Characterisation of *Botryosphaeria* species from mango in South Africa

Submitted by

René Jacobs

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

MAGISTER SCIENTIAE

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

July 2002

Supervisors: Prof. L. Korsten

Mr. B. Slippers

Prof. M.J. Wingfield

DECLARATION

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

René Jacobs

July 2002

TABLE OF CONTENTS

ACKNOWLEDGEMENTS 5

PREFACE 6

CHAPTER 1

THE INFLUENCE AND CONTROL OF MANGO DISEASES, WITH SPECIFIC REFERENCE TO DISEASES CAUSED BY *BOTRYOSPHAERIA* SPECIES 9

- INTRODUCTION 10
- *BOTRYOSPHAERIA* DISEASES OF MANGO 12
- TAXONOMY OF *BOTRYOSPHAERIA* SPECIES THAT CAUSE DISEASES OF MANGO 17
- CONTROL STRATEGIES 23
- CONCLUSIONS 27
- REFERENCES 29

CHAPTER 2

IDENTIFICATION AND CHARACTERISATION OF *BOTRYOSPHAERIA* SPECIES FROM MANGO IN SOUTH AFRICA 38

- ABSTRACT 39
- INTRODUCTION 40
- MATERIALS AND METHODS 42
- RESULTS 47
- TAXONOMY 52
- DISCUSSION 55
- REFERENCES 59
- APPENDIX 72

CHAPTER 3

PATHOGENICITY OF *BOTRYOSPHAERIA* SPECIES ON TWO MANGO CULTIVARS IN SOUTH AFRICA 94

- ABSTRACT 95
- INTRODUCTION 96
- MATERIALS AND METHODS 99
- RESULTS 101
- DISCUSSION 104
- REFERENCES 107

CHAPTER 4

DEVELOPMENT AND TESTING OF A PCR-RFLP IDENTIFICATION SYSTEM FOR *BOTRYOSPHAERIA* SPECIES FROM MANGO 120

- ABSTRACT 121
- INTRODUCTION 122
- MATERIALS AND METHODS 124
- RESULTS 126
- DISCUSSION 127
- REFERENCES 131

GENERAL DISCUSSION 149

SUMMARY 157

OPSOMMING 160

ACKNOWLEDGEMENTS

This thesis is dedicated to my husband, Francois for your unconditional love and support. You help me fulfill all my dreams and aspirations. Thank you for everything!

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

'When the going gets tough, the tough gets going', but my parents have kept me going all the time and I thank you from the bottom of my heart.

Bernard Slippers for your enthusiasm, guidance, encouragement and endless support. You have made me see the molehill in every situation when I could only see the mountain in front of me and I will be eternally grateful for that.

Mike Wingfield and Lise Korsten for helping me keep 'my eye on the ball' and for your willing support, guidance and the reviewing of this thesis.

Fanus Swart (DuRoi-UP alliance) for your time, effort and field knowledge. It is much appreciated.

For financial support, I would like to thank the National Research Foundation (NRF), South African Mango Growers Association (SAMGA) and the University of Pretoria and THRIP.

Most importantly, I thank my heavenly Farther for His eternal love and light upon my path.

PREFACE

Botryosphaeria spp. are well-known endophytes and opportunistic pathogens of many woody hosts, including mango (*Mangiferae indica* Linn.). In South Africa, *Botryosphaeria* spp. commonly cause cankers, twig die-back, blossom blight and fruit rot of mango, which result in significant economic losses annually. Limited control is available for these diseases, which is partly due to the lack of knowledge concerning the identity and epidemiology of the causal agent. There is, however, confusion regarding the taxonomy of the causal agent. This thesis aims to address the current lack of knowledge and problems with taxonomy, efficient identification and etiology of *Botryosphaeria* spp. on mango in South Africa.

In the first chapter, the literature concerning *Botryosphaeria* spp. that occur on mango is reviewed. Information is summarized and reviewed under three main themes, namely taxonomy, epidemiology and current control of *Botryosphaeria* diseases of mango. The taxonomy of *Botryosphaeria* spp. is currently in disarray due to difficulties in identifying species based on morphological characteristics. Various species have been implicated as causal agents of mango diseases previously. Due to the discrepancies over the taxonomic status of the pathogen, chritical epidemiological issues and aspects could not be clarified, thus, making the development of an effective disease management strategy difficult. This has a complicating effect on the control of the pathogen, which is currently achieved with limited success. The need for a revision of the taxonomy of the *Botryosphaeria* pathogens involved in mango diseases is emphasised, as correct identification of the pathogen is the first step in developing effective control strategies and quarantine regulations.

The taxonomy of *Botryosphaeria* is currently confusing, since various species, namely *Hendersonia creberrima*, *Dothiorella dominicana*, *Nattrassia mangiferae* and *Lasiodiplodia theobromae*, are names of fungi assumed to cause various diseases on mango. Most of these taxa are, however, not validly described and the identity of these species is suspect. These pathogens were previously identified by using only anamorph morphological characteristics. The use of morphology alone is, however, questionable due to the overlap between some species. In Chapter two, traditional morphological characterisation was combined with molecular sequence data to identify the *Botryosphaeria* spp. from mango in South Africa. This was compared and related to species affecting mango in other parts of the world.

Confirming the pathogenicity of the different *Botryosphaeria* spp. occurring on mango in South Africa will be important for implementing disease control and quarantine strategies. Little information is currently available regarding the pathogenicity and role of the four *Botryosphaeria* spp. identified from mango in South Africa. Two of these *Botryosphaeria* spp. are newly described and their pathogenicity has not been determined. The objective in the third chapter of this thesis was to utilise apple and potted tree inoculation trials, to determine pathogenicity of all *Botryosphaeria* spp. from mango in South Africa. The identity of the most and least pathogenic *Botryosphaeria* spp. on a resistant and susceptible commercial mango cultivar in South Africa was also investigated.

Identification of *Botryosphaeria* spp. is focused on combined morphological and sequence data. Morphological data is not always reliable and the use of sequence data in species identification of large numbers of isolates is impractical. For this reason, a PCR-RFLP technique was considered in Chapter four as an alternative approach to achieve rapid and reliable identifications for *Botryosphaeria* spp. from mango. This technique was then used to

identify a large number of isolates collected during a survey of *Botryosphaeria* spp. from South Africa and Australia.

CHAPTER 1

THE INFLUENCE AND CONTROL OF MANGO DISEASES, WITH SPECIFIC REFERENCE TO DISEASES CAUSED BY *BOTRYOSPHAERIA* SPECIES

INTRODUCTION

The mango (*Mangifera indica* L.) belongs to the dicotyledenous family *Anacardiaceae.* This tree is indigenous to India and southern Asia and originated from the Indian/Burmese border region where it has been cultivated for many centuries (Kwee & Chang, 1985). Today, mangoes are cultivated in most tropical and subtropical parts of the world where they are commonly eaten fruits (Prakash & Srivastava, 1987; Schroeder, 1990). Countries that cultivate mangoes commercially, but primarily for local consumption, include India, Pakistan, Indonesia, Mexico, Brazil and the Philippines. The most important mango exporting countries are Australia, South Africa, Israel, Egypt and the United States of America (Johnson, 1992).

The conditions under which mango trees are cultivated, often favour disease development. Mango trees are able to adapt to harsh environments that are normally not conducive to growth of other fruit trees (Wolstenholme *et al.*, 1995). These sub-optimal environmental conditions, however, often cause stress, which reduces the tree's ability to elicit an active defense response to pathogen infection and invasion (Schoeneweiss, 1984). Mango trees, therefore, experience different levels of stress in different environments, which together with varying levels of pathogen inoculum pressure, can trigger symptom development and result in disease expression (Finnemore, 2000).

In South Africa, as with many other countries, mango fruit mainly develop and ripen during the rainy season when prevailing weather conditions are warm with a high humidity, which makes fruit prone to attack by various microorganisms (Reckhaus, 1987; Ramos *et al.*, 1991; Lonsdale, 1993a). A wide diversity of pathogens attack various parts of nursery- and adult mango trees. Anthracnose, blossom blight, powdery mildew, flower malformation, cankers,

twig dieback and bacterial black spot are some of the main problems faced by mango producers world-wide (Prakash & Srivastava, 1987; Wolstenholme *et al.*, 1995). Of the diseases, those caused by fungi contribute the most to production and economic losses (Singh, 1960; Prakash & Srivastava, 1987; Johnson, 1992).

Fungi generally affect mango production through disease development and *Botryosphaeria* spp. are amongst the most common and destructive of these fungi (Johnson, 1992). Anamorphs of *Botryosphaeria* spp., commonly associated with mango, are *Dothiorella* spp., *Nattrassia* spp., *Fusicoccum* spp. and *Lasiodiplodia* spp. (Ramos *et al.*, 1991; Darvas, 1991; Johnson *et al.*, 1991; Johnson, 1992). There is, however, great confusion regarding the taxonomy, classification and identification of these anamorph species (Johnson, 1992; Jacobs & Rehner, 1998; Crous & Palm, 1999). The morphological criteria for identification is generally not enough to differentiate between these species (Jacobs & Rehner, 1998; Denman *et al.*, 2000). For this reason, the naming, synonymy, occurrence and importance of these anamorph species of *Botryosphaeria* from mango have not been clarified yet. Such clarification is, however, needed to assess pathogen epidemiology and efficient future control.

