resulted in a θ-value of 0.44 (P<0.001). The θ-values obtained from the partition test for the *Syzygium*/*Tibouchina* and the *Eucalyptus*/*Tibouchina* populations, was supported by significant P-values, resulting in $\frac{1}{\theta}$-values of 0 for both data sets and indicating low levels of gene flow. The test performed on the *Syzygium*/*Eucalyptus* populations, however, resulted in a higher $\frac{1}{\theta}$-value of 2.27. These findings correlate with gene flow estimated from G_{st}, which gives an indication of the number of migrants per generation between populations (Fig. 3).

DISCUSSION

In this study we compared the genetic structure of three populations of *C. cubensis* isolates originating from two exotic (*Eucalyptus* and *Tibouchina* spp.), and one native (*Syzygium* spp.) host in South Africa. We were able to show that the two exotic hosts have been colonised more recently than the native host, and possibly by isolates originating from the native host. We have also shown that South African *C. cubensis* isolates have a high level of genetic diversity, strongly suggesting that the fungus is native to this country.

When genetic variation (G_{ST}) was re-sampled per locus for each population of *C. cubensis*, it was shown that for both the *Eucalyptus* and *Tibouchina* populations, the maximum amount of genetic diversity had been sampled. The same was, however, not true for the population from *Syzygium*. This result is surprising as the *Syzygium* population consisted of the highest number of isolates. This population was also sampled over a greater geographic area than the *Eucalyptus* or *Tibouchina* populations, which were from plantations or ornamental trees. *Syzygium* trees that were sampled occurred abundantly over a wide area, whereas the *Tibouchina* trees were in a relatively limited area, and mostly from the town of KwaMbonambi. The *Eucalyptus* isolates were also from trees in a small area in comparison to the distribution of *Syzygium* isolates. The low diversity for *C. cubensis* from *Syzygium* spp., could be due to ecological homeostasis (Leppik 1970), which results in low infection levels. *Eucalyptus* and *Tibouchina* trees are being selected for advantageous traits such as wood quality, growth vigour, flower colour and many others. This could lead to a limited genetic base and uniform distribution of variation in the host. It is, therefore, conceivable that a *C. cubensis* epidemic is much more likely on the exotic hosts than on the native host, and can be more easily sampled.

The *C. cubensis* population from *Syzygium* had a relatively low level of mean allelic diversity ($\overline{H}_s$ = 0.17)at the investigated loci calculated from SSR data. In contrast, isolates on the exotic *Eucalyptus* and *Tibouchina* spp. displayed gene diversity ($\overline{H}_s$) as high as 0.43 in the former and 0.57 in the latter group. This might suggest that the centre of diversity for the pathogen is not the native host included in this study. Following the view that native populations generally tend to have high levels of genetic diversity (Tsutsui *et al.* 2000), it appears that the host of origin of *C. cubensis* in South Africa has not yet been found. Despite this, the high diversities obtained for the *Eucalyptus* and *Tibouchina* populations refute the notion that the fungus was introduced into the country, as originally proposed (Wingfield *et al.* 1989, Wingfield 1990).

Based on the number of unique alleles in each of the three populations of *C. cubensis* considered in this study, it appears that the founder population of the pathogen might be most closely related to the fungus on *Syzygium* spp. This is supported by the fact

that the population on *Syzygium* spp. possesses the smallest number of unique alleles when compared with those on *Eucalyptus* and *Tibouchina* spp. Isolates from *Syzygium*, therefore, have most of their genetic background in common with those populations. The population on *Eucalyptus* had the highest number of unique alleles (13). This suggests that it most likely originated from multiple colonisation events from different hosts. In contrast, the population present on *Tibouchina* possessed only five unique alleles. This could be due to the fact that *Tibouchina* is an ornamental tree in South Africa, and can be easily and cheaply reproduced by vegetative propagation. The host would, therefore, have a very uniform genetic base and would subsequently have been colonised by a limited number of *C. cubensis* genotypes due to natural selection (Brasier 1999). This is consistent with results obtained from the gametic disequilibrium tests for the various groups of isolates. Only the population of *C. cubensis* from *Tibouchina* had alleles that were not randomly associated, indicating that random mating does not occur on this host.

It is possible that outcrossing occurs in the population of *C. cubensis* from *Syzygium* spp., but that the resultant genotypes are not environmentally fit to colonise *Tibouchina* trees. This would be similar to the situation for *Microbotryum violaceum* (Pers.) G. Deml & Oberw. as described by Delmotte, Buchelli & Shykoff (1999). However, the test for gametic disequilibrium included only those isolates that are present on this host and not isolates from other populations, resulting in an increased association of alleles between loci. Another possibility is that the homothallic mating strategy of *C. cubensis* (Hodges & Reis, 1974) is governed by mating between closely related individuals, and subsequently leads to recombination between different alleles at a highly reduced frequency (Elliott 1994, Milgroom 1996).

Outcrossing was found to be present in populations of *C. cubensis* on *Eucalyptus* and *Syzygium* spp. Also, the partitions between the three investigated populations correlate strongly with the mode of reproduction. The strongest partitions were found between the isolates on *Tibouchina* spp., which are apparently clonal, and populations on *Eucalyptus* and *Syzygium* spp., that appear to be outcrossing. However, partitioning between the latter two groups of isolates was relatively weak (G_{st} = 0.3). It is, therefore, not surprising that gene flow exists between the two outcrossing populations.

The genotypic properties of the *C. cubensis* populations studied were similar to those for the phenotypic tests. Five VCGs are shared between the *Eucalyptus* and *Syzygium* populations, whereas only one VCG was shared between the *Syzygium* and *Tibouchina* isolates. The fact that no VCGs are shared between *C. cubensis* on *Eucalyptus* and *Tibouchina* suggests that the population on *Eucalyptus* did not originate from the population on *Tibouchina*.

This study provided the first comparison of genotypic and phenotypic characters for three different populations of *C. cubensis* in South Africa. It also represents the first consideration of the population biology of *C. cubensis* in South Africa, using DNA based tools. Results clearly show that, in contrast to previous hypothesis (Van Heerden & Wingfield 2001), *C. cubensis* in South Africa has a high diversity and is most likely native to the country. This is also consistent with results of recent studies showing that *C. cubensis* in South Africa is distinct from *C. cubensis* in the rest of the world (Myburg *et al.* 2002, Myburg *et al.* 2003). All three of the currently known hosts for *C. cubensis* in South Africa were represented in this study. Although we have shown that the fungus has been on *Syzygium* spp. for the longest period of time, we could not show that this is the host of origin for *C. cubensis* in South Africa. Our results, however, clearly indicate that identical genotypes of *C. cubensis* from *Syzygium*, occur on *Tibouchina* and *Eucalyptus* spp. The unique allele observed in the *Syzygium* spp. population could be a result of infection of isolates from the founder population on a yet undiscovered host. Further and more detailed surveys for *C. cubensis* on native Myrtales will be required to resolve this intriguing question. They will, however, be hampered by the fact that the fungus is difficult to find on native trees and that these trees also occur in relatively remote and unexplored areas of South Africa.

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Table 1: Isolates of *C. cubensis* used in this study

[a]Isolate Number	Host	Region
CMW 9363	*S. cordatum*	KwaZulu Natal, KwaMbonambi
CMW 9367, 9368	..	KwaZulu Natal, Duku-Duku
CMW 9364	..	KwaZulu Natal, Moba dam
CMW 9366	..	Northern Province, Tzaneen
CMW 9934 - CMW 9935	*S. cordatum*	KwaZulu Natal, KwaMbonambi
CMW 10036 - CMW 10045	..	..
CMW 10046 - CMW 10048	..	KwaZulu Natal, Sodwana
CMW 10049	..	KwaZulu Natal, KwaMbonambi
CMW 10050 - CMW 10060	..	KwaZulu Natal, Sodwana
CMW 10061 - CMW 10066	..	KwaZulu Natal, Amanzimgwenia
CMW 10067 - CMW 10069	..	KwaZulu Natal, Sodwana
CMW 10070	..	KwaZulu Natal, Munzi region
CMW 10071 - CMW 10087	..	KwaZulu Natal, Kosi bay
CMW 10191 - CMW 10194	*S. guineense*	Mpumalanga Province, Hazyview
CMW 9327 - CMW 9340	*T. granulosa*	KwaZulu Natal, KwaMbonambi
CMW 9341, 9342	..	KwaZulu Natal, Richards bay
CMW 9343 - CMW 9362	..	KwaZulu Natal, KwaMbonambi
CMW 9370 - CMW 9374	..	KwaZulu Natal, Durban
CRY 598 – CRY 601	*Eucalyptus* spp.	KwaZulu Natal, KwaMbonambi
CRY 606 – CRY 609	..	..
CRY 611- CRY 633	..	..
CRY 783- CRY 784	..	..

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

Table 2. Six polymorphic primer pairs used to study isolates of *C. cubensis*.

Primer name	Fluorescent label	Fragment size	Primer sequence (5' – 3')
Col 3	HEX (yellow)	172	F gatgaatacgaggtttcgtctc R ccgggatgaagatcagcacgag
SA 1	HEX (yellow)	320	F ggaatcaccaccactagcgtcc R gtgtctccgttaacgcagtggt
SA 2	FAM (blue)	285	F tcatgtgctcgaggaacttctg R tcttggaatgagattaagtcac
SA 6	FAM (blue)	210	F atcgacgatcaggttctggatc R tattgcggtaacccaattttcg
SA 9	TET (green)	191	F gctcgggctgccaatccttaag R cgccgagtttctcgccaccatc
	TET (green)	196	
SA 10	HEX (yellow)	183	F gccgagccatcgctttacgaag R ccgccgatgtgcttcttggacg

"F" and "R" denote forward and reverse primers respectively (Van der Merwe *et al.* 2003)

Table 3. Comparison of maximum percentage of genotypic diversity of the *Syzygium, Eucalyptus* and *Tibouchina* populations as calculated using VC tests.

					Microsatellite analysis		VCG analysis	
Population	**Collection area**	**No. of unique alleles**	**No. of Isolates**	*Outcrossing*	$\hat{G}$	$\overline{H}_s$	$\hat{G}$	H_s
Eucalyptus	narrow	13	34	Yes	45%[1]	0.43[1]	0.4%	55%
Tibouchina	narrow	5	37	No	33%[2]	0.57[2]	22%	24%
Syzygium	broad	2	62	Yes	5%[3]	0.17[3]	26%	36%

[1]Based on 100 isolates (Van Heerden *et al.* 2001)

[2]Based on 64 isolates

[3]Based on 38 isolates

Table 4. Allele ranges and number of loci amplified using the six polymorphic primers.

[a]Primer	Allele range	Number of loci
Col 3	169-173	4
SA 1	275-286	4
SA 2	204-207	4
SA 6	209-211	3
SA 9.1	181-195	3
SA 9.2	165-197	5
SA 10	176-189	7

[a] Primers developed by Van der Merwe *et al.* (2003)

Figure 1. Isolates placed in a predetermined pattern on oatmeal agar used to assess vegetative compatibility. White arrows show barrage lines found between vegetatively incompatible isolates. Vegetively compatible isolates formed a confluent lawn of mycelium as shown by the black arrows.

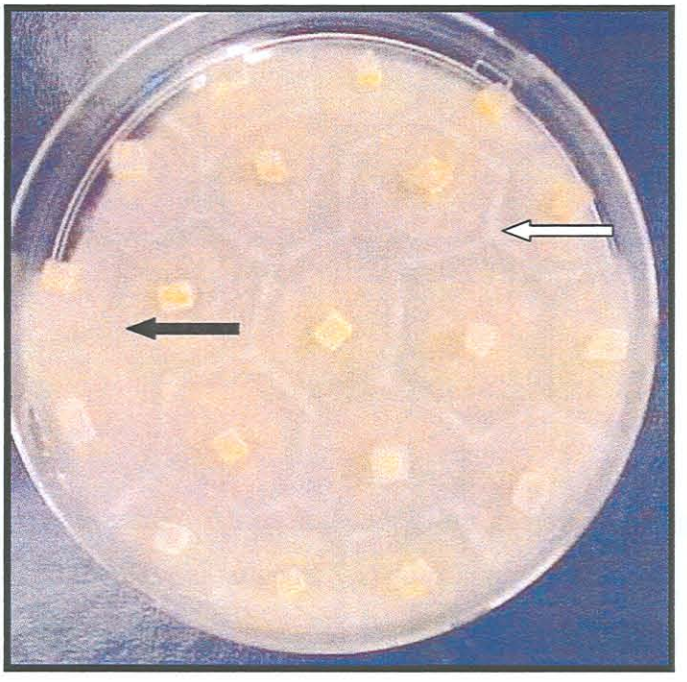
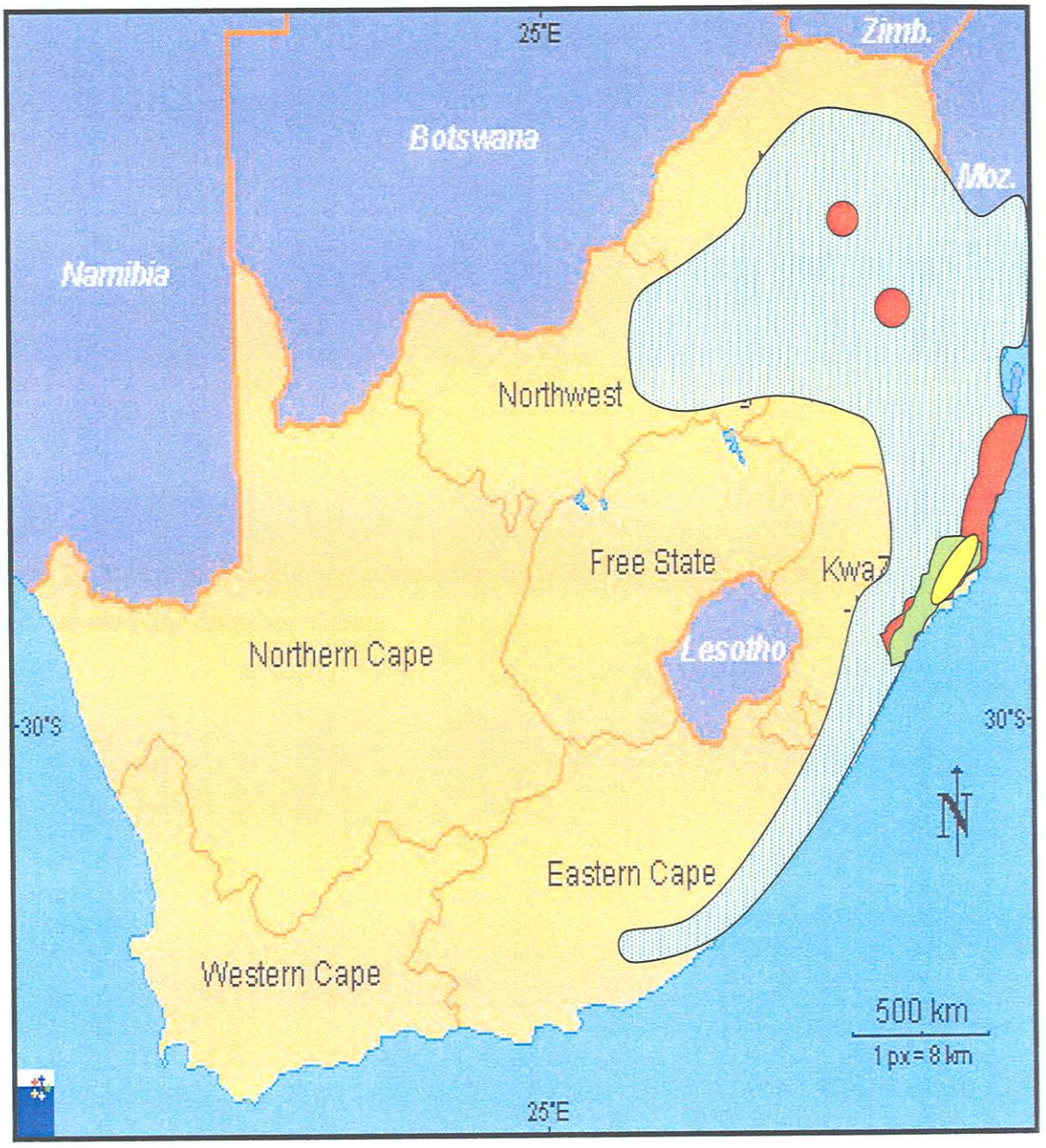


Figure 2. A map of Southern Africa indicating the geographic origin of the isolates used in the study and the natural range of *Syzygium* spp.



- Natural distributions of *Syzygium* spp.
- Isolates obtained from *Syzygium* spp.
- Isolates obtained from *Eucalyptus* spp.
- Isolates obtained from *Tibouchina urvilleana*.

Figure 3. Matrix indicating the correlation between G_{st} and the number of VCGs shared between the three populations.

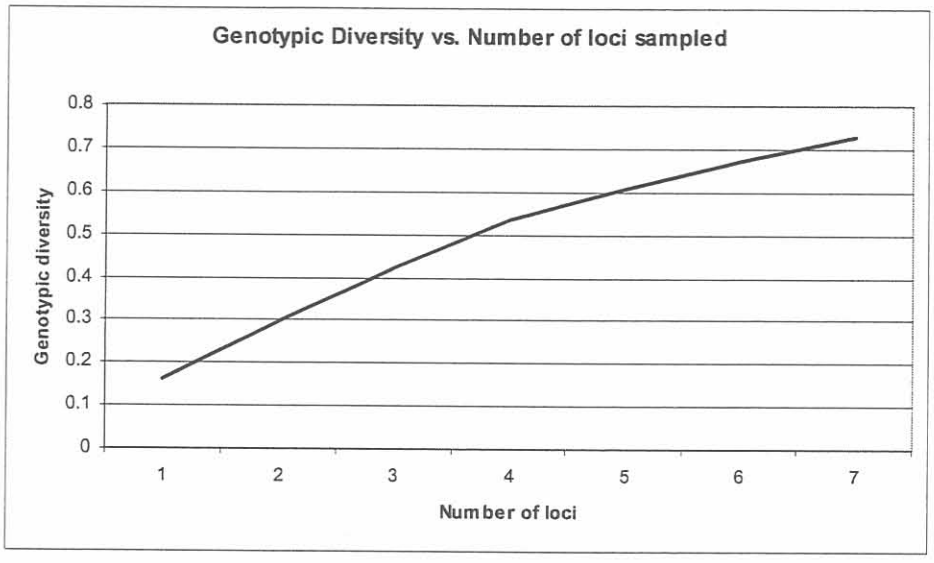
Population	*Eucalyptus*	*Tibouchina*	*Syzygium*
Eucalyptus		0	5
Tibouchina	0.5		1
Syzygium	0.3	0.4	

= G_{st}

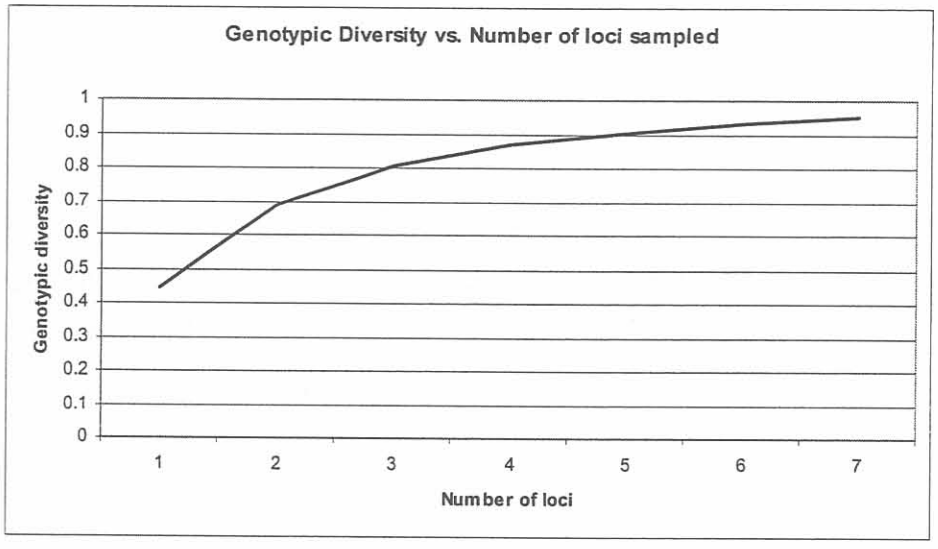
= Number of shared VCGs

Figure 4. Sigmoidal graph indicating when the diversity present in the natural population has adequately been sampled by reaching a plateau. (a) *Syzygium*, (b) *Eucalyptus* and (c) *Tibouchina* populations.