There is a lack of effective control strategies for diseases such as those caused by *Botryosphaeria* spp. associated with mango trees and fruit (Johnson *et al.*, 1991; Peterson *et al.*, 1991; Johnson, 1992), which poses a serious threat to the entire industry. To address the problems with control of *Botryosphaeria* diseases, there is a need to understand the taxonomy and biology of these fungi. This information is also crucial to develop quarantine strategies for preventing further spread of this pathogen to areas where it does not occur. The aim of this review is, therefore, to assess the current information regarding the epidemiology, identification and taxonomy, as well as the control of *Botryosphaeria* diseases.

BOTRYOSPHAERIA DISEASES OF MANGO

Botryosphaeria spp. are mainly saprophytic and endophytic, but occasionally cause extensive disease symptoms on a variety of woody hosts (Von Arx, 1987; Schoeneweiss, 1984; Denman *et al.*, 2000). These species infect through natural openings and wounds, but the infection is usually latent. The disease symptoms are commonly expressed when hosts are stressed with inactivated natural host defence mechanisms (Schoeneweiss, 1979).

Botryosphaeria spp. can attack different parts of the mango tree and fruit, resulting in pre- and postharvest diseases. The pathogen colonizes the blossom as an endophyte, often resulting in blossom blight. The infected axes, florets and fruitlets shrivel and become necrotic. If environmental conditions are favourable for the pathogen, it moves down the main axis and colonize stem tissue, causing twig dieback and extensive cankering of stems and trunks. Infection of unripe fruit in orchards remains latent until fruit start to ripen after harvest. At this stage, the pathogen invasion continues and fruit is colonized, giving rise to a soft brown rot (SBR), a typical body rot and stem end rot (SER) (Johnson *et al.*, 1992; Lonsdale, 1993b).

Preharvest diseases

Blossom blights are common in most mango-growing countries (Kwee & Chang, 1985). Inflorescences are extensively colonised by *Botryosphaeria* species, especially during the rainy season (Darvas, 1991). The early symptoms of blossom blight are inflorescence wilting and production of minute black spots, which later enlarge and coalesce, resulting in shedding of flowers and shriveling and drying of the flower axes (Lonsdale, 1992; Lonsdale, 1993a). The severity of blossom blight is greatly dependent on environmental factors contributing to

induced stress on trees during inflorescence development (Kwee & Chang, 1985; Lonsdale, 1992; Lonsdale, 1993a).

Twig dieback poses a major preharvest problem in various mango producing countries. Infected twigs and stems turn brown, dry out and become necrotic from the tips, backwards. The pathogen most frequently associated with twig dieback of mangoes in Australia closely resembles *Botryosphaeria dothidea* (Johnson, 1992). Ramos *et al.* (1991) investigated mango tip dieback in Florida and found the primary organism responsible to be *Botryosphaeria ribis* Gross. & Duggar or the anamorphs associated with it.

Cankers usually appear as longitudinal cracks in the bark with a brown to black discoloration of the infected area. Latex exudation from the collars is seen in severe cases (Jayasinghe & Silva, 1994). Developing cankers often have a zonate pattern of dark and lighter regions (Maas & Uecker, 1984). Cankerous lesions often develop around and beneath the nodes and later spread above this area (Jayasinghe & Silva, 1994). Conidiomata of the fungus are scattered sub-epidermally throughout the cankers, becoming erumpent with exposed ostioles.

Postharvest infections

A serious threat to the mango industry is postharvest decay. Postharvest losses may be due to various factors, including physiological changes, physical damage, chemical injury or residues and pathological decay (Swart, 1999). When anthracnose, caused by *Colletotrichum gloeosporiodes* (Penzig) Penzig & Sacc. is well controlled, the most economically important postharvest decay of mango in various countries is SER or SBR (Johnson *et al.*, 1991; Sanchote, 1991; Johnson, 1992; Johnson & Sanchote, 1994; Lonsdale, 1993b).

Stem end rot and SBR has been reported from all major mango-growing regions of the world. The term "stem end rot" has been used to describe lesions that develop at the pedicel end of the fruit after harvest, eventually leading to complete fruit decay (Johnson *et al.*, 1991). On the body of mango fruit, decay caused by *Botryosphaeria* spp. is referred to as SBR, which is in essence the same disease as SER. The variation in the incidence of SER and SBR can be related to overall tree health and age, pruning history, fruit maturity at harvest, preharvest spray schedules, postharvest handling and storage conditions and postharvest fungicidal treatments (Johnson, 1992; Johnson & Sanchote, 1994; Wolstenholme & Whiley, 1995; Cooke *et al.*, 1998; Sanchote, 1993b).

Fruit rot lesions appear as water-soaked tissue irregularly radiating from the stem ends or infected areas on the fruit body, which quickly darken and coalesce into irregular circular lesions. Superficial white fungal mycelium may be seen protruding from the pedicel end of fruit. A watery fluid drains from the stem end or ruptures of the fruit surface. As fruit decay and begin to desiccate, fungal fruiting bodies is observed on the surfaces in some instances (Darvas, 1991; Sanchote, 1991; Johnson, 1992; Johnson *et al.*, 1992; Lonsdale, 1993b).

Botryosphaeria spp. can quickly spread from infected to healthy adjacent fruit in a carton (Kruger *et al.*, 1995). This causes significant problems for exporters that usually only detect rotten fruit at the end of the export chain, resulting in significant financial losses (Lonsdale, 1993a; Saaiman, 1996). Since mangoes from South Africa are exported by sea to mainly European countries, fruit are exposed to long cold storage conditions. This makes effective pre- and postharvest control of the pathogen essential to minimize losses at the retail end.

Epidemiology

To formulate an effective control strategy for diseases caused by *Botryosphaeria* spp. it is essential to understand the infection processes and epidemiology of the pathogen (Johnson & Sanchote, 1994). The exact mode of entry of *Botryosphaeria* on mango trees is not known, but natural openings and wounds caused by pruning, insects and sunburn is considered the most likely route of infection (Maas & Uecker, 1984; Johnson 1992; Johnson, 1994; Lonsdale, 1992). Fruit invasion by the pathogen is through the stem ends causing latent infections. After latency is broken, systemic spread of the pathogen can occur (Johnson, 1992; Lonsdale, 1993b). During ripening, levels of natural anti-fungal substances in the fruit are depleted to an extent where the pathogen can easily invade the fruit peel and tissue (Prusky & Keen, 1993), leading to SER or SBR symptom development.

High humidity and movement of water is generally responsible for the release and dispersal of *Botryosphaeria* conidia from limbs of various woody hosts (Weaver, 1979; Sutton, 1981; Creswell & Milholland, 1988). Creswell and Milholland (1988) found that conidia are present in rainwater all year, indicating the importance of rain as a mechanism of pathogen spread. Fruiting structures of *Botryosphaeria* spp. are often produced on old mango tree litter, enabling easy spore dispersal by means of rain splash and wind. As the ostioles open, conidia are easily released and can be spread by splashing raindrops, wind and direct contact with uninfected host tissue (Sutton, 1981; Creswell & Milholland, 1988; Sutton & Davidson, 1983; Maas & Uecker, 1984; Johnson, 1992). Darvas (1991) and Johnson (1992) also commonly detected stem end rot fungi in dead twigs, branches and fallen fruit. The teleomorph stage of the fungus is, however, not often encountered, probably because orchard sanitation programs include the regular removal of fallen twig and leaf litter under trees (Sutton, 1981; Pusey, 1989).

Botryosphaeria spp. can occur endophytically in healthy plant tissue and in plant debris and soil. They can colonise plant tissue through stomata, lenticells and directly on stems (Maas & Uecker, 1984). In many hosts, invasion through lenticels leads to localized infections manifested as sunken necrotic lesions and gum exudation on trunks and limbs. The pathogen resides in lenticels and invades the cortical tissue beneath lenticels when moisture stress develops (Pusey, 1989). The pathogen also has the ability to invade the vascular system of woody hosts (Ramos *et al.*, 1991). Once the pathogen enters the vascular system, it moves quickly down the stem, but with slow lateral movement. Death of the portions above the stem canker may result from tyloses and mycelium clogging the xylem vessels (Maas & Uecker, 1984; Ramos *et al.*, 1991).