(a)


Genotypic Diversity vs. Number of loci sampled
Genotypic diversity
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
7
Number of loci

(b)


Genotypic Diversity vs. Number of loci sampled
Genotypic diversity
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
7
Number of loci

(c)

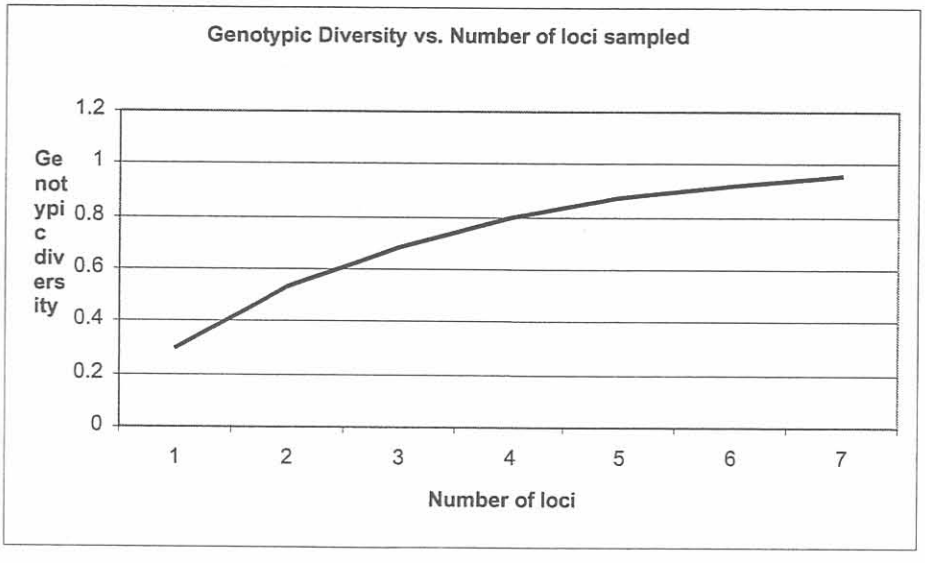
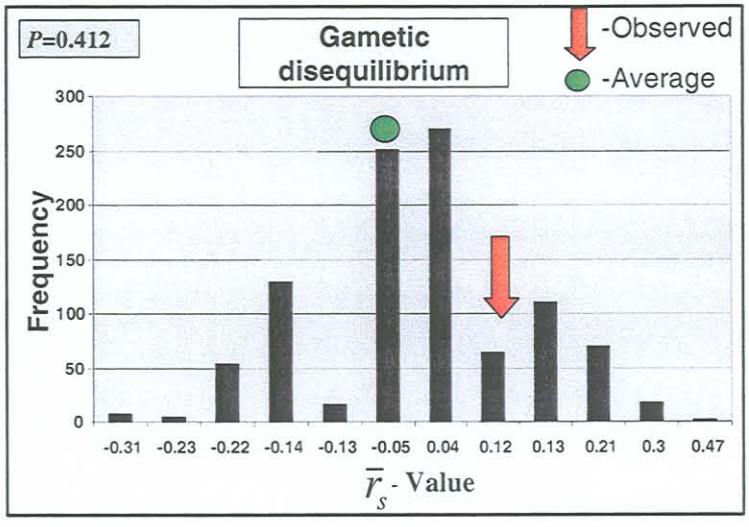
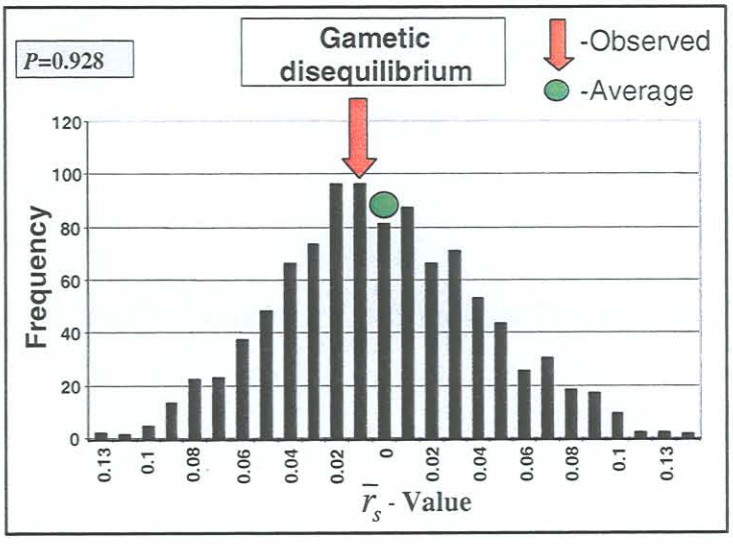

Genotypic Diversity vs. Number of loci sampled
Genotypic diversity
1.2
1
0.8
0.6
0.4
0.2
0
1
2
3
4
5
6
7
Number of loci

Figure 5. Graph illustrating $\bar{r}_s$-value against allele frequency of the (a) *Syzygium* (b) *Eucalyptus* and (c) *Tibouchina* populations. The position of the observed value relative to the ranges of the distribution for the randomised data set indicates mode of reproduction.

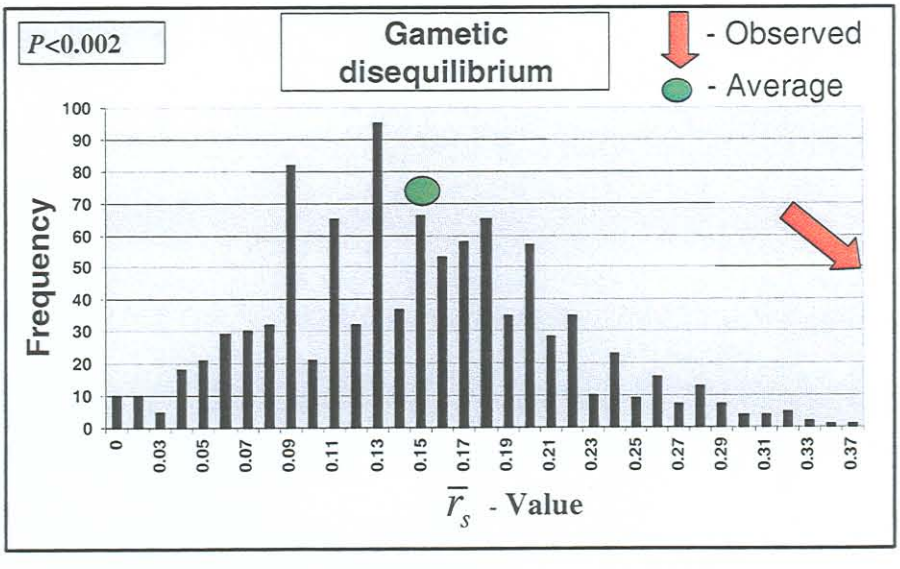
(a)



(b)



(c)



CHAPTER 4

FIRST REPORT OF AN *ENDOTHIELLA* SPECIES ON *TIBOUCHINA URVILLEANA* IN AUSTRALIA

ABSTRACT

Tibouchina spp. (family: Melastomataceae) are native to South America, but have been planted as ornamentals in many southern hemisphere countries. The Melastomataceae reside in the order Myrtales and are close relatives of the Myrtaceae, to which the genus *Eucalyptus* belongs. The recent discovery of the *Eucalyptus* canker pathogen *Cryphonectria cubensis* on *Tibouchina* spp., first in Colombia and subsequently in South Africa, has prompted a more detailed survey for other *Eucalyptus* pathogens that might occur on this tree. In this study we report on the discovery of an *Endothiella* sp. on diseased stems of *Tibouchina* spp. in Australia. Characterisation of this fungus based on morphology and on comparisons of β-tubulin gene sequences showed that the fungus represents an undescribed *Endothiella* sp. resembling the anamorph of the *Eucalyptus* pathogen *Cryphonectria eucalypti*. Greenhouse pathogenicity tests including isolates of *C. eucalypti* from Australia and South Africa showed that the undescribed *Endothiella* sp. is significantly more pathogenic than *C. eucalypti* isolates from South Africa and Australia

INTRODUCTION

Cryphonectria eucalypti M. Venter & M. J. Wingf., is a mild canker pathogen of *Eucalyptus* spp. in Australia (Walker, Old & Murray 1985, Old *et al.* 1986), Tasmania (Yuan & Mohammed 1997, Wardlaw 1999, Yuan & Mohammed 1999, 2000) and South Africa (Van der Westhuizen *et al.* 1993, Venter *et al.* 2002). Typical symptoms of *C. eucalypti* infection are basal stem cankers, kino exudation from depressed cankers and in severe cases, branch and shoot die-back in juvenile trees (Walker *et al.* 1985, Old *et al.* 1986, Van der Westhuizen *et al.* 1993). Severe cankers extending into the cambium have also been reported in Tasmania (Wardlaw 1999). Cankers caused by *C. eucalypti* are often covered in orange fruiting structures, which makes them very conspicuous on the stems of trees (Van der Westhuizen *et al.* 1993). In Australia the fungus has been reported to kill trees under stressful conditions (Walker *et al.* 1985, Old *et al.* 1986, Wardlaw 1999).

Cryphonectria eucalypti was previously known as *Endothia gyrosa* (Schw.: Fr.) Fr. (Venter *et al.* 2002), a canker pathogen of many hardwood species in the United States (Shear, Stevens & Tiller 1917, Stipes & Phipps 1971, Roane *et al.* 1974, Appel & Stipes 1986). *C. eucalypti*, however, is only known to infect *Eucalyptus* spp. in Australia, Tasmania and South Africa and is clearly distinguished from *E. gyrosa* based on morphological characteristics and DNA sequence data (Venter *et al.* 2001, Venter *et al.* 2002).

A recent and intriguing discovery has been that the serious canker pathogen *Cryphonectria cubenis* (Bruner) Hodges occurs not only on members of the Myrtaceae but also on *Tibouchina* spp., which reside in the Melastomataceae (Wingfield *et al.* 2001). Although this discovery seemed unusual at first, it is better understood in the light of recent DNA based comparisons showing that these two families in the Myrtales are closely related (Conti *et al.* 1997). The presence of *C. cubensis* on both *Eucalyptus* spp. and *Tibouchina* spp. has raised the question whether these plants might not share other pathogens.

Although the origin of *C. eucalypti* is not known for certain, its common occurence on *Eucalyptus* in Australia suggests that it is native in that area. *Tibouchina* spp. are

native to South America but relatively widely planted in cities in the eastern parts of Australia as ornamentals. The aim of this study was to consider whether these ornamental plants have become infected with the commonly occurring *C. eucalypti*.

MATERIALS AND METHODS

Fungal isolates

Tibouchina urvilleana Cogn. trees growing as ornamentals in Melbourne, Coff's Harbour and Brisbane were examined during November 2001 for stem canker symptoms. Cankers covered in structures resembling an *Endothiella* sp. were found on *T. urvilleana* trees in Melbourne and at Coff's Harbour. Isolations were made directly from fungal structures and spore masses were transferred to Malt Extract Agar (MEA) containing 10 g Malt extract and 15 g agar per litre water.

Morphological characterisation

Fruiting structures on *T. urvilleana* bark were compared with herbarium specimens of *C. eucalypti* from *Eucalyptus* spp. (Venter *et al.* 2002) (Table 1). Fruiting structures were sectioned using a Leica CM1100 cryostat and Leica embedding medium (Setpoint Technologies, Johannesburg, South Africa) at –20 °C to a thickness of 12 µm. Conidia and conidiophores were mounted in 3% KOH and measured. Ten measurements were taken of conidiophores and conidia for each collection and these are presented as (min-) (mean-SD) – (mean+SD) (-max) µm. Standard colour notations given by Rayner (1970) were used. Bark specimens bearing fruiting structures have been deposited in the herbarium of the National Collection of Fungi, Pretoria, South Africa (PREM) (Table 2).

DNA isolation and amplification

Mycelium from actively growing cultures was inoculated into 1.5 ml microcentrifuge tubes containing 750 µl, 3% (w/v) Malt Extract Broth (Merck Laboratory Supplies,

Germany). DNA was isolated from 5-day-old cultures using the method described by Murray and Thompson (1980). Two β-tubulin gene regions were amplified using primer pairs Bt1a (5'-TTT CCC CGT CTC CAC TTC TTC ATG- 3'), Bt1b (5'-GAC GAG ATC GTT CAT GTT GAA GC-3') and Bt2a (5'-GGT AAC CAA ATC GGT GCT GCT TTC-3'), Bt2b (5'-ACC CTC AGT GTA GTG ACC CTT GGC-3') (Glass & Donaldson 1995).

Each polymerase chain reaction (PCR) contained 2 ng DNA, 0.2 mM of each dNTP (Promega, Madison, Wisconsin, U.S.A.), 0.15 µM of primers Bt1a/b and Bt2a/b, 5 U/µl Taq polymerase (Roch Molecular Biochemicals, Almeda, California) and 1X Buffer containing $MgCl_2$ (10 mM Tris-HCL, 1.5 mM $MgCl_2$, 50 mM KCL). Sterile water was used to adjust the final volume to 25 µl. Amplification reactions were performed on a Perkin Elmer GeneAmp PCR System 9700 thermocycler (Perkin-Elmer Applied Biosystems Inc., Foster City, California). The conditions for all PCR reactions were: an initial denaturation at 96 °C for one min followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing for one min at 54 °C, followed by extension at 72 °C for 90 sec. A 5 sec elongation step was added with each cycle after the first twenty-five cycles. The process was completed with a final extension at 72 °C for ten minutes. PCR products were visualised on a 2% agarose-ethidium bromide gel using ultra violet light. Product sizes were estimated with a 100 bp standard size marker (Promega).

DNA sequencing and analyses

PCR products were purified using a High Pure PCR Product Purification Kit (Roche Diagnostic GmbH, Mannheim, Germany) and sequenced with the same primer pairs used in the PCR amplification reactions. An ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS (Perkin-Elmer, Warrington, United Kingdom) was used for sequencing with an ABI PRISM 3100™ automated sequencer. Sequences were aligned using ClustalX (Thompson *et al.* 1997) and manually adjusted using Sequence Navigator version 1.0.1 (Perkin-Elmer Applied BioSystems Inc., Foster City, California). All sequences obtained in this study have been deposited in GenBank (Table 1).

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

Pathogenicity

To determine the relative pathogenicity of the *Endothiella* sp. isolated from *T. urvilleana* in Australia, pathogenicity trials were performed under greenhouse conditions. The tests were performed using a complete randomised design. All trees were maintained under greenhouse conditions for two weeks to acclimatise them prior to inoculation. The greenhouse was subjected to natural day/night conditions and a temperature setting of ~25 °C. Tree diameter varied from 20 to 30 mm. The two most rapidly growing and healthy isolates of the test fungus from *T. urvilleana* (CMW 6245, CMW 6246) were each inoculated onto twenty-five *T. urvilleana* trees. Twenty-five trees were also each inoculated with the isolates of *C. eucalypti* from *Eucalyptus* in South Africa (CMW 7036) and Australia (CMW 7038), previously shown to be pathogenic (Venter *et al.* 2002). Twenty trees were inoculated with sterile MEA plugs to serve as a control.

Wounds were made on the test trees using a 10 mm diameter cork borer. Mycelial plugs of a similar size were taken from the actively growing edges of 7-day-old cultures and placed in the wounds with the mycelium facing the cambium. Wounds were sealed with laboratory film (Parafilm "M", American National Can$_{TM}$ Chicago, IL.) to protect the inoculated fungus and cambium from desiccation. After 10 weeks, lesion lengths were measured and compared. Data were subjected to analysis of variance using the General Linear Model procedure of SAS (SAS/STAT Users guide, Version 6, 1989).

RESULTS

Fungal isolates

An *Endothiella* sp. was found on five *T. urvilleana* trees. Four isolates (CMW 6244, CMW 6245, CMW 6246, CMW 6249) were obtained from trees in Melbourne. One isolate (CMW 10729) was isolated from a tree at Coff's Harbour. All isolates are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

Morphological characterisation

Only asexual fruiting bodies were found on *T. urvilleana* trees (PREM 57596). The conidiomata of the fungus resembled the *Endothiella* anamorph of *Cryphonectria* spp. Stromata were 180-330 µm wide, orange (15) (Rayner 1970), pulvinate, semi-immersed in the bark, uni- to multilocular with strongly convoluted locules (Fig. 1A-B). Long sterile hyphae, (18.5-) 31.5-60.5 x 1-1.5 (-2) µm reminiscent of the paraphyses found between conidiophores of *C. eucalypti* (Walker *et al.* 1985, Venter *et al.* 2002), were observed (Fig. 1C). Conidia were cylindrical (Fig. 1D) and similar in size (3.0-) 3.5-4.5 (-5.0) x 1.0-1.5 µm to those of *C. eucalypti* (Walker *et al.* 1985, Venter *et al.* 2002). Conidiophores (Fig. 1E) were (6.0-)8.5-18.0(-25) x 1.0-1.5(-2.0) µm and corresponded with those of *C. eucalypti* (Venter *et al.* 2002). The conidiogenous cells of the fungus on *T. urvilleana* from Australia differed from those of *C. eucalypti*. Phialides of the fungus on *T. urvilleana* had inconspicuous collarettes

and attenuated apices (Fig. 1E). Phialides of the holotype and paratype specimens of *C. eucalypti* from South Africa (PREM 56211, PREM 56212, PREM 56305, PREM 56214, PREM 56215, PREM 56216), however, had flaring collarettes and the apices of the conidiogenous cells were not as attenuated as those of the fungus from *T. urvilleana* (Fig. 1F). These differences suggest that the fungus on *Tibouchina* from Australia is different to *C. eucalypti* found on *Eucalyptus*.

DNA sequencing and analyses

PCR amplification of the four isolates from *Tibouchina* in Australia, with the two primer pairs resulted in fragments of ~540 bp (Bt1a/Bt1b) and ~490 bp (Bt2a/Bt2b), respectively. Aligned sequences of the combined data resulted in a data set of 1097 characters (Appendix 1), consisting of 566 constant characters, 424 parsimony informative characters and 107 variable characters that were parsimony uninformative. The heuristic search produced two most parsimonious trees, of which one is presented (Fig. 2). Most variation between trees occurred in the *C. eucalypti* clade. A strict Bootstrap consensus tree [tree length = 1045 steps, Consistency index (CI) = 0.820, Retention index (RI) = 0.88664, Rescaled consistency index (RC) = 0.727 and Homoplasy index (HI) = 0.180] was generated from the 107 variable characters and most branches were well supported with high bootstrap values (Fig. 2). Posterior probability values calculated for the branch nodes supported the bootstrap values. All the isolates from *T. urvilleana* from Australia grouped in a separate clade (bootstrap support 96%, posterior probability 53%) most closely related to the *C. eucalypti* clade. These clades grouped within the greater *Cryphonectria* clade separate from the *Endothia* clade (bootstrap support 100%, posterior probability 100%).

Pathogenicity

Greenhouse inoculations on *T. urvilleana* trees resulted in distinct lesions within ten weeks (Fig. 3). The control inoculations produced no lesions (Table 3). There were no significant differences in pathogenicity between the Australian (CMW 7038) and South African (CMW 7036) *C. eucalypti* isolates (P=0.1699). Isolates of the undescribed

Endothiella sp. were, however, significantly more pathogenic than the *C. eucalypti* isolates ($P<0.001$).