Botryosphaeria diseases of stems often follow stress conditions on the mango tree. Such stress is induced by various factors such as mineral deficiency, sunburn, hail, drought and freezing and other environmental factors (Pusey, 1989; Wene & Schoeneweiss, 1980; McPartland & Schoeneweiss, 1984; Schaffer *et al.*, 1988; Ramos *et al.*, 1991). Under these conditions, trees usually have low levels of resistance or tolerance and disease symptoms develop rapidly. McPartland and Schoeneweiss (1984) investigated the mechanism of plants to resist invasion by *Botryosphaeria* species on *Betula alba* and found that an increased frequency of swelling and bursting of fungal hyphal tips after infection occurs in unstressed plants, while little or no effects were observed on hyphae infecting stressed plants. This may be due to a reduction in calcium ions in stressed stems (McPartland & Schoeneweiss, 1984), since it has previously been demonstrated that calcium ions cause *in vitro* swelling and bursting of fungal hyphal tips (Dow & Rubery, 1975). This study indicate that unstressed stems have natural resistance to *Botryosphaeria*, which results from an active biochemical host defense response and that this mechanism is not active in stressed plants (McPartland & Schoeneweiss, 1984).

TAXONOMY OF *BOTRYOSPHAERIA* SPECIES THAT CAUSE DISEASES OF MANGO

The type species of the teleomorph genus *Botryosphaeria*, is *B. dothidea* Ces. & De Not (Sutton, 1980; Johnson, 1992). *Botryosphaeria dothidea* was first described by Cesati and De Notaris from *Fraxinus* sp. when the genus was established in 1863. The fungi treated under this genus have, however, undergone a number of changes since the initial description. Currently, the taxonomy of many species in this genus is unclear and is in serious need of review (Sivanesan, 1984; Rayachhetry *et al.*, 1996; Jacobs & Rehner, 1998; Denman *et al.*, 2000).

In culture and on diseased material, the anamorphs of *Botryosphaeria* is most frequently encountered. The features for species differentiation are often more distinct in the anamorph genera than the teleomorphs (Sutton, 1980; Jacobs & Rehner, 1998; Denman *et al.*, 2000). For this reason, the taxonomy of *Botryosphaeria* spp. largely depends on variation in the anamorph genera. The characters for identification of the anamorphs are, however, poorly described (Sutton, 1980; Morgan-Johnes & White, 1987; Denman *et al.*, 2000). Changes in conidial morphology with maturity also limits the identification process (Laundon, 1973; Rayachhetry *et al.*, 1996; Denman *et al.*, 2000).

Conidia obtained from mango tissue are mostly hyaline, single-celled, ellipsoid to fusoid and distinctly basally truncate (Ramos *et al.*, 1991). Formation of septa in germinating conidia has been reported for various species, but little is known concerning the factors that stimulate this process. Conidia of some species become bi-septate with the middle of the cells becoming darker with maturity, although this phenomenon is not always constant (Maas & Uecker,

1984; Pennycook & Sameuls, 1985). Due to the uncertainty concerning the taxonomic status of some of the anamorphs, many authors have chosen to use only the teleomorph name.

A detailed study of the *Botryosphaeria* spp. is long overdue and should include both morphological and molecular data (Jacobs & Rehner, 1998; Crous & Palm, 1999; Denman *et al.*, 2000; Zhou & Stanosz, 2001). Correct identification of pathogenic species provides the basis for an effective disease control strategy. Due to their importance and predominance on infected tissue, the taxonomy of the anamorphs of *Botryosphaeria* are discussed in detail in this review.

Anamorph taxonomy

Botryosphaeria produces anamorphs that have been variously assigned in the form-genera *Fusicoccum* Corda in Sturm., *Dothiorella* Sacc., *Diplodia* Fr. in Mont., *Lasiodiplodia* Ellis & Everh., *Sphaeropsis* Sacc. and *Phyllosticta* Pers. (Von Arx, 1987; Jacobs & Rehner, 1998). The anamorphs of *Botryosphaeria* commonly associated with mango fruit infection are *D. dominicana* Pet. et Cif., *D. mangiferae* H. et P. Syd. et But., *D.* 'long' (an unnamed *Dothiorella* sp.) and *L. theobromae* (Pat.) Griff. et Maubl., (Johnson, 1992). The identification and characterization of these anamorph species is generally based on differences in morphological characteristics.

The most important morphological characteristics separating *Botryosphaeria* anamorph genera are variation in pycnidia and conidia (Sutton, 1980). *Botryosphaeria* anamorphs can be separated in two distinct groups according to conidial colour. The one group includes genera with hyaline, narrow conidia and the other darker coloured, broader conidia (Jacobs & Rehner, 1998; Denman *et al.*, 2000; Zhou & Stanosz, 2001). It has thus been proposed that all

anamorphs of *Botryosphaeria* should reside in either *Fusicoccum* or *Diplodia* (Sutton, 1980; Maas & Uecker, 1984; Crous & Palm, 1999; Denman *et al.*, 2000).

(I) ***Dothiorella***

Dothiorella species are common on twigs and branches of woody plants and grasses (Von Arx, 1987). The status of the name *Dothiorella* has been in question for many years. Crous and Palm (1999) found, while comparing findings of Berkley (1860) and Saccardo (1884), that they evaluated and described the type of the genus *Dothiorella* on separate occasions. Only small differences were found between their findings (Crous & Palm, 1999). Berkley did not believe in separating the anamorph and teleomorph and treated this genus as *Botryosphaeria*. Saccardo, however, placed the emphasis on anamorphs and resurrected *Dothiorella* to its original state.

Crous and Palm (1999) challenged the validity of *Dothiorella* and synonimised the type species of *Dothiorella* with *Diplodia*. This synonymy was based on the finding that the conidiomata of *Dothiorella pyrenophora* Sacc., the type species of *Dothiorella*, are unilocular to multilocular and conidiophores are branched, septate, holoblastic and give rise to smooth or verriculous, brown, euseptate conidia. This made the *Dothiorella* type species indistinguishable from *Diplodia* (Crous & Palm, 1999). These findings emphasises that the taxa with hyaline or dark conidia, which was previously referred to as *Dothiorella,* needs careful re-evaluation for the correct taxonomic placement in *Diplodia* or *Fusicoccum* (Crous & Palm, 1999; Denman *et al.*, 1999).

Fungi resembling *Dothiorella* or *Fusicoccum* from mango have generally been placed in *Dothiorella.* Sutton (1980) and Morgan-Jones and White (1987) shared the view of Saccardo

that the name *F. aesculi* Corda was not missapplied to a group of fungi with hyaline, aseptate conidia, and that fungi classified as *Dothiorella,* should best reside in *Fusicoccum.* Johnson (1992) considered this view in detail based on Australian isolates from mango and suggested that *D. dominicana* fits the description of the *F. aesculi*, which is the anamorph of *B. dothidea* (Morug. Fr.) Ces. & de Not. Various authors suggested that other *Dothiorella* spp. should be re-evaluated and correctly incorporated in *Fusicoccum* (Sutton, 1980; Maas & Uecker, 1984; Johnson, 1992; Crous & Palm, 1999).

Some of the most important species recognized worldwide as causal agents of major pre- and postharvest losses in mango are *D. dominicana, D. mangiferae* and to a lesser extent *D. aromatica* (Johnson, 1992). The final taxonomic status of these species has not yet been clarified, but is currently being investigated (Slippers, personal communication). Because of the uncertain status of these names, they are used as per their original or translated description in this review (Table 1; p 37).

(II) ***Fusicoccum***

The genus *Fusicoccum* was first described in 1829 and the type species is *F. aesculi* Corda., but the status of *Fusicoccum* and the type has been the source of confusion for many years (Sutton, 1980; Maas & Uecker, 1984; Jacobs & Rehner, 1998; Crous & Palm, 1999; Zhou & Stanosz, 2001). Sutton's (1980) description of *Fusicoccum* suggested that it resides in the Coelomycetes with fusiform, hyaline, aseptate conidia, produced holoblastically in eustromatic conidiomata. He showed that the conidia of *Fusicoccum* are produced with a single precurrent proliferation. *Fusicoccum* was regarded as an appropriate genus for anamorphs of *B.ribis* Grossenb. & Dugg. (currently known as *B. parva*) and *B. dothidea* (Sutton, 1980; Denman *et al.*, 2000). Sutton's view of *Fusicoccum* was later shared by Maas

and Uecker (1984). Pennycook and Sameuls (1985) also agreed with this description, but stated that the original description was based on the immature state of the fungus, since all pycnidia examined were covered with host tissue. These authors also believe that most conidiogenous loci appear to produce only one holoblastic conidium. It was observed that older conidiogenous cells of *F. aesculi* were enteroblastic and proliferated precurrently at the same level. This observation was confirmed by Crous and Palm (1999).