DISCUSSION

This study presents the first report of an *Endothiella* sp., closely related to *C. eucalypti*, from *T. urvilleana* in Australia. Although initial identification suggested that the fungus was the same as *C. eucalypti*, morphological differences between the two fungi were found. These morphological differences were relatively inconspicuous. However, comparison of DNA sequences showed clearly that the fungus from *Tibouchina* was distinct from *C. eucalypti*.

The *Endothiella* sp. found on *T. urvilleana* in this study shares a number of characteristics with the anamorph of *C. eucalypti*. The two fungi can, however, easily be distinguished based on phialide morphology. The anamorph of *C. eucalypti* is recognised by conspicuous phialides with flaring collarettes (Venter *et al.* 2002). In comparison, the fungus from *T. urvilleana* has inconspicuous phialides with attenuated ends.

DNA sequence data provided strong support for morphological observations suggesting that the *Endothiella* sp. from *Tibouchina* is different to the anamorph of *C. eucalypti*. In this study we particularly chose to use β-tubulin sequences for comparison. This was because, of the various gene regions tested for this group of fungi (Venter *et al.* 2002), this region has been shown to accurately reflect phylogenetic relationships in *Cryphonectria* and allied genera (Venter *et al.* 2002). Our data, both based on morphological and DNA sequence comparisons, thus, strongly suggest that the fungus from *Tibouchina* in Australia represents the anamorph of a distinct species of *Cryphonectria*.

No teleomorph state was found associated with the *Endothiella* sp. discovered on *T. urvilleana* in Australia. However, its morphology is very similar to that of the anamorph of *C. eucalypti*. Phylogenetic analysis of DNA sequence data confirmed this relationship and we are confident that the fungus will represent a species of *Cryphonectria sensu stricto*. Although it would perhaps be logical to do so, description

of the fungus as a species of *Cryphonectria* is not permitted (ICBN section 59.2, Greuter *et al.* 2000). Description in an anamorph genus is thus required.

Currently, *Endothiella* serves as an anamorph genus for both *Endothia* and *Cryphonectria* (Barr 1978). However, *Endothiella* anamorphs of *Endothia* and *Cryphonectria* differ substantially. *Endothia* anamorphs are large, superficial, tubercular and multilocular while those of *Cryphonectria* are semi-immersed and uni- to multilocular (Micales & Stipes 1987, Myburg *et al.* 2003). An additional anamorph genus, thus, needs to be established for the genera *Endothia* and *Cryphonectria*, after which the fungus discovered on *T. urvilleana* in this study can be described.

In this study, we were able to show that the *Endothiella* sp. from *T. urvilleana* is pathogenic to this tree. We further assume that the fungus was responsible for die-back on the ornamental trees sampled. Our results have also shown that the *Endothiella* sp. is more pathogenic than *C. eucalypti* isolates from South Africa and Australia. Although it was beyond the scope of this study, it would be interesting to determine whether the *Endothiella* sp. from *Tibouchina* is pathogenic to *Eucalyptus*.

It is interesting to speculate on the possible origin of the *Endothiella* sp. occurring on *T. urvilleana* in Australia. *Tibouchina* spp. have been introduced into this country and it is possible that the fungus was introduced with these plants. Another possibility is that the fungus originated on native *Eucalyptus* and has the ability to infect *Tibouchina*. Given the wide distribution of the plants sampled, we favour the latter hypothesis. This would imply that the *Endothiella* sp. occurs on *Eucalyptus* in Australia and it has probably been masked by the widespread occurrence of the very similar *C. eucalypti*. Extensive collections of *C. eucalypti* in Australia with comparisons of isolates based on DNA sequences would resolve this question. This would also make it possible to evaluate the potential importance of the *Endothiella* sp. as a pathogen of *Eucalyptus*, both in Australia and elsewhere in the world.

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Table 1. Specimens used in the morphological comparisons.

Herbarium allocation*	Identification	Host	Origin	Collector	Date
PREM 56211	*C. eucalypti*	*E. grandis* x *camaldulensis*	Nyalazi, SA	M. Venter	1998
PREM 56212	*C. eucalypti*	*E. grandis*	Sabie, SA	J. Roux	1998
PREM 56305	*C. eucalypti*	*E. saligna*	Tzaneen, SA	M. Venter	1999
PREM 56214	*C. eucalypti*	*E. grandis*	Dukuduku, SA	M. Venter	1998
PREM 56215	*C. eucalypti*	*E. grandis*	Amangwe, SA	M. Venter	1998
PREM 56216	*C. eucalypti*	*E. grandis*	Dukuduku, SA	M. Venter	1998
PREM 57597	*Endothiella* sp.	*T. urvilleana*	Australia	M.J. Wingfield	2001

* PREM, National Collection of Fungi, Pretoria, South Africa.

Table 2. Isolates considered in DNA sequence comparisons and pathogenicity trials.

Culture no.[a]	Isolate identity	Host	Origin	Genbank accession number
CMW 1543	*C. macrospora*	*Castanopsis cuspidata*	Japan	AF1368351[c] AF36835
CMW 1651	*C. parasitica*	*Castanea dentata*	U.S.A.	AF273074[c], AF273467
CMW 1652	*C. parasitica*	*C. dentata*	U.S.A.	AF273468[c] AF27075[d]
CMW 2091	*Endothia gyrosa*	*Quercus palustris*	U.S.A.	AF368337[c] AF368336[d]
CMW 5288	*Diaporthe ambigua*	*Malus* sp.	South Africa	AF 543817[c] AF 54381
CMW 6244[b]	*Endothiella* sp.	*T. urvilleana*	Australia	AY194474
CMW 6245[b]	*Endothiella* sp.	*T. urvilleana*	Australia	AY194475
CMW 6246[b]	*Endothiella* sp.	*T. urvilleana*	Australia	AY194476
CMW 6249[b]	*Endothiella* sp.	*T. urvilleana*.	Australia	AY194477
CMW 7036	*C. eucalypti*	*Eucalyptus* sp.	South Africa	AF368341[c]; AF368340
CMW 7037	*C. eucalypti*	*Eucalyptus* sp.	Australia	AF368343[c]; AF368342
CMW 7038	*C. eucalypti*	*Eucalyptus* sp.	Australia	AF368345[c]; AF368344
CMW 7042	*E. gyrosa*	*Q. suber*	U.S.A.	AF368335[c] AF368334
CMW 7045	*E. gyrosa*	*Q. palustris*	U.S.A.	AF368339[c] AF368338
CMW 7046	*E. singularis*	-	U.S.A.	AF368333[c] AF368332
CMW 7047	*C. parasitica*	*Q. virginiana*	U.S.A.	AF273469[c], AF273073
CMW 7048	*C. parasitica*	*Q. virginiana*	U.S.A.	AF273470[c], AF273076
CMW 10729[b]	*Endothiella* sp.	*T. urvilleana*	Australia	AY194478

[a] Culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa.
[b] β-tubulin 1a/b and 2a/b sequence data generated in this study.
[c] β-tubulin 1a/b sequence data obtained from Genbank.
[d] β-tubulin 2a/2b sequence data obtained from Genbank.

Table 3. Mean lesion lengths on *Tibouchina urvilleana* trees 6 weeks after inoculation with the undescribed *Endothiella* sp. and *C. eucalypti* in the greenhouse.

Isolates	[a] Mean lesion length (mm)	Host
[b] CMW 7036	28.4	*Eucalyptus*
[b] CMW 7038	25.4	*Eucalyptus*
[c] CMW 6245	36.1	*T. urvilleana*
[c] CMW 6246	39.7	*T. urvilleana*
Control	10.0	

[a]Each value is the average of 25 measurements for each isolate

[b]*Cryphonectria eucalypti*

[c]*Endothiella* sp.

P > 0.001

CV = 27.53

R-Square = 0.65

F = 56.51

Figure. 1. Light micrographs of fruiting structures of the *Endothiella* sp. on *T. urvilleana* from Australia. (a) Conidioma on bark. (b) Longitudinal section through conidioma. (c) Paraphyses (indicated with arrow). (d) Conidia. (e) Conidiophores. (f) Ligth micrograph of conidiophores (collarettes indicated with arrow) of *C. eucalypti* from South Africa. (Scale bars of a, b 100 μm; c, d, e, f 10 μm)

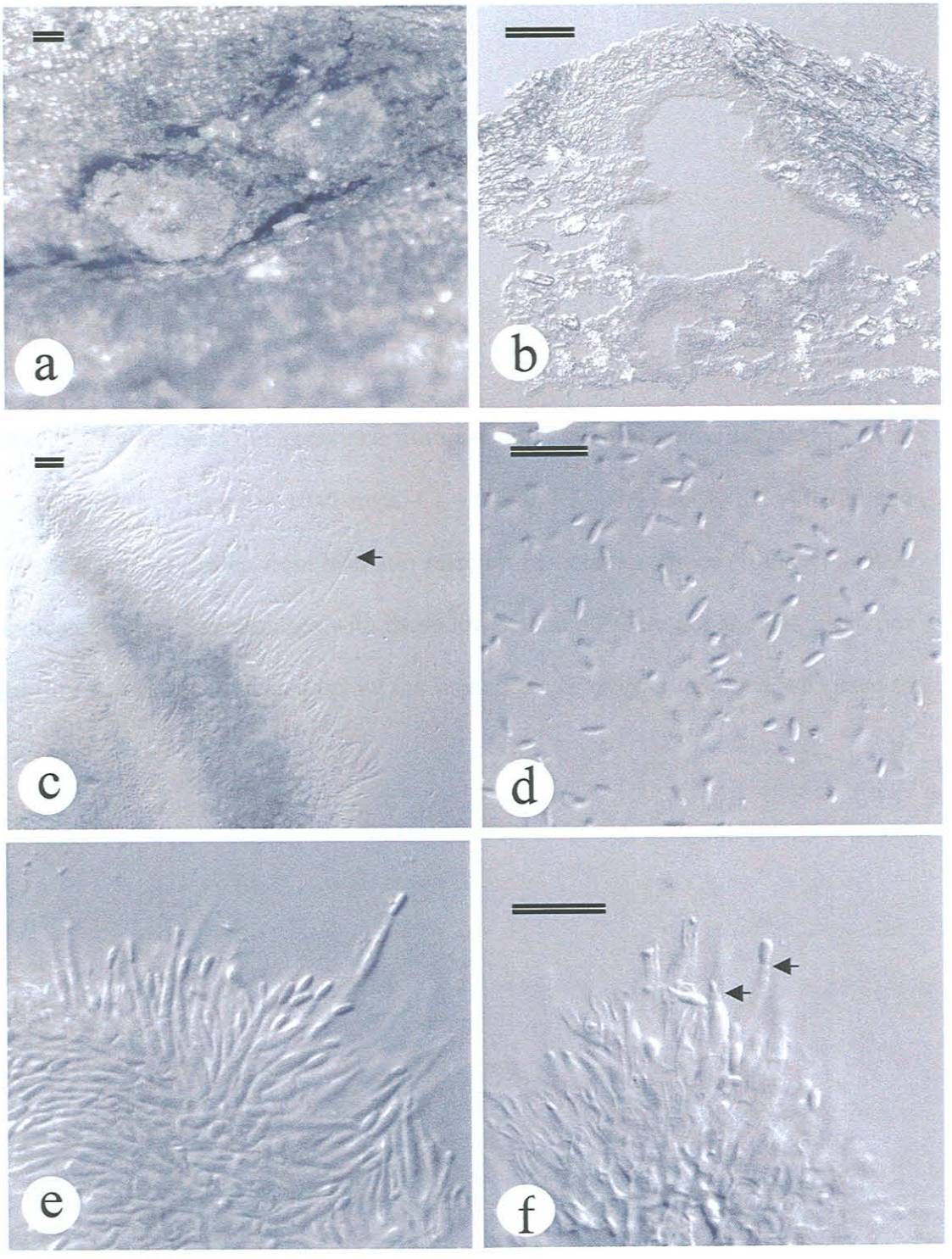

a
b
c
d
e
f

Figure. 2. Phylogenetic tree generated from β-tubulin gene sequence data set. A strict consensus tree (tree length = 1045 steps, CI = 0.820, RI = 0.0.88664, RC = 0.727 and HI = 0.180) was generated from heuristic searches. Bootstrap values (1000 replicates) are indicated above the branches with those lower than 50% are not shown. Posterior propability values are indicated below the branches. *Diaporthe ambigua* was used to root the tree.

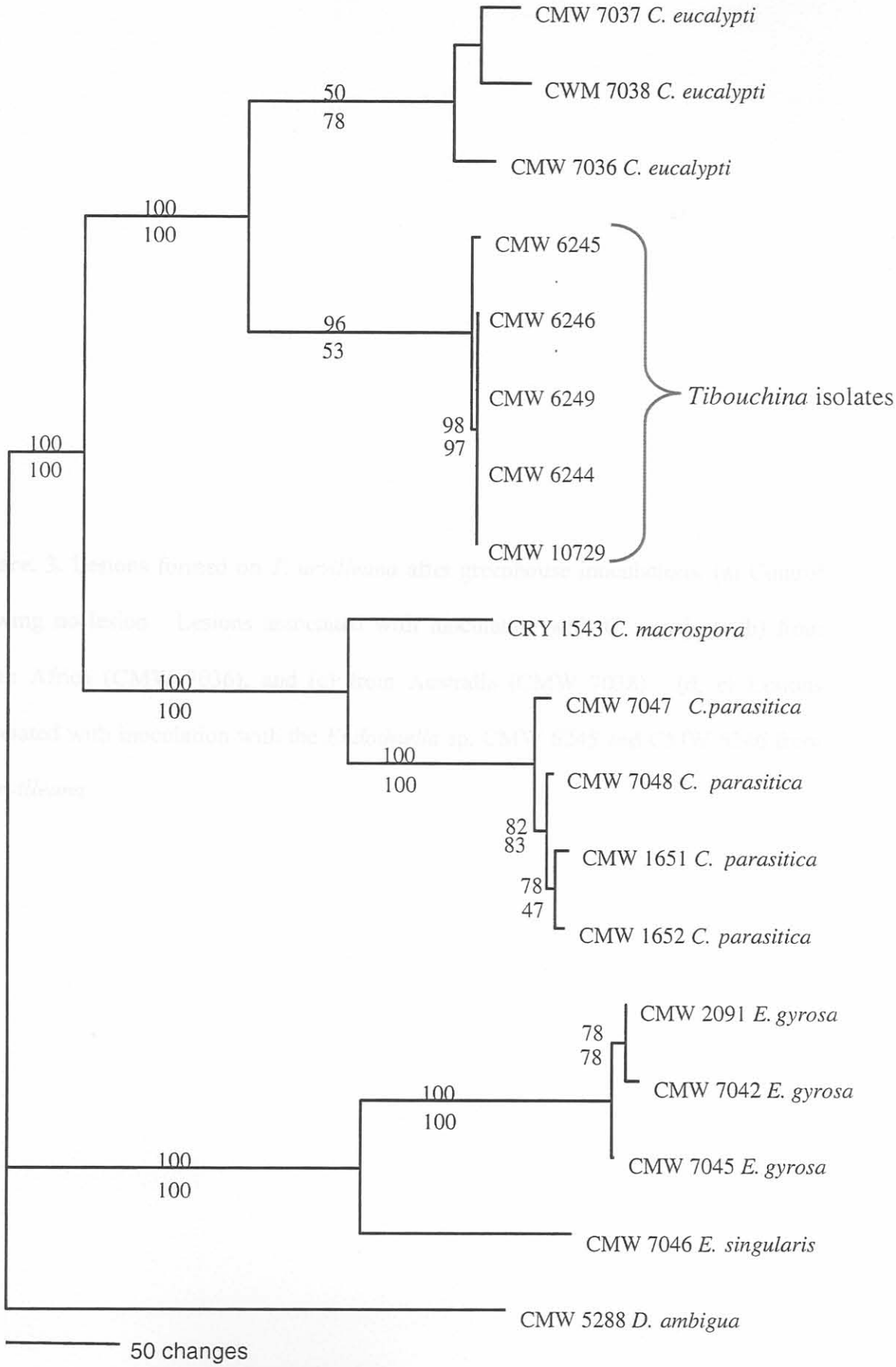


Figure. 3. Lesions formed on *T. urvilleana* after greenhouse inoculations. (a) Control showing no lesion. Lesions associated with inoculation with *C. eucalypti* (b) from South Africa (CMW 7036), and (c) from Australia (CMW 7038). (d, e) Lesions associated with inoculation with the *Endothiella* sp. CMW 6245 and CMW 6246 from *T. urvilleana.*

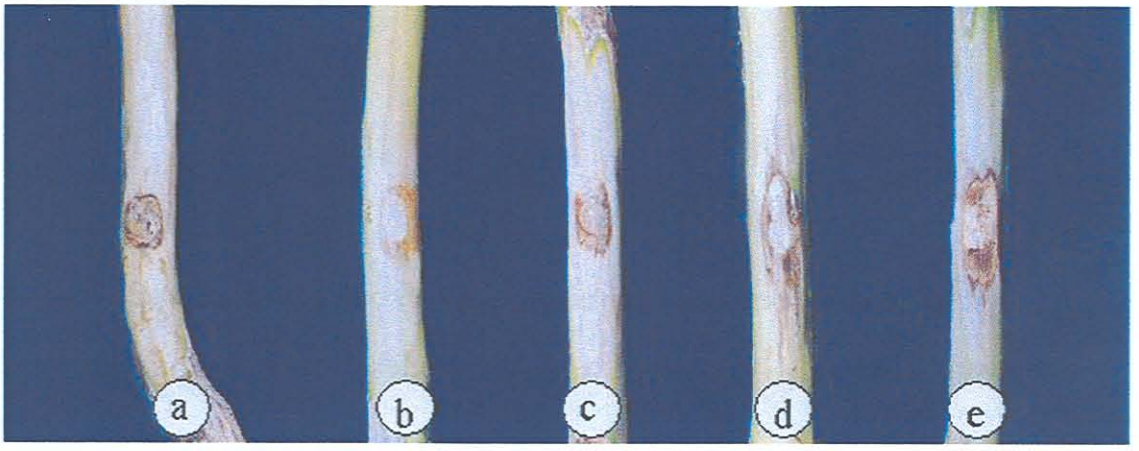
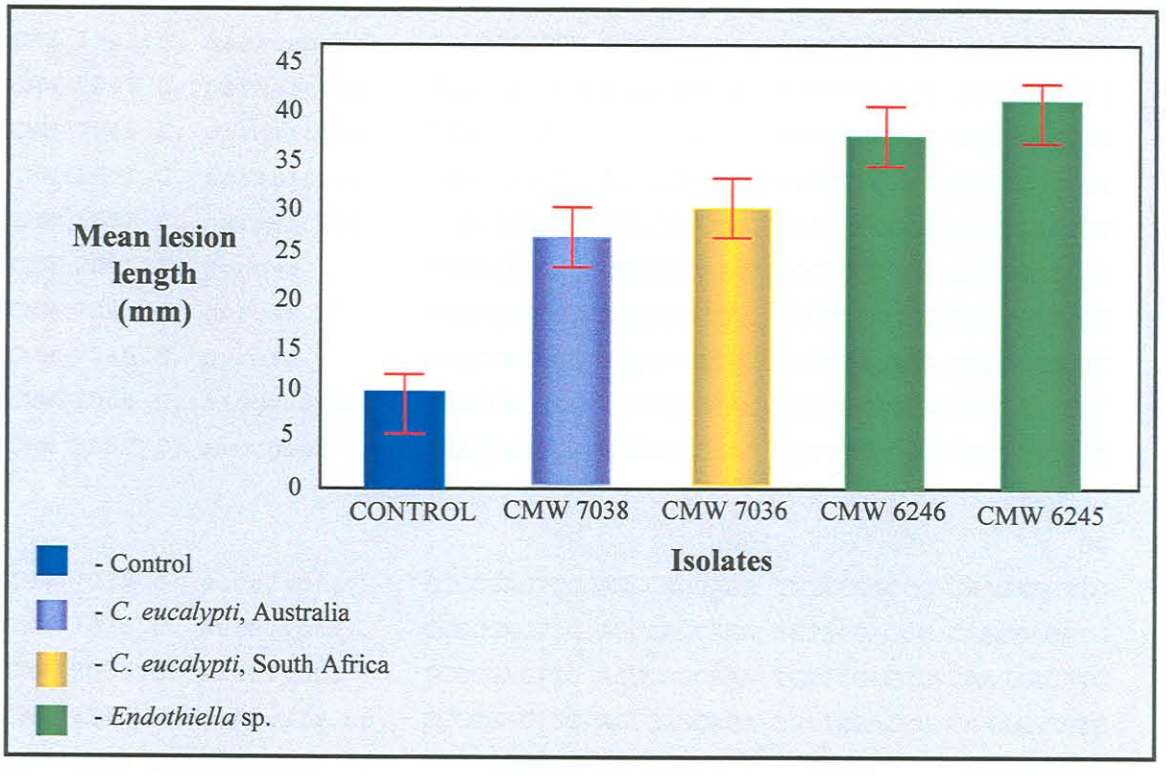


Figure. 4. Bar chart indicating the average lesion length in millimetres resulting from inoculation trials of *Endothiella* sp. and *C. eucalypti* on *Tibouchina urvilleana*. Error bars indicate 95% confidance interval of the data.


Mean lesion length (mm)
45
40
35
30
25
20
15
10
5
0
CONTROL
CMW 7038
CMW 7036
CMW 6246
CMW 6245
Isolates
- Control
- C. eucalypti, Australia
- C. eucalypti, South Africa
- Endothiella sp.

APPENDIX 1

```
                                  10         20         30         40
CMW 7037 C. eucalypti             TGACCAGCCG TGGCGCCCAC TCTTTCCGCG CCCTCACCGT
CWM 7038 C. eucalypti             TGACCAGCCG TGGCGCCCAC TCTTTCCGCG CCCTCACCGT
CMW 7036 C. eucalypti             TGACCAGCCG TGGCGCCCAC TCTTTCCGCG CCCTCACCGT
CMW 6245 Endothiella sp.          TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CCCTCACCGT
CMW 6246 Endothiella sp.          TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CCCTCACCGT
CMW 6249 Endothiella sp.          TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CCCTCACCGT
CMW 6244 Endothiella sp.          TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CCCTCACCGT
CMW 10729 Endothiella sp.         TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CCCTCACCGT
CRY 1543 C. macrospora            TGACCAGCCG TGGCGCCTAC TCCTTCCGCG CCCTCACCGT
CMW 7047 C. parasitica            TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CCCTCACCGT
CMW 7048 C. parasitica            TGACCAGCCG TGGCGCCCAC TCTTTCCGCG CCCTCACCGT
CMW 1651 C. parasitica            TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CCCTCACCGT
CMW 1652 C. parasitica            TGACCAGCCG TGGCGCCCAC TCCTTCCGCG CCCTAACCGT
CMW 2091 E. gyrosa                TGACCAGCCG TGGCGCTTAC TCCTTCCGCG CCGTCACCGT
CMW 7042 E. gurosa                TGACCAGCCG TGGCGCTTAC TCCTTCCGCG CCGTCACCGT
CMW 7045 E. gyrosa                TGACCAGCCG TGGCGCTTAC TCCTTCCGCG CCGTCACCGT
CMW 7046 E. singularis            TGACCAGCCG TGGCGCCTAC TCCTTCCGCG CCGTCACCGT
CMW 5288 D. ambigua               TGACCAGCCG CGGCGCCCAC TCCTTCCGTG CCGTCACCGT

                                  50         60         70         80
CMW 7037 C. eucalypti             GCCCGAGTTG ACCCAGCAGA TGTTCGACCC CAAGAACATG
CWM 7038 C. eucalypti             GCCCGAGTTG ACCCAGCAGA TGTTCGACCC CAAGAACATG
CMW 7036 C. eucalypti             GCCCGAGTTG ACCCAGCAAA TGTTCGACCC CAAGAACATG
CMW 6245 Endothiella sp.          GCCCGAGTTG ACCCAGCAGA TGTTCGACCC CAAGAACATG
CMW 6246 Endothiella sp.          GCCCGAGTTG ACCCAGCAGA TGTTCGACCC CAAGAACATG
CMW 6249 Endothiella sp.          GCCCGAGTTG ACCCAGCAGA TGTTCGACCC CAAGAACATG
CMW 6244 Endothiella sp.          GCCCGAGTTG ACCCAGCAGA TGTTCGACCC CAAGAACATG
CMW 10729 Endothiella sp.         GCCCGAGTTG ACCCAGCAGA TGTTCGACCC CAAGAACATG
CRY 1543 C. macrospora            TCCCGAGTTG ACCCAGCAGA TGTTCGACCC CAAGAACATG
CMW 7047 C. parasitica            CCCCGAGTTG ACCCAGCAGA TGTTCGACCC CAAGAACATG
CMW 7048 C. parasitica            CCCCGAGTTG ACCCAGCAGA TGTTCGACCC CAAGAACATG
CMW 1651 C. parasitica            CCCCGAGTTG ACCCAGCAGA TGTTCGACCC CAAGAACATG
CMW 1652 C. parasitica            CCCCGAGTTG ACCCAGCAGA TGTTCGACCC CAAGAACATG
CMW 2091 E. gyrosa                CCCCGAGTTG ACCCAGCAGA TGTTCGACCC CAAGAACATG
CMW 7042 E. gurosa                CCCCGAGTTG ACCCAGCAGA TGTTCGACCC CAAGAACATG
CMW 7045 E. gyrosa                CCCCGAGTTG ACCCAGCAGA TGTTCGACCC CAAGAACATG
CMW 7046 E. singularis            CCCCGAGTTG ACCCAGCAGA TGTTCGACCC CAAGAACATG
CMW 5288 D. ambigua               GCCCGAGCTC ACCCAGCAGA TGTTCGACCC CAAGAACATG
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                                    90         100        110        120
CMW 7037 C. eucalypti          ATGGCTGCCT CGGACTTCCG CAACGGCCGT TACCTGACGT
CWM 7038 C. eucalypti          ATGGCTGCCT CGGACTTCCG CAACGGCCGT TACCTGACGT
CMW 7036 C. eucalypti          ATGGCTGCCT CGGACTTCCG CAACGGCCGT TACCTGACGT
CMW 6245 Endothiella sp.       ATGGCCGCCT CGGACTTCCG CAACGGCCGC TACCTGACGT
CMW 6246 Endothiella sp.       ATGGCCGCCT CGGACTTCCG CAACGGCCGC TACCTGACGT
CMW 6249 Endothiella sp.       ATGGCCGCCT CGGACTTCCG CAACGGCCGC TACCTGACGT
CMW 6244 Endothiella sp.       ATGGCCGCCT CGGACTTCCG CAACGGCCGC TACCTGACGT
CMW 10729 Endothiella sp.      ATGGCCGCCT CGGACTTCCG CAACGGCCGC TACCTGACGT
CRY 1543 C. macrospora         ATGGCTGCCT CTGACTTCCG TAACGGTCGC TACCTGACAT
CMW 7047 C. parasitica         ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACAT
CMW 7048 C. parasitica         ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACAT
CMW 1651 C. parasitica         ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACAT
CMW 1652 C. parasitica         ATGGCTGCCT CTGACTTCCG CAACGGTCGC TACCTGACAT
CMW 2091 E. gyrosa             ATGGCCGCCT CTGACTTCCG CAACGGCCGT TACCTGACGT
CMW 7042 E. gurosa             ATGGCCGCCT CTGACTTCCG CAACGGCCGT TACCTGACGT
CMW 7045 E. gyrosa             ATGGCCGCCT CTGACTTCCG CAACGGCCGT TACCTGACGT
CMW 7046 E. singularis         ATGGCCGCCT CTGACTTCCG CAACGGTCGC TACCTGACGT
CMW 5288 D. ambigua            ATGGCCGCCT CTGACTTCCG TAATGGTCGC TACCTGACGT

                                   130        140        150        160
CMW 7037 C. eucalypti          GCTCTGCCAT CTTGTACG-- ----TTTTCT GCTTCTTTCT
CWM 7038 C. eucalypti          GCTCTGCCAT CTTGTACG-- ----TTTT-- ----------
CMW 7036 C. eucalypti          GCTCTGCCAT CTTGTACG-- ----TTTT-T G--TCTT-CT
CMW 6245 Endothiella sp.       GCTCTGCCAT CTTGTACG-- ----TTTTTT GCTTCTTTCT
CMW 6246 Endothiella sp.       GCTCTGCCAT CTTGTACG-- ----TTTTTT GCTTCTTTCT
CMW 6249 Endothiella sp.       GCTCTGCCAT CTTGTACG-- ----TTTTTT GCTTCTTTCT
CMW 6244 Endothiella sp.       GCTCTGCCAT CTTGTACG-- ----TTTTTT GCTTCTTTCT
CMW 10729 Endothiella sp.      GCTCTGCCAT CTTGTACG-- ----TTTTTT GCTTCTTTCT
CRY 1543 C. macrospora         GCTCTGCCAT CTTGTAAGTT TTCTATA--- ----------
CMW 7047 C. parasitica         GCTCTGCCAT CTTGTAAG-- ----TTTTCT TGTCTTTTCC
CMW 7048 C. parasitica         GCTCTGCCAT CTTGTAAG-- ----TTTTCT TGTCTTTTCC
CMW 1651 C. parasitica         GCTCTGCCAT CTTGTAAG-- ----TTTTCT TGCCTTTTCC
CMW 1652 C. parasitica         GCTCTGCCAT CTTGTAAG-- ----TTTTCT TGTCTTTTCC
CMW 2091 E. gyrosa             GCTCTGCCAT CTTGTAAGTG GT---TCCCC CC--ACAC--
CMW 7042 E. gurosa             GCTCTGCCAT CTTGTAAGTG GT---TCCCC CC--ACAC--
CMW 7045 E. gyrosa             GCTCTGCCAT CTTGTAAGTG GT---TCCCC CC--ACAC--
CMW 7046 E. singularis         GCTCTGCCAT CTTGTAAGTG GTC--TCCCC CCCAACACCC
CMW 5288 D. ambigua            GCTCTGCCAT CTTGTAAGT- ---------- -------CCC
```

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                                  170        180        190        200
CMW 7037 C. eucalypti        CT----ATCT C-ACAAA-TC -TCGGATCCA CCTCTC----
CWM 7038 C. eucalypti        -T----GTCT C-ACAAA-TC -TCGGATCCA CCTCTC----
CMW 7036 C. eucalypti        CT----GTCT C-ACACA-TC -TCGGATCCA CCTCTC----
CMW 6245 Endothiella sp.     CT----GTCT C-ACAAA-CC -TCGGATCCA CCTCTC----
CMW 6246 Endothiella sp.     CT----GTCT C-ACAAA-CC -TCGGATCCA CCTCTC----
CMW 6249 Endothiella sp.     CT----GTCT C-ACAAA-CC -TCGGATCCA CCTCTC----
CMW 6244 Endothiella sp.     CT----GTCT C-ACAAA-CC -TCGGATCCA CCTCTC----
CMW 10729 Endothiella sp.    CT----GTCT C-ACAAA-CC -TCGGATCCA CCTCTC----
CRY 1543 C. macrospora       ------CCTT CTCGCAA-GC CTC-GATGAA CATCTC----
CMW 7047 C. parasitica       TCGCAGGTCT AGACAAA--- ---------- CGTTTC----
CMW 7048 C. parasitica       TCGCAGGTCT CGACAAA--- ---------- CGTCTT----
CMW 1651 C. parasitica       TCGCAAGTCT CGACGAA--- ---------- CGTCTT----
CMW 1652 C. parasitica       TCGCAAGTCT CGACGAA--- ---------- CGTCTT----
CMW 2091 E. gyrosa           -----ACCCG CTGG-CGCCT TTGGGGGGCT GTCA------
CMW 7042 E. gurosa           -----ACCCG CTGG-CGCCT TTGGGGGGCT GTCA------
CMW 7045 E. gyrosa           -----ACCCG TTGG-CGCCT TTGGGGGGCT GTCA------
CMW 7046 E. singularis       C--AGACCCC CTGGGCGCCT TTGGCGGGAG AGGGCTGTCA
CMW 5288 D. ambigua          CTGAGCATCT ----CCACAC GACCCAAGT- ----------

                                  210        220        230        240
CMW 7037 C. eucalypti        GGGCTTGTTT TT--GCTAA- CCCTG-CTTT -CCTCTCTCC
CWM 7038 C. eucalypti        GGGCTTGTTT TT--GCTAA- CCCTG-CTTT -CCTCTCTCC
CMW 7036 C. eucalypti        GGGCTTGTTT TT--GCTAA- CCCTG-CTTT -CCTCTCTCC
CMW 6245 Endothiella sp.     GGGCTTGTTT TT--GCTAA- CCCTG-CTTT -CCTCTCTCC
CMW 6246 Endothiella sp.     GGGCTTGTTT TT--GCTAA- CCCTG-CTTT -CCTCTCTCC
CMW 6249 Endothiella sp.     GGGCTTGTTT TT--GCTAA- CCCTG-CTTT -CCTCTCTCC
CMW 6244 Endothiella sp.     GGGCTTGTTT TT--GCTAA- CCCTG-CTTT -CCTCTCTCC
CMW 10729 Endothiella sp.    GGGCTTGTTT TT--GCTAA- CCCTG-CTTT -CCTCTCTCC
CRY 1543 C. macrospora       GGGCTTCTTG ----GCTAA- CCCCACGTTT --CTCTCTTT
CMW 7047 C. parasitica       GGGCTGTTGG ----GCTAA- CCCTGTCTTT --CTCTCTTC
CMW 7048 C. parasitica       GGGCTGTTTG ----GCTAA- CCCTGTCTTT --CTCTCTTC
CMW 1651 C. parasitica       GGGCTGTTTG ----GCTAA- CCCTGTCTTT --CTCTCTTC
CMW 1652 C. parasitica       GGGCTGTTTG ----GCTAA- CCCTGTCTTT --CTCTCTTC
CMW 2091 E. gyrosa           GGGCTTGTTT TTT-GCTGA- ---------- -CCCCTATCC
CMW 7042 E. gurosa           GGGCTGGTTT TTT-GCTGA- ---------- -CCCCTATCC
CMW 7045 E. gyrosa           GGGCTTGTTT TTT-GCTGA- ---------- -CCCCTATCC
CMW 7046 E. singularis       GGGCTTGTTT CT--GCTAA- ---------- -CCCCTATCC
CMW 5288 D. ambigua          ------GTTT GCGCGCTGAC AC-TGTCT-- ----------
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                                  250        260        270        280
CMW 7037 C. eucalypti      CC-----TAC AGCCGTGGCA AGGTCTCCAT GAAGGAGGTC
CWM 7038 C. eucalypti      CC-----TAC AGCCGGGGCA AGGTCTCCAT GAAGGAGGTC
CMW 7036 C. eucalypti      CC-----TAC AGCCGTGGCA AGGTCTCCAT GAAGGAGGTC
CMW 6245 Endothiella sp.   CC-----TAC AGCCGTGGCA AGGTCTCCAT GAAGGAGGTC
CMW 6246 Endothiella sp.   CC-----TAC AGCCGTGGCA AGGTCTCCAT GAAGGAGGTC
CMW 6249 Endothiella sp.   CC-----TAC AGCCGTGGCA AGGTCTCCAT GAAGGAGGTC
CMW 6244 Endothiella sp.   CC-----TAC AGCCGTGGCA AGGTCTCCAT GAAGGAGGTC
CMW 10729 Endothiella sp.  CC-----TAC AGCCGTGGCA AGGTCTCCAT GAAGGAGGTC
CRY 1543 C. macrospora     CC-----TCT AGCCGTGGCA AGGTCTCCAT GAAGGAGGTC
CMW 7047 C. parasitica     CCCT---TCT AGCCGGGGTA AGGTCTCCAT GAAGGAAGTC
CMW 7048 C. parasitica     CCCTTC-TTT AGCCGTGGTA AGGTCTCCAT GAAGGAAGTC
CMW 1651 C. parasitica     CCCTTC-TCA AGCCGTGGTA AGGTCTCCAT GAAGGAAGTC
CMW 1652 C. parasitica     CCCTTC-TCT AGCCGTGGTA AGGTCTCCAT GAAGGAAGTC
CMW 2091 E. gyrosa         C------TCC AGCCGTGGCA AGGTCTCCAT GAAGGAGGTC
CMW 7042 E. gurosa         C------TCC AGCCGTGGCA AGGTCTCCAT GAAGGAGGTC
CMW 7045 E. gyrosa         C------TCC AGCCGTGGCA AGGTCTCCAT GAAGGAGGTC
CMW 7046 E. singularis     CC-----TCC AGCCGTGGCA AGGTCTCCAT GAAGGAGGTC
CMW 5288 D. ambigua        -------TCT AGCCGTGGAA AGGTCTCCAT GAAGGAGGTC
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                                  290        300        310        320
CMW 7037 C. eucalypti      GAGGACCAGA TGCGCAACGT CCAGAGCAAG AACTCGTCCT
CWM 7038 C. eucalypti      GAGGACCAGA TGCGCAACGT CCAGAGCAAG AACTCGTCCT
CMW 7036 C. eucalypti      GAGGACCAGA TGCGCAACGT CCAGAGCAAG AACTCGTCCT
CMW 6245 Endothiella sp.   GAGGACCAGA TGCGCAACGT CCAGAGCAAG AACTCGTCCT
CMW 6246 Endothiella sp.   GAGGACCAGA TGCGCAACGT CCAGAGCAAG AACTCGTCCT
CMW 6249 Endothiella sp.   GAGGACCAGA TGCGCAACGT CCAGAGCAAG AACTCGTCCT
CMW 6244 Endothiella sp.   GAGGACCAGA TGCGCAACGT CCAGAGCAAG AACTCGTCCT
CMW 10729 Endothiella sp.  GAGGACCAGA TGCGCAACGT CCAGAGCAAG AACTCGTCCT
CRY 1543 C. macrospora     GAGGACCAGA TGCGCAACGT CCAGAGCAAG AACTCGTCCT
CMW 7047 C. parasitica     GAGGACCAGA TGCGCAACGT CCAGAGCAAG AACTCGTCCT
CMW 7048 C. parasitica     GAGGACCAGA TGCGCAACGT CCAGAGCAAG AACTCGTCCT
CMW 1651 C. parasitica     GAGGACCAGA TGCGCAACGT CCAGAGCAAG AACTCGTCCT
CMW 1652 C. parasitica     GAGGACCAGA TGCGCAACGT CCAGAGCAAG AACTCGTCCT
CMW 2091 E. gyrosa         GAGGACCAAA TGCGCAACGT CCAGAGCAAG AACTCGTCCT
CMW 7042 E. gurosa         GAGGACCAGA TGCGCAACGT CCAGAGCAAG AACTCGTCCT
CMW 7045 E. gyrosa         GAGGACCAGA TGCGCAACGT CCAGAGCAAG AACTCGTCCT
CMW 7046 E. singularis     GAGGACCAGA TGCGCAACGT CCAGAGCAAG AACTCGTCCT
CMW 5288 D. ambigua        GAGGACCAGA TGCGCAACGT CCAGAGCAAG AACTCGTCCT
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                                    330        340        350        360
CMW 7037 C. eucalypti          ACTTCGTCGA GTGGATCCCC AACAACGTCC AGACCGCCCT
CWM 7038 C. eucalypti          ACTTCGTCGA GTGGATCCCC AACAACGTCC AGACCGCCCT
CMW 7036 C. eucalypti          ACTTCGTCGA GTGGATCCCC AACAACGTCC AGACCGCCCT
CMW 6245 Endothiella sp.       ACTTCGTCGA GTGGATCCCC AACAACGTCC AGACCGCCCT
CMW 6246 Endothiella sp.       ACTTCGTCGA GTGGATCCCC AACAACGTCC AGACCGCCCT
CMW 6249 Endothiella sp.       ACTTCGTCGA GTGGATCCCC AACAACGTCC AGACCGCCCT
CMW 6244 Endothiella sp.       ACTTCGTCGA GTGGATCCCC AACAACGTCC AGACCGCCCT
CMW 10729 Endothiella sp.      ACTTCGTCGA GTGGATCCCC AACAACGTCC AGACCGCCCT
CRY 1543 C. macrospora         ACTTCGTCGA ATGGATCCCC AACAACGTCC AGACCGCCCT
CMW 7047 C. parasitica         ACTTCGTCGA GTGGATTCCC AACAACGTCC AGACCGCCCT
CMW 7048 C. parasitica         ACTTCGTCGA GTGGATTCCC AACAACGTCC AGACCGCCCT
CMW 1651 C. parasitica         ACTTCGTCGA GTGGATTCCC AACAACGTCC AGACCGCCCT
CMW 1652 C. parasitica         ACTTCGTCGA GTGGATTCCC AACAACGTCC AGACCGCCCT
CMW 2091 E. gyrosa             ACTTCGTCGA GTGGATCCCC AACAACGTCC AGACCGCCCT
CMW 7042 E. gurosa             ACTTCGTCGA GTGGATCCCC AACAACGTCC AGACCGCCCT
CMW 7045 E. gyrosa             ACTTCGTCGA GTGGATCCCC AACAACGTCC AGACCGCCCT
CMW 7046 E. singularis         ACTTCGTCGA GTGGATCCCC AACAACGTCC AGACCGCCCT
CMW 5288 D. ambigua            ACTTCGTCGA ATGGATTCCC AACAACGTCC AGACCGCCCT