Sutton (1980) examined Petrak's description of *Fusicoccum* (Petrak & Cifferi, 1930) and found that he referred to the *Fusicoccum*-like species as *Dothiorella*, citing the species as the conidial state of *B. berengeriana*. This view of Petrak is believed to have triggered the confusion regarding the taxonomy of *Fusicoccum*, *Dothiorella* and other *Botryosphaeria* anamorphs with hyaline conidia (Sutton, 1980; Denman *et al.*, 2000). The appropriate genus name for hyaline conidial anamorphs under *Botryosphaeria* should be *Fusicoccum* rather than *Dothiorella*, since the older name should take priority (Sutton, 1980; Johnson, 1992; Jacobs & Rehner, 1998; Crous & Palm, 1999; Denman *et al.*, 2000). According to further findings by Sutton (1980), the generic concept of *Fusicoccum* should be expanded to include septate, darker conidia, since *Fusicoccum* is an older name than *Diplodia,* which also includes *Botryosphaeria* anamorphs. Pennycook and Samuels (1985) examined Saccardo's specimen of *F. aesculi* and described three species of *Fusicoccum,* of which all three had conidiogenous cells proliferating precurrently, with the first formed conidia appearing to be formed holoblastically. They associated *F. aesculi* with the broad description of *Diplodia* except that *F. aesculi* was reportedly not becoming brown and septate with age. Crous and Palm (1999), however, re-evaluated the taxonomic status of *Botryosphaeria, Dothiorella* and *Fusicoccum* and provided a new description for the type of *F. aesculi* Corda (Table 1; p37).

(III) ***Nattrassia***

Nattrassia mangiferae (Nattrass) Sutton et Dyko is the only known species of this genus. The genus was first described from plum, apricot and apple isolates by Nattrass, but has since been reported from many woody hosts in various tropical and subtropical countries worldwide (Sutton & Dyko, 1989). The arthric synamorph is known as *Scytaldium dimidiatum* Pesante, and mainly causes dermatological disease in humans (Frankel & Rippon, 1989).

Sutton and Dyko (1989) examined differences between *Hendersonula toruloidea* Nattrass, *Fusicoccum eucalypti* da Camara, *Hendersonia cypia* Nattrass and *Dothiorella mangiferae* and reduced them to synonymy with *N. mangiferae* (Sutton & Dyko, 1989; Johnson, 1992). Johnson (1992), however, suggested that *Nattrassia* and *D. mangiferae* might be a synonym of *Fusicoccum.* This synonymy was justified based on the similarity between conidia and conidiogenous cells of *N. mangiferae, D. mangiferae* and a *Fusicoccum* sp.

In culture, *N. mangiferae* produces colonies of greyish to black fluffy mycelium with gregarious, partly immersed, discrete conidiomata on oatmeal agar. A radially dendritic, dark gray mycelium is found when cultures are grown on potato dextrose agar (PDA). Sutton and Dyko (1989) provided a description for the type species *N. mangiferae,* which is referred to in this review (Table 1; p 37).

(IV) ***Lasiodiplodia***

The fungus, *Lasiodiplodia theobromae* Pat., is commonly known as a saprophyte and wound invading pathogen of many tropical and sub-tropical crops, causing pre- and postharvest problems in many countries (Punithalingam, 1979; Punithalingam, 1980; Sutton, 1980; Von

Arx; 1987). *Lasiodiplodia theobromae* infection of mango has been reported on from the early 1900's (Punithalingam, 1980).

Lasiodiplodia has been referred to under various genera and synonyms were drawn to it by various authors (Punithalingam, 1976; Punithalingam, 1980). It was previously also known as *Botryodiplodia theobromae* Pat. (Punithalingam, 1976; Punithalingam, 1980; Von Arx, 1987; Crous & Palm, 1999), however, *Botryodiplodia* was synonomized with *Lasiodiplodia* by Petrak & Sydow (Sutton, 1980; Von Arx, 1987). The characteristics of the anamorph species justify the synonymy of *Diplodia* and *Botryodiplodia* (Punithalingham, 1976; Punithalingam, 1980; Crous & Palm, 1999) (Table 1; p37). *Lasiodiplodia theobromae* has previously been reported as the anamorph of *Physalospora rhodina* Berk. & Curt. apud Cooke (Punithalingam, 1980; Sutton, 1980). It is, however, now generally excepted to be the anamorph of *Botryosphaeria rhodina* (Cooke) Von Arx (Von Arx, 1987).

CONTROL STRATEGIES

Infection of mango trees and fruit by *Botryosphaeria* spp. can result in many different disease symptoms of which blossom blight, twig and stem dieback, cankering and fruit rots are of major importance. The development of control for economically important pre- and postharvest diseases caused by these fungi should include a focus on pathogen epidemiology. The fungi exist endophytically in the mango tree, spread systemically through the vascular system and expresses symptoms pre- and postharvestly if pathogen invasion and colonisation is not inhibited chemically or biologically.

Preharvest control

Disease incidence variation seems to relate to the fluctuation and extent of latent infections of *Botryosphaeria* in fruit and trees (Johnson, 1992; Lonsdale, 1993b). Latent infections can be influenced by orchard fungicide spraying, orchard sanitation, cultivar resistance, climate and tree age (Johnson *et al.*, 1992; Sangchote, 1993a; Johnson & Sanchote, 1994; Cooke *et al*, 1998). Some preharvest control measures aimed at reducing such infections, therefore, include planting for disease resistant or tolerant cultivars, reduction of potential wounds and limiting the chance of preharvest fungal inoculum deposition (Singh, 1960; Johnson & Sangchote, 1994; Sangchote, 1998b). Mismanagement and neglect of orchards is often associated with an increase in preharvest diseases.

Preharvest fungicidal sprays or the application of biocontrol agents such as *Bacillus licheniformis* (De Villiers & Korsten, 1996), and covering fruit with polyethylene caps (Kitagawa *et al.*, 1992; Johnson & Sanchote, 1994; Sanchote, 1993b), was found to reduce the incidence of fruit rots. Chemical fungicides such as flusilazol (under dryland conditions), iprodione, imazalil, prochlaraz, manganese chloride and triadimenol was shown to have a certain level of effectiveness against *Botryosphaeria* spp. causing fruit rots, but effectiveness varied with area and cultivar (Peterson *et al.*, 1991; Prusky, 1991; Johnson, 1992; Gunasekaran & Weber, 1996). Due to the reported incidence of build-up of pathogen resistance with the use of certain fungicides, most of these chemicals are either not used or alternated with copper oxychloride sprays. Copper oxychloride has to date proven to the most effective fungicide against many mango diseases (Spalding, 1982; Peterson *et al.*, 1991; Prusky, 1991; Johnson, 1992). Copper oxychloride is currently also the only preharvest fungicide registered for use on fruit destined for export from South Africa (Boshoff *et al.*, 1994).

Postharvest control

The most effective postharvest disease control strategy usually starts with an effective preharvest protection program. Preharvest practices, however, does not achieve consistent disease control. This makes it necessary to use postharvest fruit treatments to effectively control SER and SBR (Prusky, 1991; Johnson, 1992; Johnson & Sanchote, 1994). Such postharvest approaches are focussed on the delay of symptom development.

In recent years, the emphasis has been on the development and improvement of postharvest practices such as irradiation, warm water treatments and controlled atmosphere and low temperature storage (Pelser & Lesar, 1989; Johnson *et al.*, 1990; Medlicott *et al.*, 1990; Prusky, 1991; Johnson, 1992). The alternate use of increased CO_2 levels has proven to be useful in controlling postharvest pathogens during long term, low-temperature storage, but only with certain cultivars (Pelsar & Lesar, 1989; Prusky, 1991; Kobiler *et al.*, 1998; Meiburg *et al.*, 1998). Dipping of fruit in hot water (55°C) amended with registered chemicals such as prochloraz, can adequately control most of the superficial infections and prevent transmission of inoculum (Pelsar & Lesar, 1989; Johnson, 1992; Johnson & Sangchote, 1994). Prochloraz is, however, currently not registered for use on fruit destined for the European markets due to product clearance not given by countries such as France. Similarly, exposure of fruit to short wave infrared radiation, for three minutes has been shown to be effective in controlling SBR, however, this can result in lenticell damage and this technique is therefore not utilised commercially (Johnson *et al.*, 1990; Prusky, 1991; Johnson, 1992; Saaiman, 1995). Of all these control measures, only hot water fruit dips are currently commercially used in packhouses in South Africa (Saaiman, 1995).

Biological control as an alternative postharvest control measure is at an early stage of commercialisation (Gunasekaran & Weber, 1996). A warm water dip with *B. lichiformis*, followed by reduced concentrations of procloraz was found to effectively control various mango diseases, including fruit rots (De Villiers & Korsten, 1996). Even more effective control was achieved when 10% ethanol was used before applying the antagonist (De Villiers & Korsten, 1996). The main problem facing commercialisation of biological control is inconsistency in the level of control, which needs to be addressed through more effective product formulations (Korsten *et al.*, 1993).

Integrated control

With increased public concern over health risks, environmental pollution and the possibility of pathogen resistance developing against chemicals, it has become important to explore alternative measures of control (Johnson & Sanchote, 1994). Levels of endophytic colonisation in trees have been effectively reduced when commercial pruning programs in mango orchards have been synchronized with preharvest control measures (Cooke *et al.*, 1998). Canker formation can be minimized by preventing wounding and by pruning cankered or dead limbs of mango trees in the orchard. The trees respond well with vigorous growth after pruning with the addition of protective fungicidal sprays (Johnson, 1992; Johnson & Sanchote, 1994). This reduces pathogen inoculum and assists the tree to outgrow pathogen infection. Tree manipulation strategies will, however, only succeed if stress is minimized during all critical growth and dormancy periods (Johnson, 1992; Wolstenholme & Whiley, 1995). The latest focus on alternative strategies is the development of slow-ripening tropical fruit cultivars. This could facilitate long storage of fruit and subsequently delay disease development (Sangchote, 1991; Finnemore, 2000).