                                    370        380        390        400
CMW 7037 C. eucalypti          CTGCTCCATC CCCCCCAAGG GCCTCAAGAT GTCCTCCACC
CWM 7038 C. eucalypti          CTGCTCCATC CCCCCCAAGG GCCTCAAGAT GTCCTCCACC
CMW 7036 C. eucalypti          CTGCTCCATC CCCCCCAAGG GCCTCAAGAT GTCCTCCACC
CMW 6245 Endothiella sp.       CTGCTCCATC CCCCCCAAGG GCCTCAAGAT GTCCTCCACC
CMW 6246 Endothiella sp.       CTGCTCCATC CCCCCCAAGG GCCTCAAGAT GTCCTCCACC
CMW 6249 Endothiella sp.       CTGCTCCATC CCCCCCAAGG GCCTCAAGAT GTCCTCCACC
CMW 6244 Endothiella sp.       CTGCTCCATC CCCCCCAAGG GCCTCAAGAT GTCCTCCACC
CMW 10729 Endothiella sp.      CTGCTCCATC CCCCCCAAGG GCCTCAAGAT GTCCTCCACC
CRY 1543 C. macrospora         CTGCTCCATC CCCCCCAAGG GCCTCAAGAT GTCCTCTACC
CMW 7047 C. parasitica         TTGCTCCATT CCCCCCAGGG GCCTAAAGAT GTCCTCGACC
CMW 7048 C. parasitica         CTGCTCCATT CCCCCCAGGG GCCTCAAGAT GTCCTCGACC
CMW 1651 C. parasitica         CTGCTCCATT CCCCCCAAGG GCCTCAAGAT GTCCTCGACC
CMW 1652 C. parasitica         CTGCTCCATT CCCCCCAAGG GCCTCAAGAT GTCCTCGACC
CMW 2091 E. gyrosa             CTGCTCCATC CCCCCCAGGG GTCTCAAGAT GTCCTCCACC
CMW 7042 E. gurosa             TTGCTCCATC CCCCCCAGGG GTCTAAAGAT GTCCTCCACC
CMW 7045 E. gyrosa             CTGCTCCATC CCCCCCAGGG GTCTCAAGAT GTCCTCCACC
CMW 7046 E. singularis         TTGCTCCATC CCCCCCAAGG GTCTCAAGAT GTCCTCCACC
CMW 5288 D. ambigua            GTGCTCGATC CCTCCCAAGG GTCTCAAGAT GTCCTCTACC
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                                    410        420        430        440
CMW 7037 C. eucalypti       TTTGTCGGCA ACTCCACCGC CATCCAGGAG CTCTTCAAGC
CWM 7038 C. eucalypti       TTTGTCGGCA ACTCCACCGC CATCCAGGAG CTCTTCAAGC
CMW 7036 C. eucalypti       TTTGTCGGCA ACTCCACCGC CATCCAGGAG CTCTTCAAGC
CMW 6245 Endothiella sp.    TTTGTCGGCA ACTCCACCGC CATCCAGGAG CTCTTCAAGC
CMW 6246 Endothiella sp.    TTTGTCGGCA ACTCCACCGC CATCCAGGAG CTCTTCAAGC
CMW 6249 Endothiella sp.    TTTGTCGGCA ACTCCACCGC CATCCAGGAG CTCTTCAAGC
CMW 6244 Endothiella sp.    TTTGTCGGCA ACTCCACCGC CATCCAGGAG CTCTTCAAGC
CMW 10729 Endothiella sp.   TTTGTCGGCA ACTCCACCGC CATCCAGGAG CTCTTCAAGC
CRY 1543 C. macrospora      TTTGTCGGCA ACTCCACCGC CATCCAGGAG CTCTTCAAGC
CMW 7047 C. parasitica      TTTGTCGGCA ACTCCACCGC CATCCAGGAG CTCTTAAAGC
CMW 7048 C. parasitica      TTTGTCGGCA ACTCCACCGC CATCCAGGAG CTCTTCAAGC
CMW 1651 C. parasitica      TTTGTCGGCA ACTCCACCGC CATCCAGGAG CTCTTCAAGC
CMW 1652 C. parasitica      TTTGTCGGCA ACTCCACCGC CATCCAGGAG CTCTTCAAGC
CMW 2091 E. gyrosa          TTTGTTGGCA ACTCTACCGC CATCCAGGAG CTTTTCAAGC
CMW 7042 E. gurosa          TTTGTTGGCA ACTCTACCGC CATCCAGGAG CTCTTCAAGC
CMW 7045 E. gyrosa          TTTGTTGGCA ACTCTACCGC CATCCAGGAG CTTTTCAAGC
CMW 7046 E. singularis      TTTGTTGGCA ACTCCACCGC TATCCAGGAG CTCTTCAAGC
CMW 5288 D. ambigua         TTCGTTGGTA ACTCGACTGC TATCCAGGAG CTGTTCAAGC

                                    450        460        470        480
CMW 7037 C. eucalypti       GTGTTGGCGA GCAGTTCACC GCCA-G---- --TTCCGGCG
CWM 7038 C. eucalypti       GTGTTGGCGA GCAGTTCACC GCCATGT--- --TTCCGGCG
CMW 7036 C. eucalypti       GTGTTGGCGA GCAGTTCACC GCCATG---- --TTCCGGCG
CMW 6245 Endothiella sp.    GTGTTGGCGA GCAGTTCACC GCCATG---- --TTCCGGAG
CMW 6246 Endothiella sp.    GTGTTGGCGA GCAGTTCACC GCCATG---- --TTCCGGCG
CMW 6249 Endothiella sp.    GTGTTGGCGA GCAGTTCACC GCCATG---- --TTCCGGCG
CMW 6244 Endothiella sp.    GTGTTGGCGA GCAGTTCACC GCCATG---- --TTCCGGCG
CMW 10729 Endothiella sp.   GTGTTGGCGA GCAGTTCACC GCCATG---- --TTCCGGCG
CRY 1543 C. macrospora      GTGTCGGCGA GCAGTTCACC GCCATGTCTA TGTTCCGTCG
CMW 7047 C. parasitica      GTGTCGGCGA GCAGTTCACC GCCATG---- --TTCCGGCG
CMW 7048 C. parasitica      GTGTCGGCGA GCAGTTTACC GCCATG---- --TTCCGGCG
CMW 1651 C. parasitica      GTGTCGGCGA GCAGTTTACC GCCATG---- --TTCCGGCG
CMW 1652 C. parasitica      GTGTCGGCGA GCAGTTTACC GCCATG---- --TTCCGGCG
CMW 2091 E. gyrosa          GCGTTGGCGA GCAGTTCACC GCCATG---- --TTCCGTCG
CMW 7042 E. gyrosa          GCGTTGGCGA GCAGTTCACC GCCATG---- --TTCCGTCG
CMW 7045 E. gyrosa          GCGTTGGCGA GCAGTTCACC GCCATG---- --TTCCGTCG
CMW 7046 E. singularis      GCGTTGGCGA GCAGTTCACC GCCATG---- --TTCCGTCG
CMW 5288 D. ambigua         GTGTCGGCGA GCAGTTCACT GCCATG---- --TTCAGGCG
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                                    490        500        510        520
CMW 7037 C. eucalypti       CAAGGCTTTC TTGCATTGGT ACACTGG-GA CCCGATACGG
CWM 7038 C. eucalypti       CAAGGCTTTC TTGCATTGGT ACACTGG-GA CCCGATACGG
CMW 7036 C. eucalypti       CAAGGCTTTC TTGCATTGGT ACACTGG-GA CACGATACGG
CMW 6245 Endothiella sp.    CAAGGCTTTC TTGCATTGGT ACACTGGTGA GGGGATACGG
CMW 6246 Endothiella sp.    CAAGGCTTTC TTGCATTGGT ACACTGGTGA GGGGATGGAC
CMW 6249 Endothiella sp.    CAAGGCTTTC TTGCATTGGT ACACTGGTGA GGGGATGGAC
CMW 6244 Endothiella sp.    CAAGGCTTTC TTGCATTGGT ACACTGGTGA GGGGATGGAC
CMW 10729 Endothiella sp.   CAAGGCTTTC TTGCATTGGT ACACTGGTGA GGGGATGGAC
CRY 1543 C. macrospora      CAAGGCTTTC TTGCATTGGT ACACTGGG-A CACGATACAA
CMW 7047 C. parasitica      CAAGGCTTTC TTGCATTGGT ACACTGGTTT TCGACACGAT
CMW 7048 C. parasitica      CAAGGCTTTC TTGCATTGGT ACACTGGTTT TCGACACGAT
CMW 1651 C. parasitica      CAAGGCTTTC TTGCATTGGT ACACTGGTTT TCGACACGAT
CMW 1652 C. parasitica      CAAGGCTTTC TTGCATTGGT ACACTGGTTT TCGACACGAT
CMW 2091 E. gyrosa          CAAGGCCTTC TTGCATTGGT ACACCGGTGA GGGTATGGAC
CMW 7042 E. gyrosa          CAAGGCCTTC TTGCATTGGT ACACCGGTGA GGGTATGGAC
CMW 7045 E. gyrosa          CAAGGCCTTC TTGCATTGGT ACACCGGTGA GGGTATGGAC
CMW 7046 E. singularis      CAAGGCCTTC TTGCATTGGT ACACCGGTGA GGGTATGG--
CMW 5288 D. ambigua         CAAGGCTTTC TTGCATTGGT ACACTGG--- ----------

                                    530        540        550        560
CMW 7037 C. eucalypti       C--GATGT-- CG----ACC- ---TCGTGCA GCAGAC----
CWM 7038 C. eucalypti       C--GATGT-- CG----ACC- ---TCGTGCA GCAGAC----
CMW 7036 C. eucalypti       C--GATGT-- CG----ACC- ---TCGTGCA GCAGAC----
CMW 6245 Endothiella sp.    C--GATGT-- CG----ACC- ---TCGTGCG GCAGAC----
CMW 6246 Endothiella sp.    G--GATGT-- CG----ACC- ---TCGTGCG GCAGAC----
CMW 6249 Endothiella sp.    G--GATGT-- CG----ACC- ---TCGTGCG GCAGAC----
CMW 6244 Endothiella sp.    G--GATGT-- CG----ACC- ---TCGTGCG GCAGAC----
CMW 10729 Endothiella sp.   G--GATGT-- CG----ACC- ---TCGTGCG GCAGAC----
CRY 1543 C. macrospora      C--GATAT-- CG----ACA- ---TCGTGCA GCAGAC----
CMW 7047 C. parasitica      A--CAGCT-- AC----ATCG ACATCGTGCA GCAGAC----
CMW 7048 C. parasitica      A--CAGCT-- AC----ATCG ACATCGTGCA GCAGAC----
CMW 1651 C. parasitica      A--CAGCT-- AC----ATCG ACATCGTGCA GCAGAC----
CMW 1652 C. parasitica      A--CAGCT-- AC----ATCG ACATCGTGCA GCAGAC----
CMW 2091 E. gyrosa          G---AGATGG AGTTC-ACCG AGGCCGAGTT CAACATGAAC
CMW 7042 E. gyrosa          G---AGATGG AGTTC-ACCG AGGCCGAGTT CAACATGAAC
CMW 7045 E. gyrosa          G---AGATGG AGTTC-ACCG AGGCCGAGTT CAACAT----
CMW 7046 E. singularis      --------TA TTTTCGACTC GATA------ CAAC------
CMW 5288 D. ambigua         ---------- ---------- ---------- ----------
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                                570        580        590        600
CMW 7037 C. eucalypti     ----TTGGGT GCTGACCTCG -ACGCCAGGC AAACCATT-T
CWM 7038 C. eucalypti     ----TTGGGT GCTGACCTCG -ACCGCAGGC AAACCATT-T
CMW 7036 C. eucalypti     ----TTGGGT GCTGACCTCG -ACCGCAGGC AAACCATC-T
CMW 6245 Endothiella sp.  ----TTGGGT GCTGACCTCG -ACCGCAGGC AAACCATC-T
CMW 6246 Endothiella sp.  ----TTGGGT GCTGACCTCG -ACCGCAGGC AAACCATC-T
CMW 6249 Endothiella sp.  ----TTGGGT GCTGACCTCG -ACCGCAGGC AAACCATC-T
CMW 6244 Endothiella sp.  ----TTGGGT GCTGACCTCG -ACCGCAGGC AAACCATC-T
CMW 10729 Endothiella sp. ----TTGGGT GCTGACCTCG -ACCGCAGGC AAACCATC-T
CRY 1543 C. macrospora    ----TTGGAT GCTAATCTCG -ACCACAGGC AAACCATC-T
CMW 7047 C. parasitica    ----TTGGAT GCTGACCTCG -ACAATAGGC AAACCATC-T
CMW 7048 C. parasitica    ----TTGGAT GCTGACCTCG -ACAATAGGC AAACCATC-T
CMW 1651 C. parasitica    ----TTGGAT GCTGACCTCG -ACAATAGGC AAACCATC-T
CMW 1652 C. parasitica    ----TTGGAT GCTGACCTCG -ACAATAGGC AAACCATC-T
CMW 2091 E. gyrosa        GA---TGGCA A--CCACCGA CGCGTGT--- -CGAC-GTTG
CMW 7042 E. gyrosa        GAATTTGGCA A--CCACCGA CGCGTGT--- -CGAC-GTTG
CMW 7045 E. gyrosa        -----GGGCA A--CCACCGA CGCGTGT--- -CGAC-GTTG
CMW 7046 E. singularis    ---------- --------GA TATCGA---- ----------
CMW 5288 D. ambigua       ---------- ---------- ---------C AAACCATC-T

                                610        620        630        640
CMW 7037 C. eucalypti     CGGGCGAGCA CGGCCTCGAC AGCAATGGCG TGTATGTACC
CWM 7038 C. eucalypti     CGGGCGAGCA CGGCCTCGAC AGCAATGGCG TGTATGTACC
CMW 7036 C. eucalypti     CGGGCGAGCA CGGCCTCGAC AGCAATGGCG TGTATGTACC
CMW 6245 Endothiella sp.  CGGGCGAGCA CGGCCTCGAC AGCAATGGCG TGTATGTACC
CMW 6246 Endothiella sp.  CGGGCGAGCA CGGCCTCGAC AGCAATGGCG TGTATGTACC
CMW 6249 Endothiella sp.  CGGGCGAGCA CGGCCTCGAC AGCAATGGCG TGTATGTACC
CMW 6244 Endothiella sp.  CGGGCGAGCA CGGCCTCGAC AGCAATGGCG TGTATGTACC
CMW 10729 Endothiella sp. CGGGCGAGCA CGGCCTCGAC AGCAATGGCG TGTATGTACC
CRY 1543 C. macrospora    CCGGCGAGCA TGGTCTCGAC AGCAATGGCG TGTATGTACC
CMW 7047 C. parasitica    CCGGCGAGCA CGGCCTCGAC AGCAATGGCG TGTACGTACC
CMW 7048 C. parasitica    CCGGCGAGCA CGGCCTCGAC AGCAATGGCG TGTACGTACC
CMW 1651 C. parasitica    CCGGCGAGCA CGGCCTCGAC AGCAATGGCG TGTACGTACC
CMW 1652 C. parasitica    CCGGCGAGCA CGGCCTCGAC AGCAATGGCG TGTACGTACC
CMW 2091 E. gyrosa        CCGGCCTCGT GGTGGCTTCG GTGC----TG ACCTCGGCTG
CMW 7042 E. gyrosa        CCGGCCTCGT GGTGGCTTCG GTGC----TG ACCTCGGCTG
CMW 7045 E. gyrosa        CCGGCCTCGT GGTGGCTTCG GTGC----TG ACCTCGGCTG
CMW 7046 E. singularis    -CATCGTGCA GCAGACTTGG ATGC----TG ACCTCGACCA
CMW 5288 D. ambigua       CTGGCGAGCA CGGTCTCGAC AGCAATGGCG TGTACGTACC
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                                650         660         670         680
CMW 7037 C. eucalypti      --ACACCATA CCCT------ --ACACGGCG GCCCAC-GCA
CWM 7038 C. eucalypti      --ACACCATA CCCT------ --ACACGGCG GCCCAC-GCA
CMW 7036 C. eucalypti      --ACACCATA CCCT------ --ACACGGCG GCCCAC-GCA
CMW 6245 Endothiella sp.   --ACACCATA CCCT------ --GCACGGCG GCCCAC-GCA
CMW 6246 Endothiella sp.   --ACACCATA CCCT------ --GCACGGCG GCCCAC-GCA
CMW 6249 Endothiella sp.   --ACACCATA CCCT------ --GCACGGCG GCCCAC-GCA
CMW 6244 Endothiella sp.   --ACACCATA CCCT------ --GCACGGCG GCCCAC-GCA
CMW 10729 Endothiella sp. --ACACCATA CCCT------ --GCACGGCG GCCCAC-GCA
CRY 1543 C. macrospora     --CTACCTCG GCTT------ CCCCAGCAAG ATAGAC-GCG
CMW 7047 C. parasitica     --CTATCTCG GCTT------ CCCAAGCAAG ACAGAC-GCG
CMW 7048 C. parasitica     --CTATCTCG GCTT------ CCCAAGCAAG ACAGAC-GCG
CMW 1651 C. parasitica     --CTATCTCG GCTT------ CCCAAGCAAG ACAGAC-GCG
CMW 1652 C. parasitica     --CTATCTCG GCTT------ CCCAAGCAAG ACAGAC-GCG
CMW 2091 E. gyrosa         CAGGCAAACC ATCTCCGGCG A-GCACGGCC -TCGACAGCG
CMW 7042 E. gyrosa         CAGGCAAACC ATCTCCGGCG A-GCACGGCC -TCGACAGCG
CMW 7045 E. gyrosa         CAGGCAAACC ATCTCCGGCG A-GCACGGCC -TCGACAGCG
CMW 7046 E. singularis     CAGGCAAACC ATCTCCGGCG A-GCATGGCC -TCGACAGCA
CMW 5288 D. ambigua        --TCGTATCC CCT------G CCCACTGGTC TCGTCC----
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                                690         700         710         720
CMW 7037 C. eucalypti      AGATGGACGC GGCTCG-G-G CTCTCCT--- ----------
CWM 7038 C. eucalypti      AGATGGACGC GGCCCG-G-G CTCTCCT--- ----------
CMW 7036 C. eucalypti      AGATGGACGC GGCTCG-G-G CTTTCCT--- ----------
CMW 6245 Endothiella sp.   AGATGGACGC GGCTCG-G-G CTTTCCT--- ----------
CMW 6246 Endothiella sp.   AGATGGACGC GGCTCG-G-G CTTTCCT--- ----------
CMW 6249 Endothiella sp.   AGATGGACGC GGCTCG-G-G CTTTCCT--- ----------
CMW 6244 Endothiella sp.   AGATGGACGC GGCTCG-G-G CTTTCCT--- ----------
CMW 10729 Endothiella sp. AGATGGACGC GGCTCG-G-G CTTTCCT--- ----------
CRY 1543 C. macrospora     ACTTG----- ------TGAG CTTTCCT--- ----------
CMW 7047 C. parasitica     ACTT------ -------GAG CTTTCCT--- ----------
CMW 7048 C. parasitica     ACTT------ -------GAG CTTTCCT--- ----------
CMW 1651 C. parasitica     ACTT------ -------GAG CTTTCCT--- ----------
CMW 1652 C. parasitica     ACTT------ -------GAG CTTTCCT--- ----------
CMW 2091 E. gyrosa         ATGGCGTGTA T------GTT GTACCCTACT GCTGCCCGGC
CMW 7042 E. gyrosa         ATGGCGTGTA T------GTT GTACCCTACT GCTGCCCGGC
CMW 7045 E. gyrosa         ATGGCGTGTA T------GTT GTACCCTACT GCTGCCCGGC
CMW 7046 E. singularis     ATGGCGTGTA T----GTACT CTACC-T-CG GCTTCCCCAG
CMW 5288 D. ambigua        ---------- ---------- TCTCCCT-CG GCTT------
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	730	740	750	760
CMW 7037 *C. eucalypti*	----------	----------	-------GCT	GACCA--CCC
CWM 7038 *C. eucalypti*	----------	----------	-------GCT	GACCA--CCC
CMW 7036 *C. eucalypti*	----------	----------	-------GCT	AACCA--CCC
CMW 6245 *Endothiella* sp.	----------	----------	-------GCT	GACCA--CCC
CMW 6246 *Endothiella* sp.	----------	----------	-------GCT	GACCA--CCC
CMW 6249 *Endothiella* sp.	----------	----------	-------GCT	GACCA--CCC
CMW 6244 *Endothiella* sp.	----------	----------	-------GCT	GACCA--CCC
CMW 10729 *Endothiella* sp.	----------	----------	-------GCT	GACCA--CCC
CRY 1543 *C. macrospora*	----------	----------	-------GCT	GACGA---CC
CMW 7047 *C. parasitica*	----------	----------	-------GCT	GACCA---CC
CMW 7048 *C. parasitica*	----------	----------	-------GCT	GACCA---CC
CMW 1651 *C. parasitica*	----------	----------	-------GCT	GACCA---CC
CMW 1652 *C. parasitica*	----------	----------	-------GCT	GACCA---CC
CMW 2091 *E. gyrosa*	CGACGCGCTC	GGGCCCTTCC	CC-----GCT	GACCA---CC
CMW 7042 *E. gyrosa*	CGACGCGCTC	GGGCCCTTCC	CC-----GCT	GACCA---CC
CMW 7045 *E. gyrosa*	CGACGCGCTC	GGGCCCTTCC	CC-----GCT	GACCA---CC
CMW 7046 *E. singularis*	CAAGATAGAC	GCG-ACTTGT	GAGACTTCCT	GCTGATCACC
CMW 5288 *D. ambigua*	----------	----------	-------GGC	ACTGACAACT

	770	780	790	800
CMW 7037 *C. eucalypti*	GCATAGC---	----------	---TACAACG	GCACCTCCGA
CWM 7038 *C. eucalypti*	GCATAGC---	----------	---TACAACG	GCACCTCCGA
CMW 7036 *C. eucalypti*	GCGTAGC---	----------	---TACAACG	GCACCTCCGA
CMW 6245 *Endothiella* sp.	GCATAGC---	----------	---TACAACG	GCACCTCCGA
CMW 6246 *Endothiella* sp.	GCATAGC---	----------	---TACAACG	GCACCTCCGA
CMW 6249 *Endothiella* sp.	GCATAGC---	----------	---TACAACG	GCACCTCCGA
CMW 6244 *Endothiella* sp.	GCATAGC---	----------	---TACAACG	GCACCTCCGA
CMW 10729 *Endothiella* sp.	GCATAGC---	----------	---TACAACG	GCACCTCCGA
CRY 1543 *C. macrospora*	ACATAGC---	----------	---TACAACG	GCACCTCCGA
CMW 7047 *C. parasitica*	ACATAGC---	----------	---TACAACG	GCACCTCCGA
CMW 7048 *C. parasitica*	ACATAGC---	----------	---TACAACG	GCACCTCCGA
CMW 1651 *C. parasitica*	ACATAGC---	----------	---TACAACG	GCACCTCCGA
CMW 1652 *C. parasitica*	ACATAGC---	----------	---TACAACG	GCACCTCCGA
CMW 2091 *E. gyrosa*	GCACAGC---	----------	---TACAACG	GCACCTCCGA
CMW 7042 *E. gyrosa*	GCACAGC---	----------	---TACAACG	GCACCTCCGA
CMW 7045 *E. gyrosa*	GCACAGC---	----------	---TACAACG	GCACCTCCGA
CMW 7046 *E. singularis*	ACATAGC---	----------	---TACAACG	GCACCTCCGA
CMW 5288 *D. ambigua*	GCACAGT---	----------	---TACAACG	GCACTTCCGA

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                                  810        820        830        840
CMW 7037 C. eucalypti      GCTCC-AGCT CGAGCGCATG AACGTCTACT TCAACGAGGT
CWM 7038 C. eucalypti      GCTCC-AGCT CGAGCGCATG AACGTCTACT TCAACGAGGT
CMW 7036 C. eucalypti      GCTCC-AGCT CGAGCGCATG AACGTCTACT TCAACGAGGT
CMW 6245 Endothiella sp.   GCTCC-AGCT CGAGCGCATG AACGTCTACT TCAACGAGGT
CMW 6246 Endothiella sp.   GCTCC-AGCT CGAGCGCATG AACGTCTACT TCAACGAGGT
CMW 6249 Endothiella sp.   GCTCC-AGCT CGAGCGCATG AACGTCTACT TCAACGAGGT
CMW 6244 Endothiella sp.   GCTCC-AGCT CGAGCGCATG AACGTCTACT TCAACGAGGT
CMW 10729 Endothiella sp. GCTCC-AGCT CGAGCGCATG AACGTCTACT TCAACGAGGT
CRY 1543 C. macrospora     GCTCC-AGCT CGAGCGCATG AACGTCTACT TCAACGAGGT
CMW 7047 C. parasitica     GCTCC-AGCT CGAGCGCATG AACGTCTACT TTAACGAGGT
CMW 7048 C. parasitica     GCTCC-AGCT CGAGCGCATG AACGTCTACT TTAACGAGGT
CMW 1651 C. parasitica     GCTCC-AGCT CGAGCGCATG AACGTCTACT TTAACGAGGT
CMW 1652 C. parasitica     GCTCC-AGCT CGAGCGCATG AACGTCTACT TTAACGAGGT
CMW 2091 E. gyrosa         GCTCC-AGCT CGAGCGCATG AACGTCTACT TCAACGAGGT
CMW 7042 E. gyrosa         GCTCC-AGCT CGAGCGCATG AACGTCTACT TCAACGAGGT
CMW 7045 E. gyrosa         GCTCC-AGCT CGAGCGCATG AACGTCTACT TCAACGAGGT
CMW 7046 E. singularis     GCTCC-AGCT CGAGCGCATG AACGTCTACT TCAACGAGGT
CMW 5288 D. ambigua        GCTCC-AGCT CGAGCGCATG AACGTCTACT TCAACGAGGT

                                  850        860        870        880
CMW 7037 C. eucalypti      ATGTC-TTAT CGGGTGAC-C AGGCC-TCGA GCCATCATCC
CWM 7038 C. eucalypti      ATGTC-TTAT CGGGTGAC-C AGGCC-TCGA GCCAGCATCC
CMW 7036 C. eucalypti      ATGTC-TTGT CGGCTGAC-C AGGCC-TCCA GCCATCATCC
CMW 6245 Endothiella sp.   ATGTC-TTAT CGGCTGGC-C AGGCC-TCGA GCCATCATCC
CMW 6246 Endothiella sp.   ATGTC-TTAT CGGCTGGC-C AGGCC-TCGA GCCATCATCC
CMW 6249 Endothiella sp.   ATGTC-TTAT CGGCTGGC-C AGGCC-TCGA GCCATCATCC
CMW 6244 Endothiella sp.   ATGTC-TTAT CGGCTGGC-C AGGCC-TCGA GCCATCATCC
CMW 10729 Endothiella sp. ATGTC-TTAT CGGCTGGC-C AGGCC-TCGA GCCATCATCC
CRY 1543 C. macrospora     ATGTC-TTAT CGGGTGAC-C AGGC--TCGA GCATCCATCT
CMW 7047 C. parasitica     ATGTC-TTAT CGGGTGAT-C AAGC--TCAA GCTTCCA-CC
CMW 7048 C. parasitica     ATGTC-TTAT CGGGTGAT-C AAGC--TCAA GCTTCCA-CC
CMW 1651 C. parasitica     ATGTC-TTAT CGGGTGATAC AAGC--TCAA GCTTCCA-CC
CMW 1652 C. parasitica     ATGTC-TTAT CGGGTGAT-C AAGC--TCAA GCTTC-A-CC
CMW 2091 E. gyrosa         ATGTC--TAT GGGGG-AC-C AGGCCCTGGC GTGGCCCC--
CMW 7042 E. gyrosa         ATGTC--TAT GGGGG-AC-C AGGCCCTGGC GTGGCCCC--
CMW 7045 E. gyrosa         ATGTC--TAT GGGGG-AC-C AGGCCCTGGC GTGGCCCC--
CMW 7046 E. singularis     ATGTC-TTAT CGGGTGAC-C AGGC--TCGA GCATCCATCT
CMW 5288 D. ambigua        AAGTC----- ------AA-C AG-CCACGTC GTCAATTCAA
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                                    890        900        910        920
CMW 7037 C. eucalypti       TGCCTCCTCC CTCCTCATCC CC--TCGGGG CTTTTGTGGC
CWM 7038 C. eucalypti       TGCCTCCTGC CTCCTCATCC CC--TCGGGG CTTTTGTGGC
CMW 7036 C. eucalypti       TGCCTCCTGC CTCCTCCTTC CA--TCGGGA CTTCTGTGGC
CMW 6245 Endothiella sp.    TGCCTCCTGC CTCCTCCTTC CC--TCGGGA CTTTTGTGGC
CMW 6246 Endothiella sp.    TGCCTCCTGC CTCCTCCTTC CC--TCGGGA CTTTTGTGGC
CMW 6249 Endothiella sp.    TGCCTCCTGC CTCCTCCTTC CC--TCGGGA CTTTTGTGGC
CMW 6244 Endothiella sp.    TGCCTCCTGC CTCCTCCTTC CC--TCGGGA CTTTTGTGGC
CMW 10729 Endothiella sp.   TGCCTCCTGC CTCCTCCTTC CC--TCGGGA CTTTTGTGGC
CRY 1543 C. macrospora      CAA----CCC CCCCCCCCTC C----CAAAT CCCGGGCCCC
CMW 7047 C. parasitica      TCGG-CCACC CCCCCCCCCC C-TTTCCGGG G-CCCTC---
CMW 7048 C. parasitica      TCGG-CCAAC CCCCCCCCCC CCTTTCCGGG G-CCCTC---
CMW 1651 C. parasitica      TGGG-CCAAC CCCCCCCCCC C-TTTCCGGG G-CCTTC---
CMW 1652 C. parasitica      TCGG--CAAC CCCCCCCCCC C-TTTCCGGG G-CCTT----
CMW 2091 E. gyrosa          -GCCCGCGGC CCC------- ---------- -TGGCGTG-A
CMW 7042 E. gyrosa          -GCCCGCGGC CCC------- ---------- -TGGCGTG-A
CMW 7045 E. gyrosa          -GCCCGCGGC CCC------- ---------- -TGGCGTG-A
CMW 7046 E. singularis      ------CAGC CCACCCCTGT TCCCTC-CAC TTCTGGTACA
CMW 5288 D. ambigua         ATTTGACAAC CTACGGCATG G-TTTCCCGC CGTCG-----
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                                    930        940        950        960
CMW 7037 C. eucalypti       CT-GACC--- ---------G AGCTTGCCCT TCTGACGCGT
CWM 7038 C. eucalypti       CT-GACC--- ---------G AGCCTGCCCT TCTGACGCGT
CMW 7036 C. eucalypti       CT-GACC--- ---------G AGCTTGCCCT TCTGACGCGT
CMW 6245 Endothiella sp.    CT-GACC--- ---------G AGCTTGCCCT TCTGACGCGT
CMW 6246 Endothiella sp.    CT-GACC--- ---------G AGCTTGCCCT TCTGACGCGT
CMW 6249 Endothiella sp.    CT-GACC--- ---------G AGCTTGCCCT TCTGACGCGT
CMW 6244 Endothiella sp.    CT-GACC--- ---------G AGCTTGCCCT TCTGACGCGT
CMW 10729 Endothiella sp.   CT-GACC--- ---------G AGCTTGCCCT TCTGACGCGT
CRY 1543 C. macrospora      TC-GACTTCT GGCATAGGCG AGCTTCCTCT TCTGACGCGC
CMW 7047 C. parasitica      ---GACTTCT GGTATAGGCG AGCTTCCTCT TCTGACGCGC
CMW 7048 C. parasitica      ---GACTTCT GGTATAGGCG AGCTTCCTCT TCTGACGCGC
CMW 1651 C. parasitica      -T-GACTTCT GGTATAGGCG AGCATCCTCT TCTGACGCGC
CMW 1652 C. parasitica      ---GACTTCT GGTATAGGCG AGCTTCCTCT TCTGACGCGC
CMW 2091 E. gyrosa          CC-------- ---------G AGCTCCCA-- -CTGACGCGC
CMW 7042 E. gyrosa          CC-------- ---------G AGCTCCCA-- -CTGACGCGC
CMW 7045 E. gyrosa          CC-------- ---------G AGCTCCCA-- -CTGACGCGC
CMW 7046 E. singularis      GGC------- ---------G AGCTTCCTCT TCTGACGCGC
CMW 5288 D. ambigua         -----CCAAG GCTTG----- ---------- -CTAACGCAT
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                                    970        980        990        1000
CMW 7037 C. eucalypti               TTCTCGTCCA GGCCTCCGGC AACAAGTATG TTCCCCGCGC
CWM 7038 C. eucalypti               TTCTCGTCCA GGCCTCCGGC AACAAGTATG TTCCCCGCGC
CMW 7036 C. eucalypti               TTCTCGTCCA GGCCTCCGGC AACAAGTATG TTCCCCGCGC
CMW 6245 Endothiella sp.            TTCTCGTCCA GGCCTCCGGC AACAAGTATG TCCCCCGCGC
CMW 6246 Endothiella sp.            TTCTCGTCCA GGCCTCCGGC AACAAGTATG TCCCCCGCGC
CMW 6249 Endothiella sp.            TTCTCGTCCA GGCCTCCGGC AACAAGTATG TCCCCCGCGC
CMW 6244 Endothiella sp.            TTCTCGTCCA GGCCTCCGGC AACAAGTATG TCCCCCGCGC
CMW 10729 Endothiella sp.           TTCTCGTCCA GGCCTCCGGC AACAAGTATG TCCCCCGCGC
CRY 1543 C. macrospora              TTCTTATCCA G-CCTCCAGC AACAAGTATG TTCCCCGCGC
CMW 7047 C. parasitica              TTCTTGTCCA GGCCTCCGGC AACAAGTATG TTCCCCGCGC
CMW 7048 C. parasitica              TTCTTGTCCA GGCCTCCGGC AACAAGTATG TTCCCCGCGC
CMW 1651 C. parasitica              TTCTTGTCCA GGCCTCCGGC AACAAGTATG TTCCCCGCGC
CMW 1652 C. parasitica              TTCTTGTCCA GGCCTCCGGC AACAAGTATG TTCCCCGCGC
CMW 2091 E. gyrosa                  TCCT-GTCCA GGCCTCCGGC AACAAGTATG TCCCCCGCGC
CMW 7042 E. gyrosa                  TCCT-GTCCA GGCCTCCGGC AACAAGTATG TCCCCCGCGC
CMW 7045 E. gyrosa                  TCCT-GTCCA GGCCTCCGGC AACAAGTATG TCCCCCGCGC
CMW 7046 E. singularis              TTCTTATCCA GGCCTCCAGC AACAAGTATG TTCCCCGCGC
CMW 5288 D. ambigua                 TTATCGCCCA GGCCTCCGGC AACAAGTATG TGCCTCGCGC