CONCLUSIONS

The export value of fresh mango fruit and its importance in the diet of people in many developing countries makes mango one of the most important fruit crops in the world. Due to the popularity of the crop and its wide distribution, mango is commonly cultivated under sub-optimal environmental conditions, often resulting in stress conditions conducive to pathogen attack. The high temperature and humid condition during fruit development favours infection and colonisation of fungal pathogens. Mango production is, therefore, seriously threatened by fungi that attack mango trees, flowers or fruit.

Currently, the most economically important diseases of mango trees and fruit are caused by *Botryosphaeria* species. These species are recognised endophytes of mango trees, however, the endophytes can become pathogenic and cause diseases of all the tree and fruit parts. The pathogenic nature of *Botryosphaeria* spp. is easily induced when trees are predisposed to stress conditions such as water stress, sunburn and mineral deficiencies. *Botryosphaeria* spp. infects through natural openings and wounds in the host tissue. After infection, the pathogen can remain quiescent or quickly enter the vascular system, causing vein discoloration and clogging of vessels. The restricted nutrient flow and rapid tissue invasion initiates the expression of disease symptoms such as blossom blights, twig diebacks, cankering and fruit rots of mango.

Various anamorph genera of *Botryosphaeria* are readily encountered on mango trees and fruit and the identification and characterisation of the *Botryosphaeria* spp. are based on morphological characteristics of the anamorphs. Due to the similarities between these anamorphs, considerable confusion has surrounded the taxonomy and epidemiology of the

Botryosphaeria spp. infecting mango world-wide. Many different species in *Hendersonia*, *Dothiorella*, *Nattrassia*, *Fusicoccum* and *Lasiodiplodia* have previously been identified as mango pathogens and the current generic concepts are, therefore, in need of urgent revision.

Limited success in controlling mango diseases caused by *Botryosphaeria* spp., emphasise the need and importance of developing effective alternative control strategies. The lack of tolerance in the more than 100 mango cultivars world-wide to *Botryosphaeria* infection, is a factor for major concern. Furthermore, there has recently been an emphasis on quarantine to prevent the further spread of new or exotic pathogens to foreign countries. This emphasises the need for revision of the taxonomy of the *Botryosphaeria* pathogens involved in mango diseases, as identification is the first step in developing effective control strategies and quarantine regulations.

REFERENCES

Berkley, M.J. 1860. Outlines of British Fungology – Lovell Reeve, London, UK.

Boshoff, M., Slabbert, M.J. & Korsten, L. 1995. Effect of detergent sanitizers and postharvest diseases of avocado. *South African Growers' Association Yearbook* 18: 96–98.

Cesati, V. & De Notaris, G. 1863. Schema di classificazione degli sferiacei italici aschigeri piu o meno apparleneti al genere *Sphaeria* nell antico significato attribuitogli da Persoon. *Commentario della Societa Crittogamologica Italiana* 1: 177-240. (English translation).

Creswell, T.C. & Milholland, R.D. 1988. Spore release and infection periods of *Botryosphaeria dothidea* on blueberry in North Carolina. *Plant Disease* 72: 342–346.

Cooke, A.W., Van der Kruyssen, A. & Johnson, G.I. 1998. The effect of commercial pruning on colonization of mango by endophytic *Dothiorella* species. In: Post-harvest handling of tropical fruit. Proceedings of an International Congress, Chaing Mai, Thailand. Champ, B.R., Highley, E. and Johnson, G.I. (Eds.). pp. 294- 298. Australian Centre for International Agricultural Research, Canberra.

Crous, P.W. & Palm, M.E. 1999. Reassessment of the anamorph genera *Botriodiplodia, Dothiorella* and *Fusicoccum*. *Sydowia* 51: 167–175.

Darvas, J.M. 1991. *Dothiorella dominicana*, a new mango pathogen in South Africa. *Phytophylactica* 23: 295-298.

Denman, S., Crous, P.W., Taylor, J.E., Kang, J., Pascoe, I. & Wingfield, M.J. 2000. An overview of the taxonomic history of *Botryosphaeria*, and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. *Studies in Mycology* 45: 129-140.

De Villiers, E.E. & Korsten, L. 1996. Alternative strategies for the control of mango fruit diseases. *South African Mango Growers' Association Yearbook* 16: 61-64.

Dow, J.M. & Rubery, P.H. 1975. Hyphal tip bursting in *Mucor rouxii:* Antagonistic effects of calcium ions and acid. *Journal of General Microbiology* 91: 425–428.

Finnemore H J. 2000. An overview of the South African mango industry (past and future) 1-11 pp. http://students.washington.edu/melliott/arbutus/natt.htm

Frankel, D.H. & Rippon, J.W. 1989. *Hendersonula toruloidea* infection in man. *Mycopathologia* 105: 175–186.

Gunasekaran, M. & Weber, D.J. 1996. Molecular Biology of the Biological Control of Pests and Diseases of Plants. pp. 31-35. CRC Press, Inc.

Jacobs, K.A. & Rehner, S.A. 1998. Comparison of cultural and morphological characters and ITS sequencing in anamorphs of *Botryosphaeria* and related taxa. *Mycologia* 90: 601–610.

Jayasinghe, C.K. & Silva, W.P.K. 1994. Foot canker and sudden wilt of *Hevea brasiliensis* associated with *Nattrassia mangiferae*. *Plant Pathology* 43: 938–940.

Johnson, G.I. 1992. Biology and control of stem end rot pathogens of mango. Ph.D. thesis. University of Queensland, Queensland, Australia.

Johnson, G.I., Sanchote, S. & Cooke, A.W. 1990. Control of stem end *rot (Dothoirella dominicana*) and other postharvest diseases of mangoes (cv. Kensington Pride) during short and long-term storage. *Tropical Agriculture* (*Trinidad*) 67: 183-187.

Johnson, G.I., Cooke, A.W., Mead, A.J. & Wells, I.A. 1991. Stem-end rot of mangoes in Australia: Causes and control. *Acta Horticulturae* 291: 288-295.

Johnson, G.I., Mead, A.J., Cooke, A.W. & Dean, J.R. 1992. Mango stem end rot pathogens – Fruit infection by endophytic colonisation of the inflorescence and pedicel. *Annals of Applied Biology* 120: 225–234.

Johnson, G.I. 1994. Part III. Mango: Stem-end rot. In: Compendium of tropical fruit diseases. Ploetz, R.C., Zentmyer, G.A., Nishijima, W.T., Rohrbach, K.G. & Ohr, H.D. (Eds.). pp. 36-37. APS Press, St Paul, Minnesota.

Johnson, G.I. & Sangchote, S. 1994. Control of post-harvest diseases of tropical fruits: Challenges for the 21st century. In: Post-harvest handling of tropical fruit. Proceedings of an International Congress, Chaing Mai, Thailand. Champ, B.R., Highley, E. and Johnson, G.I. (Eds.). pp. 140-161. Australian Centre for International Agricultural Research, Canberra.

Kitagawa, H., Manabe, K. & Esguerra, E.B. 1992. Bagging of fruit on the tree to control disease. *Acta Horticulturae* 321: 871-875.

Kobiler, I., Reved, R., Artez, L. & Prusky, D. 1994. Antifungal compounds regulating quiescent disease in mango. In: Post-harvest handling of tropical fruit. Proceedings of an International Congress, Chaing Mai, Thailand. Champ, B.R., Highley, E. and Johnson, G.I. (Eds.). pp. 109–114. Australian Centre for International Agricultural Research, Canberra.

Korsten, L., De Villiers, E.E. & Lonsdale, J.H. 1993. Biological control of mango postharvest diseases in the packhouse. *South African Mango Growers' Association Yearbook* 13: 117-121.

Kruger, F.J., Goodchild, L., Schroeder, W. & Nel, A. 1995. Current mango research initiatives of the post-harvest section at the research institute for tropical and subtropical crops. *South African Mango Growers' Association Yearbook* 15: 63-66.

Kwee, L.T. & Chang, K.K. 1985. Diseases and disorders of mango in Malaysia. pp. 1-23. Tropical Press, Malaysia.

Laundon, G.F. 1973. *Botryosphaeria obtusa, B. stevensii* and *Otthia spiraeae* in New Zealand. *Transactions of the British Mycological Society* 6: 369-374.

Lonsdale, J.H. 1992. Etiology and control of some mango blossom diseases in Transvaal. MSc thesis. University of Pretoria, Pretoria.

Lonsdale, J.H. 1993a. Mango diseases in South Africa. *South African Mango Growers' Association Yearbook* 13: 89-92.