                                         1010       1020       1030       1040
CMW 7037 C. eucalypti               CGTCCTCGTC GATCTCGAGC CCGGTACCAT GGATGCCGTC
CWM 7038 C. eucalypti               CGTCCTCGTC GATCTCGAGC CCGGTACCAT GGATGCCGTC
CMW 7036 C. eucalypti               CGTCCTCGTC GATCTCGAGC CCGGTACCAT GGATGCCGTC
CMW 6245 Endothiella sp.            CGTCCTCGTC GATCTCGAGC CCGGTACCAT GGATGCCGTC
CMW 6246 Endothiella sp.            CGTCCTCGTC GATCTCGAGC CCGGTACCAT GGATGCCGTC
CMW 6249 Endothiella sp.            CGTCCTCGTC GATCTCGAGC CCGGTACCAT GGATGCCGTC
CMW 6244 Endothiella sp.            CGTCCTCGTC GATCTCGAGC CCGGTACCAT GGATGCCGTC
CMW 10729 Endothiella sp.           CGTCCTCGTC GATCTCGAGC CCGGTACCAT GGATGCCGTC
CRY 1543 C. macrospora              AGTCCTCGTC GATCTCGAGC CCGGTACCAT GGATGCCGTC
CMW 7047 C. parasitica              CGTCCTCGTC GATCTCGAGC CCGGTACCAT GGATGCCGTC
CMW 7048 C. parasitica              CGTCCTCGTC GATCTCGAGC CCGGTACCAT GGATGCCGTC
CMW 1651 C. parasitica              CGTCCTCGTC GATCTCGAGC CCGGTACCAT GGATGCCGTC
CMW 1652 C. parasitica              CGTCCTCGTC GATCTCGAGC CCGGTACCAT GGATGCCGTC
CMW 2091 E. gyrosa                  CGTCCTCGTC GATCTCGAGC CCGGTACCAT GGACGCCGTC
CMW 7042 E. gyrosa                  CGTCCTCGTC GATCTCGAGC CCGGTACCAT GGACGCCGTC
CMW 7045 E. gyrosa                  CGTCCTCGTC GATCTCGAGC CCGGTACCAT GGACGCCGTC
CMW 7046 E. singularis              TGTCCTCGTC GATCTCGAGC CCGGTACCAT GGACGCCGTC
CMW 5288 D. ambigua                 CGTCCTCGTC GATCTCGAGC CCGGTACCAT GGACGCCGTC
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	1050	1060	1070	1080
CMW 7037 *C. eucalypti*	CGCGCCGGCC	CCTTCGGCCA	GCTGTTCCGT	CCCGACAACT
CWM 7038 *C. eucalypti*	CGCGCCGGCC	CCTTCGGCCA	GCTGTTCCGT	CCCGACAACT
CMW 7036 *C. eucalypti*	CGCGCCGGCC	CCTTCGGCCA	GCTGTTCCGT	CCCGACAACT
CMW 6245 *Endothiella* sp.	CGCGCCGGCC	CCTTCGGCCA	GCTGTTCCGT	CCCGACAACT
CMW 6246 *Endothiella* sp.	CGCGCCGGCC	CCTTCGGCCA	GCTGTTCCGT	CCCGACAACT
CMW 6249 *Endothiella* sp.	CGCGCCGGCC	CCTTCGGCCA	GCTGTTCCGT	CCCGACAACT
CMW 6244 *Endothiella* sp.	CGCGCCGGCC	CCTTCGGCCA	GCTGTTCCGT	CCCGACAACT
CMW 10729 *Endothiella* sp.	CGCGCCGGCC	CCTTCGGCCA	GCTGTTCCGT	CCCGACAACT
CRY 1543 *C. macrospora*	CGCGCCGGCC	CCTTTGGTCA	GCTGTTCCGT	CCCGACAACT
CMW 7047 *C. parasitica*	CGCGCTGGCC	CCTTTGGTCA	GCTGTTCCGT	CCCGACAACT
CMW 7048 *C. parasitica*	CGCGCTGGCC	CCTTTGGTCA	GCTGTTCCGT	CCCGACAACT
CMW 1651 *C. parasitica*	CGCGCTGGCC	CCTTTGGTCA	GCTGTTCCGT	CCCGACAACT
CMW 1652 *C. parasitica*	CGCGCTGGCC	CCTTTGGTCA	GCTGTTCCGT	CCCGACAACT
CMW 2091 *E. gyrosa*	CGCGCCGGCC	CCTTCGGCCA	GCTGTTCCGC	CCCGACAACT
CMW 7042 *E. gyrosa*	CGCGCCGGCC	CCTTCGGCCA	GCTGTTCCGC	CCCGACAACT
CMW 7045 *E. gyrosa*	CGCGCCGGCC	CCTTCGGCCA	GCTGTTCCGC	CCCGACAACT
CMW 7046 *E. singularis*	CGCGCCGGCC	CCTTTGGCCA	GCTGTTCCGT	CCCGACAACT
CMW 5288 *D. ambigua*	CGTGCCGGTC	CCTTCGGCCA	GCTGTTCCGC	CCCGACAACT

	1090	1097
CMW 7037 *C. eucalypti*	TCGTCTTCGG	CCAGTCC
CWM 7038 *C. eucalypti*	TCGTCTTCGG	CCAGTCC
CMW 7036 *C. eucalypti*	TCGTCTTCGG	CCAGTCC
CMW 6245 *Endothiella* sp.	TCGTCTTCGG	CCAGTCC
CMW 6246 *Endothiella* sp.	TCGTCTTCGG	CCAGTCC
CMW 6249 *Endothiella* sp.	TCGTCTTCGG	CCAGTCC
CMW 6244 *Endothiella* sp.	TCGTCTTCGG	CCAGTCC
CMW 10729 *Endothiella* sp.	TCGTCTTCGG	CCAGTCC
CRY 1543 *C. macrospora*	TCGTCTTTGG	CCAGTCC
CMW 7047 *C. parasitica*	TCGTCTTTGG	CCAGTCC
CMW 7048 *C. parasitica*	TCGTCTTTGG	CCAGTCC
CMW 1651 *C. parasitica*	TCGTCTTTGG	CCAGTCC
CMW 1652 *C. parasitica*	TCGTCTTTGG	CCAGTCC
CMW 2091 *E. gyrosa*	TCGTCTTCGG	CCAGTCC
CMW 7042 *E. gyrosa*	TCGTCTTCGG	CCAGTCC
CMW 7045 *E. gyrosa*	TCGTCTTCGG	CCAGTCC
CMW 7046 *E. singularis*	TCGTCTTTGG	CCAGTCC
CMW 5288 *D. ambigua*	TCGTCTTCGG	CCAGTCC

CHAPTER 5

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

ABSTRACT

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

INTRODUCTION

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

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

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

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

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

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

The fact that *C. cubensis* is a pathogen of both *Eucalyptus* spp. and *Tibouchina* spp. has led us to question whether other *Eucalyptus* pathogens might occur on *Tibouchina* spp. *Tibouchina* spp. produce attractive flowers and are commonly planted in southern hemisphere countries such as South Africa and Australia as ornamentals. These trees

are also commonly found in areas where *Eucalyptus* spp. occur as natives or are grown in plantations. The aim of this study was, thus, to determine whether *Botryosphaeria* spp. occur on *Tibouchina* spp. and if so, whether these fungi represent similar species to those on *Eucalyptus* spp. in the same areas.

MATERIALS AND METHODS

Disease symptoms and collection of samples

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

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

Morphological characterisation

Cultures resembling those of *Botryosphaeria* spp. were induced to sporulate by transferring them to water agar (WA) (20 g Biolab Agar, 1 litre distilled water), to which sterile pine needles or *Tibouchina* twigs had been added to the agar surface. Cultures were incubated at 25 °C for 2 weeks under near UV light (280 nm). Fruiting structures produced on the pine needles or *Tibouchina* twigs were dissected by hand to expose conidia or ascospores. Spores were mounted in lactophenol and examined

using Nomarski differentiation interference contrast microscopy. Isolates were grouped based on conidial morphology. For each isolate the average length and width (upper third of the spore) of 25 conidia was measured using a light microscope (Carl Zeiss) and these are presented as (min-)(mean-SD) - (mean+SD)(-max) µm. Images were captured electronically with an Axiovision digital camera system (Carl Zeiss).

DNA isolation and amplification

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

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

Restriction Fragment Length Polymorphisms (RFLPs)

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

DNA sequencing and analyses

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

Data analyses were performed using PAUP* (Phylogenetic Analysis Using Parsimony) version 4.0b (Swofford 1998). Analyses were done using the heuristic search option with TBR (tree-bisection-reconnection) branch swapping. Gaps inserted during sequence alignment, were treated as fifth base (NEWSTATE). A bootstrap analysis (50% majority rule, 1000 replications) was done to determine the confidence levels of the tree branching points (Felsenstein 1995). Previously published sequences of

various commonly known *Botryosphaeria* spp. as well as those from Slippers *et al.* (2003) were included for comparative purposes (Table 2). *Mycosphaerella africana* Crous & Wingf., a genus known to be closely related to *Botryosphaeria* spp., was used as a monophyletic outgroup taxon to root the phylogenetic tree. The stringency of the branch nodes were tested using Markov Chain Monte Carlo Algorithms (MCMC) (Larget & Simon 1999) in Bayesian Analysis (Lutzoni, Pagel & Reeb 2001). Random trees were obtained through 100 000 generations with every 10th tree sampled. The first 1500 trees were discarded as the burnin period. A general time reversal model was used and four MCMC chains were run simultaneously in the analysis. The sampled trees were summarised in a consensus tree showing posterior probabilities of the branches.

Pathogenicity

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

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

RESULTS

Disease symptoms and collection of samples

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

Morphological characterisation

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

Restriction Fragment Length Polymorphisms (RFLPs)

Digestion of isolates with restriction enzyme *Cfo* I supported the separation of isolates into two groups (Fig. 2). Thus, all but one isolate from South Africa showed banding patterns corresponding to those of *B. parva*, with bands at 121 bp and 182 bp. A single isolate produced a banding pattern that could not be assigned to a species, but

corresponded with patterns produced by *F. indigoticum* Jacobs, Slippers & Wingf. (prov. nom.), *Dothiorella mangiferae* Syd. & P. Syd. (Slippers *et al.* 2001), *F. aesculi* Corda and *F. bacilliforme* Jacobs, Slippers & Wingf. (prov. nom.) with fragment sizes of 121 bp, 148 bp and 182 bp respectively (Jacobs 2002).

DNA sequencing and analyses

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

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

Pathogenicity

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

DISCUSSION

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

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

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

indicate that this tree is not a preferred host of the fungus, but it also might be related to the relatively small sample of *Botryosphaeria* isolates collected in this study.

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

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

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

Both *Botryosphaeria* spp. collected in this study were isolated from symptomatic as well as asymptomatic tissue. The single *D. mangiferae* isolate was isolated from asymptomatic tissue and various isolates of *B. parva* were also from healthy tree parts. This indicates that both species are able to exist as latent pathogens. This is consistent

with the biology of other *Botryosphaeria* spp. that are known to exist as endophytes and latent pathogens (Toole 1963, Schoeneweiss 1965, Neely 1968, Pusey 1989, Ramos *et al.* 1991, Johnson 1994, Johnson *et al.* 1992, Smith *et al.* 1994, Stanoz *et al.* 1997). The endophytic nature of the fungi on these hosts could have facilitated their spread to new environments, as they would have been difficult to detect using typical quarantine systems.

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

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

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

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

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

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

[a]CMW represents the culture collection of the Forestry and Agricultural Biotechnology Institute ATCC represents the American Type Culture Collection.

[b] Sequences obtained from GenBank.

[c] Sequences generated in this study.

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

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

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

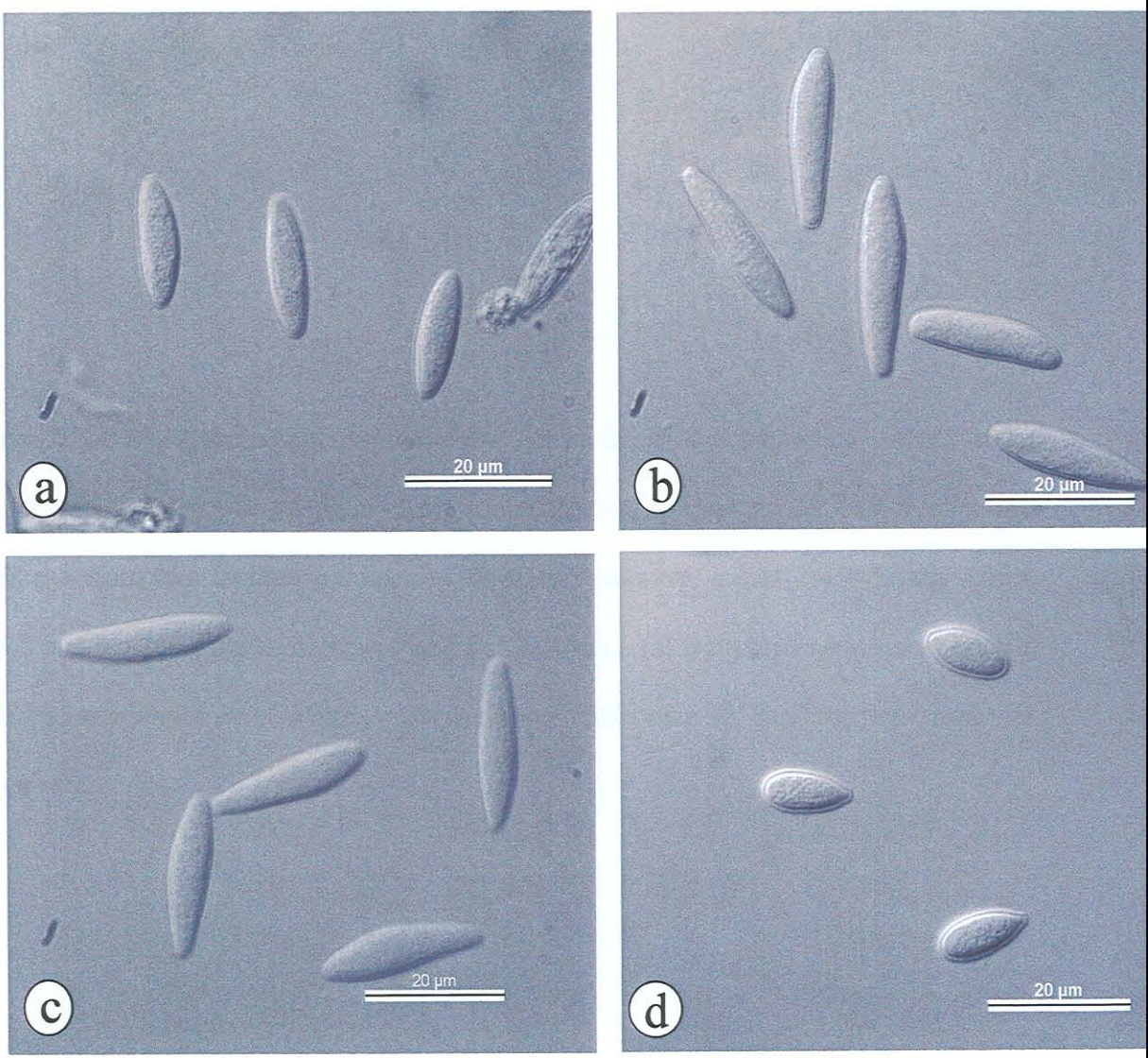


Figure 2. A 3% Agarose-ethidium bromide gel of the different banding paterns of the *Cfo* I digested ITS amplicons. Lane 1 represents the 100 bp marker. Lanes 2-8 represents amplicons from two isolates per species from each country. Lanes 2-4 = South Africa, lanes 5 and 6 = New Zealand, lanes 7 and 8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

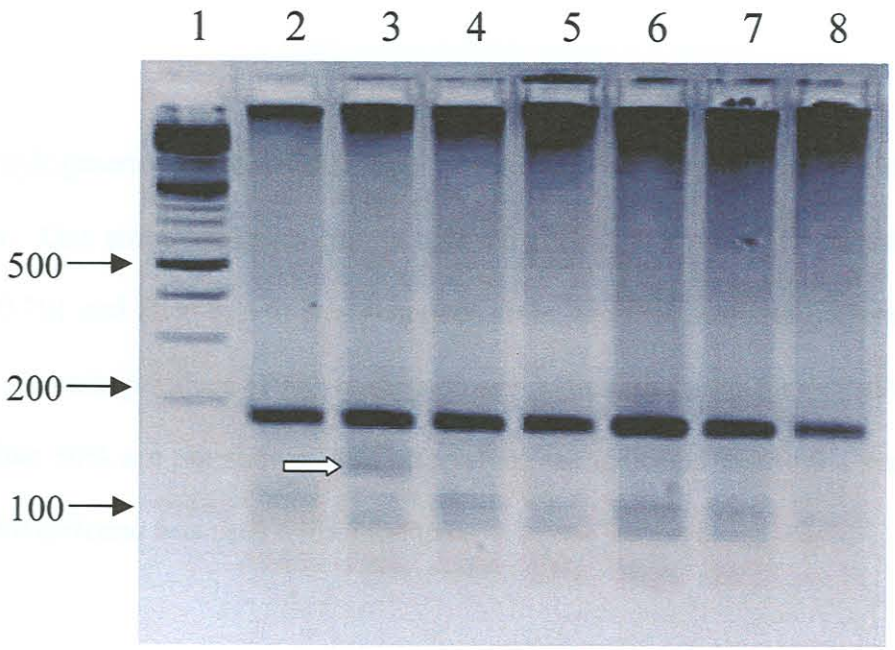


Figure 3. Phylogenetic tree generated from a data set including ITS and 5.8S gene sequence data. One strict consensus tree (length of tree = 217 steps, CI = 0.806, RI = 0.869, RC = 0.701 and HI = 0.194) was generated from heuristic searches performed on the data set. Bootstrap values (1000 replicates)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

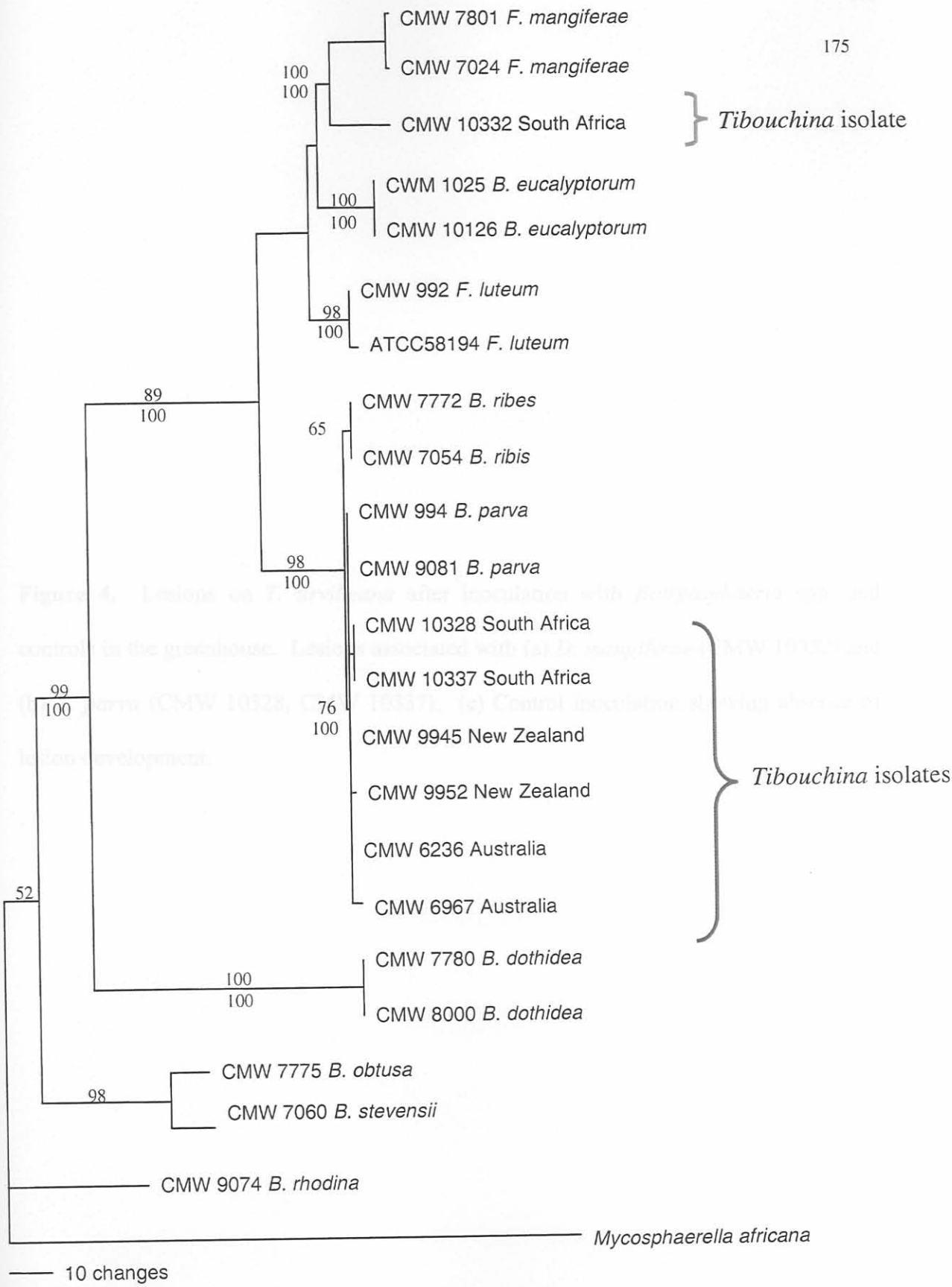


Figure 4. Lesions on *T. urvilleana* after inoculation with *Botryosphaeria* spp. and controls in the greenhouse. Lesions associated with (a) *D. mangiferae* (CMW 10332) and (b) *B. parva* (CMW 10328, CMW 10337). (c) Control inoculation showing absence of lesion development.