Lonsdale, J.H. 1993b. Preliminary results on the mode of infection of *Nattrassia mangiferae* in mango. *South African Mango Growers' Association Yearbook* 13: 97-99.

Maas, J.L. & Uecker, F.A. 1984. *Botryosphaeria dothidea* cane canker of thornless blackberry. *Plant Disease* 68: 720–726.

McPartland, J.M. & Schoeneweiss, D.F. 1984. Hyphal morphology of *Botryosphaeria dothidea* in vessels of unstressed and drought-stressed stems of *Betula alba*. *Phytopathology* 74: 358-362.

Medlicott, A.P., Sigrist, J.M.M. & Sy, O. 1990. Ripening of mango following low temperature storage. *Journal of the American Society of Horticultural Science* 115: 430-444.

Meiburg, G.F., Hofman, P.J., Smith, L.G., Cooke, A.W. & Baker, J.A. 1998. Quality of Kensington mangoes after short duration exposure to high carbon dioxide concentrations. In: Post-harvest handling of tropical fruit. Proceedings of an International Congress, Chaing Mai, Thailand. Champ, B.R., Highley, E. and Johnson, G.I. (Eds.). pp. 55-60. Australian Centre for International Agricultural Research, Canberra.

Morgan-Jones, G. & White, J.F. Jr. 1987. Notes on *Coelomycetes*. II. Concerning the *Fusicoccum* anamorph of *Botryosphaeria ribis*. *Mycotaxon* 30: 117–125.

Pelser, P. du T & Lesar, K. 1989. Decay control in South African mangoes by flusilazol, penconazole and procloraz during simulated shipment to Europe. *Aspects of Applied Biology* 20: 41-48.

Pennycook, S.R. & Samuels, G.J. 1985. *Botryosphaeria* and *Fusicoccum* species associated with ripe fruit rot of *Actinidia deliciosa* (kiwifruit) in New Zealand. *Mycotaxon* 24: 445–458.

Peterson, R.A., Johnson, G.I., Schipke, L.G. & Cooke, A.W. 1991. Chemical control of stem end rot in mango. *Acta Horticulturae* 291: 304-307.

Petrak, F. & Cifferi, R. 1930. Fungi dominicani. *Annales Mycologici* 28: 411.

Prakash, O.M. & Srivastava, K.C. 1987. Mango diseases. In: Mango diseases and their management. pp. 1. Today and Tomorrow's Printers and Publishers. New Deli.

Prusky, D. 1991. Mango diseases: An overview. *Acta Horticultura* 291: 279-287.

Punithalingam, E. 1976. *Botryodiplodia theobromae. CMI Description of pathogenic fungi and bacteria* no. 519.

Punithalingam, E. 1979. Plant diseases attributed to *Botryosphaeria theobromae*. pp 123. J.Cramer, Germany.

Punithalingam, E. 1980. Plant diseases attributed to *Botryodiplodia theobromae* Pat. pp. 42-43. J. Cramer. Germany.

Prusky, D. & Keen, N.T. 1993. Involvement of preformed antifungal compounds in the resistance of subtropical fruits to fungal decay. *Plant Disease* 77: 114-119.

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

Ramos, L.J., Lam, S.P., McMillan, R.J. & Narayanan, K.R. 1991. Tip dieback of mango (*Mangiferae indica*) caused by *Botryosphaeria ribis. Plant Disease* 75: 315–318.

Rayachhetry, M.B., Blakeslee, G.M., Webb, R.S. & Kimbrough, J.W. 1996. Characteristics of the *Fusicoccum* anamorph of *Botryosphaeria ribis*, a potential biological control agent for *Melaleuca quinquenervia* in South Florida. *Mycologia* 88: 239-248.

Reckhaus, P. 1987. *Hendersonula* dieback of mango in Niger. *Plant Disease* 71: 1045.

Saaiman, W. C. 1995. Short wave infra-red radiation as an alternative to the hot water bath for the control of post-harvest diseases in mango. *South African Mango Growers' Association Yearbook* 15: 86–89.

Saaiman, W.C. 1996. Preliminary report on the time of infection of the soft brown rot pathogen *Nattrassia mangiferae* in mango. *South African Mango Growers' Association Yearbook* 16: 55–57.

Saccardo, P.A. 1884. *Sylloge Fungorum onium hucusque cognitorum* 3: 1–860.

Sangchote, S. 1991. *Botryodiplodia* stem end rot of mango and its control. *Acta Horticulturae* 291: 296–304.

Sangchote, S. 1993a. Fruit rots of mangosteen and their control. In: Post-harvest handling of tropical fruit. Proceedings of an International Congress, Chaing Mai, Thailand. Champ, B.R., Highley, E. and Johnson, G.I. (Eds.). pp. 81-86. Australian Centre for International Agricultural Research, Canberra.

Sangchote, S. 1993b. Effect of fruit bagging, fruit position, cultivar and post-harvest treatment on post-harvest disease of mangoes. In: Post-harvest handling of tropical fruit. Proceedings of an International Congress, Chaing Mai, Thailand. Champ, B.R., Highley, E. and Johnson, G.I. (Eds.). pp. 63-66. Australian Centre for International Agricultural Research, Canberra.

Schaffer, B., Larson, K.D., Snyder, G.H. & Sanchez, C.A. 1988. Identification of mineral deficiencies associated with mango decline by DRIS. *Hortscience* 23: 617-619.

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

Schoeneweiss, D.F. 1984. The role of environmental stress in diseases of woody plants. *Plant Disease* 56: 308–314.

Schroeder, W. 1990. Origin of the mango. In: Mangoes. pp. 100-102. Citrus and Subtropical Fruit Research Institute, Nelspruit.

Singh, L.B. 1960. Mango. In: The Mango. pp 320-343. World Crop Series. Interscience Publishers Inc., New York.

Sivanesan, A. 1984. The bitunicate ascomycetes and their anamorphs. pp 127-139. J. Cramer, Germany.

Spalding, D.H. 1982. Resistance of mango pathogens to fungicides used to control postharvest diseases. *Plant Disease* 66: 1185-1186.

Sutton, B.C. 1980. The *Coelomycetes*. Fungi Imperfecti with pycnidia, acervuli and stroma. pp. 32, 41. CMI, Kew, Surrey, England.

Sutton, B.C. & Davidson, E.M. 1983. Three stromatic coelomycetes from Western Africa. *Transactions of the British Mycological Society* 81: 291-301.

Sutton, B.C. & Dyko, B.J. 1989. Revision of *Hendersonula. Mycological Research* 93: 466–488.

Sutton, T.B. 1981. Production and dispersal of ascospores and conidia of *Physalospora obtusa* and *Botryosphaeria dothidea* in apple orchards. *Phytopathology* 71: 584-589.

Swart, G.M. 1999. Comparative studies of *Colletotrichum gloeosporoides* from avocado and mango. Ph.D. Thesis. University of Pretoria, Pretoria.

Sydow, H. & Sydow, P. 1919. Mykologische mitteilungen. *Annales Mycologici* 17: 33-47.

Von Arx, J.A. 1987. Plant-pathogenic fungi. 203–204 pp. J. Cramer, Berlin, Germany.

Weaver, D.J. 1979. Role of conidia of *Botryosphaeria dothidea* in the spread of peach tree gummosis. *Phytopathology* 69: 330–334.

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

Wolstenholme, B.N. & Whiley, A.W. 1995. Ecophysiology of the mango tree as a basis for pre-harvest management. *South African Mango Growers' Association Yearbook* 15: 10-17.

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

Table 1. Description of *Botryosphaeria* found on mango

	F.aesculi Crous & Palm (1999)	*D. dominicana* Petrak & Cifferi (1930) Johnson (1992)	*D. mangiferae* Sydow & Sydow (1919) Johnson (1992)	*D. aromatica* Darvas (1991) (Translation)	*N. mangiferae* Sutton & Dyko (1989)	*L. theobromae* Punithalingam (1980)
Conidiomata						
* Stroma	Eustromatic	Eustromatic	Eustromatic	Eustromatic	Eustramatic	Eustromatic
* Locule	Uni- to multiloculate	Uni- to multiloculate	Uni- to multiloculate	Uni- to multiloculate	Uni- to multiloculate	Uni- to multiloculate
	Locules ostiolar	Locules ostiolar	Locules ostiolar	Locules ostiolar	Locules ostiolar	Ostioles absent
* Size	100 - 300um diameter	250um diameter				
* Paraphysis	Absent	Absent			Absent	Cylindrical, sepate
Conidiogenous						
Cells						
* Shape	Cylindrical	Cylindrical	Filiform	Cylindrical	Lageniform to ampuliform	Cylindricqal
* Conidiophore	Conidiophore simple			Conidiophore simple	Conidiophores absent	Conidiophores absent
* Colour	Hyaline, smooth	Hyaline	Hyaline	Hyaline	Hyaline	Hyaline
* Septation	0 - 1 septate	Aseptate	Aseptate	Aseptate	Aseptate	Aseptate
* Cell size	22 um	5 - 10um	5 - 8um	6 - 16 (-20) um		
* Base size	1.5 - 2.5 um	2 - 2.5 um	2um	2 um		
Conidia						
*Shape	Fusiform to elipsoid	Fusiform to clavate	Fusiform to elipsoid	Fusiform to clavavate	Fusiform to ellipsoid	Ellipsoid
	Straight	Straight to slightly curved	Slightly curved	Straight to slightly curved	Straight to slightly curved	Straight
* Apex	Subobtuse	Rounded	Rounded	Rounded		Truncate
* Base	Truncate	Truncate	Tapered to flat	Tapered		Truncate
	Smooth	Granular		Granular	Smooth	Longitudinal striations
* Cell wall	Thin	Thin	Thin	Thin	Thin	Thick
* Immature	Hyaline	Aseptae	Aseptate	Aseptate	Aseptate	Aseptate
	Aseptate	Hyaline	Hyaline	Hyaline	Hyaline	Hyaline
* Mature	Uni- to biseptate	Uni- to bisepate			Uni- to biseptate	Uniseptate
	Vericulouse	Vericulouse			Verucolouse	Dark brown
* Length	18 - 25 (-30) um	13 - 16.2 (15.6) um	9 - 14 (12.8) um	16 - 23 (22.8) um	10 - 16 (21) µm	18 - 30 um
* Width	4 - 4.5 (-5) um	4.5 - 4.7 um	3.5 - 5.5 (5.0) um	3.9 - 5.5 (4.6) um	3.5 - 6.5 µm	10 - 15 um

CHAPTER 2

IDENTIFICATION AND CHARACTERISATION OF *BOTRYOSPHAERIA* SPECIES FROM MANGO IN SOUTH AFRICA

ABSTRACT

Botryosphaeria spp. are well known endophytes and pathogens of many tropical and subtropical fruit crops, including mango. The identity of these species is difficult to determine due to overlapping morphological characteristics of both the teleomorphs and anamorphs. The purpose of this study was to determine the identity of *Botryosphaeria* spp. infecting mango in South Africa. Isolates were obtained from diseased mango plants in the Northern Province of South Africa. They were cultured on potato dextrose agar and cultural and conidial morphology was evaluated. DNA was isolated and the internally transcribed spacer (ITS) and β-tubulin gene regions were amplified and sequenced. Four morphological groups (MGs) were identified among all isolates, based on cultural and conidial morphology. These MGs directly corresponded to four distinct clades with combined ITS and β-tubulin sequence data. The species in these groups were identified as *Fusicoccum* parvum, *Lasiodiplodia theobromae* and two new species, *F. indigiticum* and *F. bacilliforme*, which are described in this study.

INTRODUCTION

Botryosphaeria species are known world-wide for the damage that they cause to various woody hosts (Sutton, 1980; Punithalingham, 1980). These fungi are well known as the causal agents of branch and stem cankers, twig dieback and blossom blight in most of the trees they infect. *Botryosphaeria* diseases also cause severe fruit rots and are responsible for extensive losses to industries that rely on fruit crop export (McPartland & Schoeneweiss, 1984; Pennycook & Samuels, 1985; Ramos *et al.*, 1991; Johnson, 1992).

Mangoes, *Mangiferae indica* Linn., can be severely damaged due to invasion and colonisation by *Botryosphaeria* spp. These fungi cause a variety of preharvest disease symptoms, which are usually expressed when trees are subjected to environmental stress (McPartland & Schoeneweiss, 1984; Ramos *et al.*, 1991; Johnson, 1992; Ploetz, 1994). Recent outbreaks of tree die-back in orchards as well as substantial export losses due to soft brown rot (SBR) and stem end rot (SER) diseases of mango fruit in South Africa, have renewed interest in the taxonomy and epidemiology of *Botryosphaeria* spp. in this country.

A number of different *Botryosphaeria* spp. have been reported to occur on mango, but the taxonomy of these fungi is confusing. Although these fungi are recognised as being *Botryosphaeria* spp. by anamorph association, teleomorph structures have not been recorded on mango (Johnson, 1992; Slippers *et al.*, 2001). As with other *Botryosphaeria* spp., identification of isolates from mango has chiefly been based on morphological characteristics of the anamorphs (Sutton, 1980; Pennycook & Samuels, 1985; Jacobs & Rehner, 1998; Crous & Palm, 1999; Smith & Stanosz, 2001). Much confusion, however, also surrounds the classification of these anamorphs and this has further complicated

accurate treatment of these pathogens, on mango and other crops (Sutton, 1980; Jacobs & Rehner, 1998; Crous & Palm, 1999; Denman *et al.*, 2000). Anamorph genera that have been documented as causal agents of SER and SBR of mango include *Dothiorella dominicana* Pet. et Cif., *D. mangiferae* H. et P. Syd. But., an unnamed fungus that has been referred to as *Dothiorella* 'long', *Nattrassia mangiferae* (Nattrass) Sutton et Dyko and *Lasiodiplodia theobromae* (Pat.) Griff. et Maubl. (Johnson, 1992).

The simplest morphological distinction between *Botryosphaeria* anamorphs is based on the production of either hyaline or pigmented and fusiform to ellipsoid conidia, with or without septation at maturity (Pennycook & Samuels, 1985; Crous & Palm, 1999; Denman *et al.*, 2000). Most species with pigmented conidia are generally treated in the genus *Diplodia* Fr. and those with hyaline conidia in *Fusicoccum*. There are, however, some limitations to the use of anamorph morphological characteristics for identification of *Botryosphaeria* spp. The fact that many species are morphologically similar and that it is sometimes difficult to induce strains to sporulate in culture, has resulted in confusion in the delimitation of species (Pennycook & Samuels, 1985; Smith & Stanosz, 2001; Zhou & Stanosz, 2001).

DNA sequencing data has begun to provide valuable insights into the natural classification of fungi where traditional characters have been shown to be insufficient for this purpose (Bruns *et al.*, 1991; Mitchell *et al.*, 1995). Recent studies on *Botryosphaeria* using DNA sequence data have provided considerable insight into the taxonomy of these fungi (Jacobs & Rehner, 1998; Denman *et al.*, 2000; Zhou & Stanosz, 2001). For example, where *B. dothidea* and *B. ribis* (now known as *B. parva*) had previously been reduced to synonymy based on morphology, these fungi have clearly been shown to be distinct species based on

sequence data sets for a number of genes (Jacobs & Rehner, 1998; Zhou & Stanosz, 2001; Slippers *et al.*, 2001).

Parts of the rDNA operon have been most useful in resolving taxonomic and phylogenetic questions pertaining to fungi (Hillis & Huesenbeck, 1992; O'Donnell, 1992; Carbone & Kohn, 1993). Thus, the internally transcribed spacers (ITS 1 and ITS 2) of the rDNA operon have been successfully employed to analyse interspecific relationships in various fungi, including *Botryosphaeria* spp. (Smith *et al.*, 1994; Jacobs & Rehner, 1998; Zhou & Stanosz, 2001). Jacobs and Rehner (1998) used ITS sequence and morphological characteristics to relegate several anamorphs to *Botryosphaeria*. Various researchers have, however, warned against basing phylogenies on a single DNA region (O'Donnell & Cigelnik, 1995; Taylor *et al.*, 2000).

The aim of this study was to identify and characterise the *Botryosphaeria* spp. associated with mango diseases in South Africa. Both morphological and molecular data were used to compare isolates from this region with those from other parts of the world where they have been collected from canker, dieback and mango fruit rot symptoms. Sequence data from two gene regions, the ITS and ß-tubulin regions, were used for molecular analysis. Conidial and cultural characteristics are considered for morphological comparisons.

MATERIALS AND METHODS

Collection and isolation of fungal isolates

Botryosphaeria spp. used in this study were isolated from mango trees and fruit cultivated in Mpumalanga and the Northern province, South Africa. Isolations were made from

asymptomatic and symptomatic material from various parts of trees and fruit. Prior to isolation, whole twigs, leaves and fruit were surface disinfested twice with 70% (v/v) ethanol and left to air dry for five minutes between treatments. Surface disinfested wood chips ($2mm^2$) and discs ($2 - 3mm^2$) from the edges of lesions on fruit were cut in half and placed on potato dextrose agar (PDA) (Biolab) amended with 100mg chloramphenicol (Centaur Laboratories). Cultures were incubated at 25°C for seven to twelve days. All cultures, with a peripheral morphology resembling that of *Botryosphaeria* spp. were transferred to clean Petri dishes containing PDA.

Twenty isolates were identified from a larger collection, based on colony colour and conidial morphology, to be representative of the *Botryosphaeria* spp. found in the mango industry of South Africa (Table 1; p63-64). All isolates were collected between 1999–2001. Reference isolates obtained from mango in Australia (supplied by Dr. G.I. Johnson) and other hosts were included for comparative purposes (Table 1; p63-64). All the *Botryosphaeria* isolates used in this study are maintained in the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa (Table 1; p63-64).