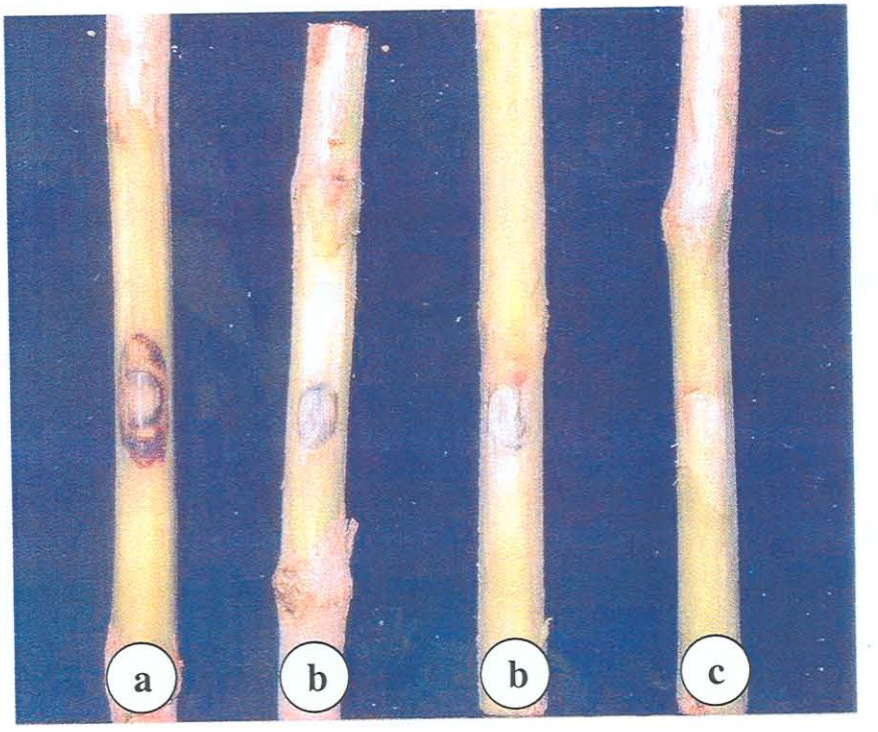
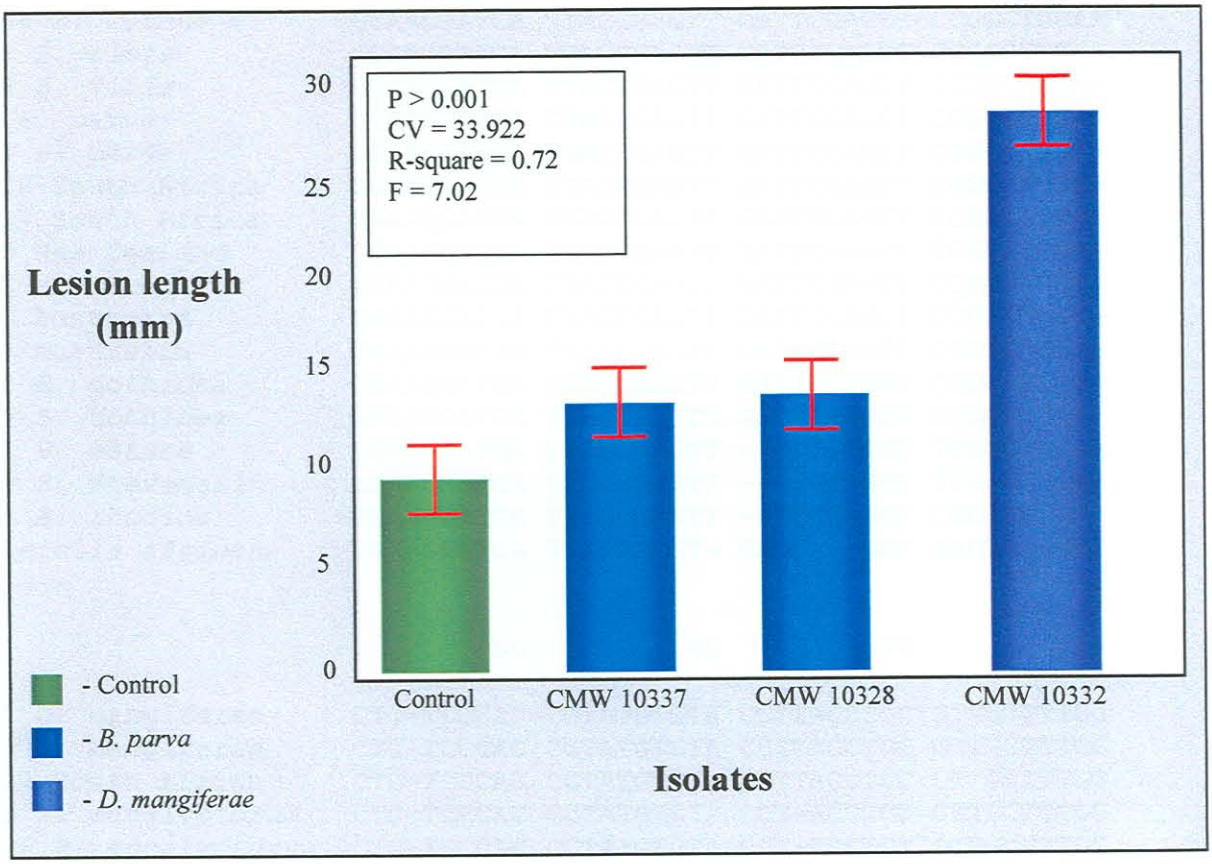


Fig. 5. Bar chart indicating the average lesion lengths in millimetres resulting from inoculation trials of *B. parva* and *D. mangiferae* on *Tibouchina urvilleana*. Error bars indicate standard deviations of the data.



APPENDIX 1

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

                                  50         60         70         80
CMW 7801 D. mangiferae            CTC-TCCCAC CCTATGTGTA CCTTACCTCC GTTGCTTTGG
CMW 7024 D. mangiferae            CTC-TCCCAC CCTATGTGTA CCTTACCTCC GTTGCTTTGG
CMW 10322 South Africa            CTC-TCCCAC CCTATGTGTA CCTTACCTCC GTTGCTTTGG
CWM 1025 B. eucalyptorum          CTC-TCCCAC CCTATGTGTA CCT-ACCTCT GTTGCTTTGG
CMW 10126 B. eucalyptorum         CTC-TCCCAC CCTATGTGTA CCT-ACCTCT GTTGCTTTGG
CMW 992 F. luteum                 CTC-TCCCAC CCCATGTGTA CCT-ACCTCT GTTGCTTTGG
ATCC58194 F. luteum               CTC-TCCCAC CCCATGTGTA CCT-ACCTCT GTTGCTTTGG
CMW 7772 B. ribis                 CTC-TCCCAC CCAATGTGTA CCT-ACCTCT GTTGCTTTGG
CMW 7054 B. ribis                 CTC-TCCCAC CCAATGTGTA CCT-ACCTCT GTTGCTTTGG
CMW 994 B. parva                  CTC-TCCCAC CCAATGTGTA CCT-ACCTCT GTTGCTTTGG
CMW 9081 B. parva                 CTC-TCCCAC CCAATGTGTA CCT-ACCTCT GTTGCTTTGG
CMW 10328 South Africa            CTC-TCCCAC CCAATGTGTA CCT-ACCTCT GTTGCTTTGG
CMW 10337 South Africa            CTC-TCCCAC CCAATGTGTA CCT-ACCTCT GTTGCTTTGG
CMW 9945 New Zealand              CTC-TCCCAC CCAATGTGTA CCT-ACCTCT GTTGCTTTGG
CMW 9952 New Zealand              CTC-TCCCAC CCAATGTGTA CCT-ACCTCT GTTGCTTTGG
CMW 6236 Australia                CTC-TCCCAC CCAATGTGTA CCT-ACCTCT GTTGCTTTGG
CMW 6967 Australia                CTC-TCCCAC CCAATGTGTA CCT-ACCTCT GTTGCTTTGG
CMW 7780 B. dothidea              -TCCTCCCAC CCTTTGTGTA CCT-ACCTCT GTTGCTTTGG
CMW 8000 B. dothidea              -TCCTCCCAC CCTTTGTGTA CCT-ACCTCT GTTGCTTTGG
CMW 7775 B. obtusa                -TC-TCCCAC CCTTTGTGAA CAT-ACCTCT GTTGCTTTGG
CMW 7060 B. stevensii             -TC-TCCCAC CCTTTGTGAA CAT-ACCTCT GTTGCTTTGG
CMW 9074 B. rhodina               CTC-TCCCAC CCTTTGTGAA CGT-ACCTCT GTTGCTTTGG
Mycosphaerella africana           C---TCCAAC CCTTTGTGAA CCA-A-CTCT GTTGCTTCGG
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```
                                    90         100        110        120

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

                                    130        140        150        160

CMW 7801 D. mangiferae              CTGG--CCA- GCGC---CCG CCAGAGGACC ACAAAACTCC
CMW 7024 D. mangiferae              CTGG--CCA- GCGC---CCG CCAGAGGACC ACAAAACTCC
CMW 10322 South Africa              CTGG--CCA- GCGC---CCG CCAGAGGACC ACAAAACTCC
CWM 1025 B. eucalyptorum            CTGG--CCA- GCGT---CCG CCAGAGGACC ACAAAACTCC
CMW 10126 B. eucalyptorum           CTGG--CCA- GCGT---CCG CCAGAGGACC ACAAAACTCC
CMW 992 F. luteum                   CCGG--CCA- GCGC---CCG CCAGAGGACC ACAAAACTCC
ATCC58194 F. luteum                 CCGG--CCA- GCGC---CCG CCAGAGGACC ACAAAACTCC
CMW 7772 B. ribis                   CTGG--CCA- GCGC---CCG CCAGAGGACC ATAAAACTCC
CMW 7054 B. ribis                   CTGG--CCA- GCGC---CCG CCAGAGGACC ATAAAACTCC
CMW 994 B. parva                    CTGG--CCA- GCGC---CCG CCAGAGGACC ATAAAACTCC
CMW 9081 B. parva                   CTGG--CCA- GCGC---CCG CCAGAGGACC ATAAAACTCC
CMW 10328 South Africa              CTGG--CCA- GCGC---CCG CCAGAGGACC ATAAAACTCC
CMW 10337 South Africa              CTGG--CCA- GCGC---CCG CCAGAGGACC ATAAAACTCC
CMW 9945 New Zealand                CTGG--CCA- GCGC---CCG CCAGAGGACC ATAAAACTCC
CMW 9952 New Zealand                CTGG--CCA- GCGC---CCG CCAGAGGACC ATAAAACTCC
CMW 6236 Australia                  CTGG--CCA- GCGC---CCG CCAGAGGACC ATAAAACTCC
CMW 6967 Australia                  CTGG--CCA- GCGC---CCG CCAGAGGACC ATAAAACTCC
CMW 7780 B. dothidea                GTGG--CCA- GCGC---CCG CCAGAGGACC ATCAAACTCC
CMW 8000 B. dothidea                GTGG--CCA- GCGC---CCG CCAGAGGACC ATCAAACTCC
CMW 7775 B. obtusa                  CCCC--CGC- GCGCTTTCCG CCAGAGGACC TTCAAACTCC
CMW 7060 B. stevensii               CCCC--CCGT GCGCTT-CCG CCAGAGGACC TTCAAACTCC
CMW 9074 B. rhodina                 ---------- -CGG---CCG CCAAAGGACC TTCAAACTCC
Mycosphaerella africana             CGGCCC---- -------CCG ---GAGGT-C ATCAAA-CAC
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                                  170        180        190        200

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

                                  210        220        230        240

CMW 7801 D. mangiferae            CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
CMW 7024 D. mangiferae            CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
CMW 10322 South Africa            CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
CWM 1025 B. eucalyptorum          CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
CMW 10126 B. eucalyptorum         CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
CMW 992 F. luteum                 CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
ATCC58194 F. luteum               CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
CMW 7772 B. ribis                 CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
CMW 7054 B. ribis                 CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
CMW 994 B. parva                  CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
CMW 9081 B. parva                 CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
CMW 10328 South Africa            CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
CMW 10337 South Africa            CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
CMW 9945 New Zealand              CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
CMW 9952 New Zealand              CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
CMW 6236 Australia                CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
CMW 6967 Australia                CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
CMW 7780 B. dothidea              CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
CMW 8000 B. dothidea              CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
CMW 7775 B. obtusa                CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
CMW 7060 B. stevensii             CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
CMW 9074 B. rhodina               CTAAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
Mycosphaerella Africana           C-AAAACTTT CAACAACGGA TCTCTTGGTT CTGGCATCGA
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                                  250        260        270        280
CMW 7801 D. mangiferae            TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 7024 D. mangiferae            TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 10322 South Africa            TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CWM 1025 B. eucalyptorum          TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 10126 B. eucalyptorum         TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 992 F. luteum                 TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
ATCC58194 F. luteum               TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 7772 B. ribis                 TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 7054 B. ribis                 TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 994 B. parva                  TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 9081 B. parva                 TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 10328 South Africa            TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 10337 South Africa            TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 9945 New Zealand              TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 9952 New Zealand              TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 6236 Australia                TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 6967 Australia                TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 7780 B. dothidea              TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 8000 B. dothidea              TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 7775 B. obtusa                TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 7060 B. stevensii             TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
CMW 9074 B. rhodina               TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA
Mycosphaerella africana           TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA

                                  290        300        310        320
CMW 7801 D. mangiferae            GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 7024 D. mangiferae            GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 10322 South Africa            GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CWM 1025 B. eucalyptorum          GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 10126 B. eucalyptorum         GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 992 F. luteum                 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
ATCC58194 F. luteum               GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 7772 B. ribis                 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 7054 B. ribis                 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 994 B. parva                  GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 9081 B. parva                 GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 10328 South Africa            GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 10337 South Africa            GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 9945 New Zealand              GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 9952 New Zealand              GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 6236 Australia                GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 6967 Australia                GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 7780 B. dothidea              GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 8000 B. dothidea              GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 7775 B. obtusa                GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 7060 B. stevensii             GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
CMW 9074 B. rhodina               GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
Mycosphaerella africana           GAATTCAGTG AATCATCGAA TCTTTGAACG CACATTGCGC
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                                330        340        350        360

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

                                370        380        390        400

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

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

                                450        460        470        480

CMW 7801 D. mangiferae          CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 7024 D. mangiferae          CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 10322 South Africa          CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CWM 1025 B. eucalyptorum        CTTG---CCT CAAGCGTAGT AGAAAT-CAC --CTCGCTTT
CMW 10126 B. eucalyptorum       CTTG---CCT CAAGCGTAGT AGAAAT-CAC --CTCGCTTT
CMW 992 F. luteum               CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
ATCC58194 F. luteum             CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 7772 B. ribis               CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 7054 B. ribis               CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 994 B. parva                CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 9081 B. parva               CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 10328 South Africa          CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 10337 South Africa          CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 9945 New Zealand            CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 9952 New Zealand            CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 6236 Australia              CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 6967 Australia              CTTG---CCT CAAGCGTAGT AGAAAA-CAC --CTCGCTTT
CMW 7780 B. dothidea            CTTG---CCT CAAGCGTAGT AGAACA-TAC ATCTCGCTTC
CMW 8000 B. dothidea            CTTG---CCT CAAGCGTAGT AGAACA-TAC ATCTCGCTTC
CMW 7775 B. obtusa              GTTCAGCCCT CAAGCGTAGT AGAATA-CAC --CTCGCTTT
CMW 7060 B. stevensii           GTTCAGCCCT CAAGCGTAAT AGAATA-CAC --CTCGCTTT
CMW 9074 B. rhodina             GTTCAGCCCT CAAGCGTAGT AGAATA-CAC --CTCGCTTT
Mycosphaerella africana         GTTCGTCTC- TAAGCGTTGT GGCATATATT ---TCGCT--
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                                  490         500         510         520
CMW 7801 D. mangiferae            GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTTT-GAATA
CMW 7024 D. mangiferae            GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTTT-GAATA
CMW 10322 South Africa            GGAGCGCACG GAGTCACGCG GCGGACGAAC CTTT-GAATA
CWM 1025 B. eucalyptorum          GGAGCGCATG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 10126 B. eucalyptorum         GGAGCGCATG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 992 F. luteum                 GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
ATCC58194 F. luteum               GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 7772 B. ribis                 GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 7054 B. ribis                 GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 994 B. parva                  GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 9081 B. parva                 GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 10328 South Africa            GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 10337 South Africa            GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 9945 New Zealand              GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 9952 New Zealand              GGAGCGCACG GCGTCGCCCG CCGGACGAAA CTT-TGAAT-
CMW 6236 Australia                GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 6967 Australia                GGAGCGCACG GCGTCGCCCG CCGGACGAAC CTT-TGAAT-
CMW 7780 B. dothidea              GGAGCGCAGG GCGTCGCCCG CCGGACGAAC CTTCTGAAC-
CMW 8000 B. dothidea              GGAGCGCAGG GCGTCGCCCG CCGGACGAAC CTTCTGAAC-
CMW 7775 B. obtusa                GGAGCGGTTG GCGTCGCCCG CCGGACGAAC CTTCTGAAC-
CMW 7060 B. stevensii             GGAGCGGTTG GCGTCGCCCG CCGGACGAAC CTTCTGAAC-
CMW 9074 B. rhodina               GGAGCGGTTG GCGTCGCCCG CCGGACGAAC CTTCTGAAC-
Mycosphaerella africana           GAAAGAGTTC GGGACGGCTT TTGGCCG--- -TT-AAATC-

                                  530         540
CMW 7801 D. mangiferae            TTTTTTCTCA A-GGTTGACC
CMW 7024 D. mangiferae            TTTTTTCTCA A-GGTTGACC
CMW 10322 South Africa            TTTTTTCTCA A-CGTTGACC
CWM 1025 B. eucalyptorum          -T-TTTCTCA A-GGTTGACC
CMW 10126 B. eucalyptorum         -T-TTTCTCA A-GGTTGACC
CMW 992 F. luteum                 -T-TTTCTCA A-GGTTGACC
ATCC58194 F. luteum               -TATTTCTCA A-GGTTGACC
CMW 7772 B. ribis                 -TATTTCTCA A-GGTTGACC
CMW 7054 B. ribis                 -TATTTCTCA A-GGTTGACC
CMW 994 B. parva                  -TATTTCTCA A-GGTTGACC
CMW 9081 B. parva                 -TATTTCTCA A-GGTTGACC
CMW 10328 South Africa            -TATTTCTCA A-GGTTGACC
CMW 10337 South Africa            -TATTTCTCA A-GGTTGACC
CMW 9945 New Zealand              -TATTTCTCA A-GGTTGACC
CMW 9952 New Zealand              -TATTTCTCA A-GGTTGACC
CMW 6236 Australia                -TATTTCTCA A-GGTTGACC
CMW 6967 Australia                -TATTTCTCA A-GGTTGACC
CMW 7780 B. dothidea              -T-TTTCTCA A-GGTTGACC
CMW 8000 B. dothidea              -T-TTTCTCA A-GGTTGACC
CMW 7775 B. obtusa                -T-TTTCTCA A-GGTTGACC
CMW 7060 B. stevensii             -T-TTTCTCA A-GGTTGACC
CMW 9074 B. rhodina               -T-TTTCTCA A-GGTTGACC
Mycosphaerella africana           ---TTTCTTA AAGGTTGACC
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SUMMARY

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

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

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

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

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

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

This dissertation reports on two new hosts for important *Eucalyptus* pathogens, a new species of *Endothelia*, as well as presenting a first report of an important agricultural pathogen in South Africa. In a relatively limited study, I hope to have highlighted the enormous threat that the unchecked movement of any plant material has to commercial forestry and agricultural operations. This is especially clear from the discovery of an

important mango pathogen on a host in the Myrtales. Clearly many pathogens do not spread only on plants closely related to their known hosts. In order to ensure the sustainability of commercial forestry and agricultural crops, it is crucially important to drastically improve quarantine procedures in many countries. To effectively achieve this goal, fungal identification techniques will need to be improved. Furthermore, education of people regarding the threat of uncontrolled plant movement will also need to substantially augmented.