Morphological characterisation

Sporulation of putative *Botryosphaeria* isolates were induced by growing isolates on water agar (WA) (Biolab), supplemented with sterile pine needles or mango twigs. Cultures were incubated at 25°C with 12 hour near UV light/dark cycles, to induce sporulation. Conidia produced in this way were also used for morphological characterisation (Sutton, 1980; Johnson, 1992; Crous & Palm, 1999). Conidia were spread on WA and single germinating conidia transferred to PDA after 12 – 24 hours. All *Botryosphaeria* isolates derived from

single conidia were then stored at 4°C on PDA slants and in sterile water to be used for molecular characterisation.

Single spore cultures of *Botryosphaeria* spp. identified in this study, were inoculated in the middle of Petri dishes containing PDA and incubated at five different temperatures ranging from 10°C to 30°C with 5°C intervals. Two to four isolates were used for every species and a total of five replicates were included for every isolate at every temperature. Two perpendicular measurements of colony diameter were taken daily for every isolate at every temperature, from specific marked areas on the Petri dishes. Colony growth and cultural characteristics were recorded for all species.

Fruiting structures formed on mango twigs or pine needles were dissected by hand to observe pycnidia, stromatic locules in cross section, conidia and conidiogenous cells. Sections were mounted in lactophenol and examined using Nomarski differential interference contrast microscopy. Isolates were grouped according to morphological characteristics. The average size (length and width) of 30 – 50 conidia were measured for each isolate. All microscope observations and measurements were made using a light microscope (Carl Zeiss) and photographic images were captured electronically with an Axiovision digital camera system (Carl Zeiss).

Molecular characterisation

DNA isolation

Mycelium from actively growing PDA cultures was used to inoculate 100mL liquid MY (2% Malt Extract and 0.2% Yeast Extract) broth in 250mL Erlenmeyer flasks. These liquid cultures were incubated at 25°C for approximately one week. Mycelium was harvested, filtered and lyophilised. A modified version of the method of Raeder and Broda (1985)

was used for isolation of DNA. Dried mycelium was ground to a fine powder and homogenised in 800µl extraction buffer (200mM Tris-HCL pH8.0, 150mM NaCl, 25mM EDTA pH8.0, SDS 0.5%). Phenol and chloroform (ratio 5:3) was added to all samples, shaken and centrifuged (13000 rpm for 60 minutes). Thereafter, chloroform was added, centrifuged and the upper aqueous phase removed repeatedly until the interphase was clear of proteins and contaminating cell debris. Precipitation of nucleic acids was done with 3M NaAc pH5.5 (0.1 v/v) and absolute ethanol (2 v/v). After a 70% EtOH (ethanol) wash step, DNA was vacuum dried to a pellet and resuspended in 50µL sterile SABAX water. RNA was degraded by the addition of 3 - 5µL RNase (1mg/mL) to the DNA and left at 37°C for three hours, or until all RNA was degraded. DNA concentrations were estimated against a λ-marker standard on a 1.5% agarose gel.

DNA amplification and purification

A portion of the nuclear rDNA operon was amplified using primers ITS1 (5'-TTT CCG TAG GTG AAC CTG C-3') and ITS 4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (MWG Biotech, Germany) (White et al., 1990). The amplified region extended from the 3' end of the 16S (small subunit) rDNA gene, including the first ITS (ITS1), 5.8S gene, the second ITS (ITS2) region and ended at the 5' end of the 26S (large subunit) rDNA gene. Part of the ß-tubulin 2 gene region was amplified with primers Bt 2a (5'-GGT AAC CAA ATC GGT GCT TTC-3') and Bt 2b (5'-ACC CTC AGT GTA GTG ACC CTT GGC-3') (Glass & Donaldson, 1995).

Polymerase chain reactions (PCR) contained 0.2mM of each dNTP (Promega, Madison, Wisconsin, U.S.A.), 0.15µM of each primer, 0,5U Expand™ High Fidelity Taq polymerase (Roche Molecular Biochemicals, Almeda, CA), 1X Buffer and $MgCl_2$ (10mM

Tris-HCL, 1.5mM $MgCl_2$, 50mM KCl). Sterile SABAX water was used to adjust the final volume to 50µL. The following conditions were standardised for all PCR reactions: An initial denaturation at 96°C for one minute followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing for one minute at 56°C for ITS and 58°C for ß-tubulin, followed by extension at 72°C for ninety seconds. A step up of five seconds elongation was added with each cycle after the first twenty-five cycles. The process was ended with a final extension at 72°C for ten minutes. All PCR products were stored at 4°C. PCR products were visualised on a 1.5% horizontal agarose gel using a TAE buffer electrophoresis system (Maniatis *et al.*, 1982). PCR products were stained with a 0.5g/mL ethidium bromide (Merck) solution and visualised under UV illumination. PCR product sizes were estimated with a 100bp standard size marker (Promega).

DNA sequencing and analysis

Twenty isolates, representative of all morphological groups from mango in South Africa, were used for sequencing (Table 1; p63-64). All PCR products were cleaned prior to sequencing with a High Pure PCR Product Purification kit (Roche Molecular Biochemicals, Almeda, CA) according to manufacturers specifications. PCR products were sequenced in both directions using the primers ITS1, ITS4, Bt 2a or Bt 2b. Sequencing reactions were performed using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Applied Biosystems, Foster City, CA). Twenty-five sequencing PCR cycles were performed with the following standardised conditions: a denaturation step at 96°C for ten seconds, annealing of primers at 50°C for 30 seconds and elongation at 60°C for four minutes. All sequence reactions were run on an ABI PRISM 377 Autosequencer (Perkin-Elmer Applied BioSystems, Foster City, CA). Sequences were analysed using Sequence Navigator version 1.0.1™ (Perkin Elmer Applied BioSystems,

Foster City, CA) and manually aligned by inserting gaps. Phylogenetic analyses were done using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0 (Swofford, 1998). All characters were given equal weight and gaps were treated as newstate (fifth base). ITS and β-tubulin datasets were analysed separately and combined .A partition homogeneity test (PAUP 4.01b) was used to test the congruence and combinability of the ITS and β-tubulin sequence data sets (Huesenbeck *et al.*, 1996). Subsequently, the datasets were analysed together. Most parsimonious trees were identified with heuristic searches using random stepwise addition and tree bisection and reconstruction (TBR) as branch swopping algorithm. Branches with a length of zero were collapsed and all multiple equally parsimonious trees were saved. Bootstrap consensus trees were obtained with PAUP for all equally parsimonious trees saved, with 1000 bootstrap replicates (Felsenstein, 1985). Eight sequences representing the most commonly recognised *Botryosphaeria* spp. were obtained from Genbank (Table 1; p63-64). Isolates known to infect mango in Australia and other woody hosts in South Africa were also included for comparative purposes (Table 1; p63-64) (Slippers *et al.*, 2001). Trees were rooted to the outgroup *Guignardia philoprina* (Ellis) Viala & Ravaz, a genus known to be closely related to *Botryosphaeria*.

RESULTS

Morphological characterisation

All isolates included in this study had multilocular and eustromatic conidiomata. Conidiophores were hyaline, cylindrical, smooth and 0-1 septate. Conidiogenous cells were hyaline and smooth. Conidia were produced holoblastically on the conidiogenous cells. Conidia were hyaline, thin-walled, smooth and ellipsoid to fusiform. Aseptate,

(immature) and uni- to biseptate (mature) conidia were observed for all but one group of isolates, which the conidia were aseptate at all times (Fig. 1; p 65) (Table 1; p 63 - 64).

Analysis of colony morphologies and conidial dimensions for single conidial isolates on PDA, gave rise to four morphologically distinct groups which were designated as MG1 – MG4 (Fig. 2; p67) (Table 1; p63 - 64). Three of these groups of isolates resembled the characteristics of *Fusicoccum* spp. Conidia in the fourth group (Fig. 1; p65) resembled those of *L. theobromae*.

Botryosphaeria isolates residing in morphological group MG1, readily produced fluffy, white aerial mycelium, which become pale olivaceous grey (21''''d) to olivaceous grey (21''''') (Rayner, 1970) (Fig. 2; p65) from the middle of the colony within three to four days of incubation on PDA. The reverse side of the Petri dishes reflectes an olivaceous grey (21''''') to iron grey (24'''''k) colour. For this fungus, an average growth of 10.8 ±1mm per day is measured. The optimum temperature for growth was between 25°C and 30°C. Conidiomata were readily produced at the edge of the colony and were generally covered with tough greenish grey (33''''i) hyphae. Immature conidia were hyaline, smooth, aseptate and fusiform, but become uni- to biseptate and light brown pigmented with age and prior to germination (Fig. 1, A–B; p 65). Conidial apices were sub-obtuse and the bases truncate or rounded. Width of conidia was measured over the widest part of conidia (middle to upper third of conidia) (Fig. 1, A-B; p65). Average of 50 conidia per isolate was (16-)17.5 – 19.7(-22) x 4.5 – 4.7(-6.2)µm [l/w = 4.3]. Based on these morphological characteristics, isolates assigned to MG1 resembled *F. parvum* (the *Fusicoccum* anamorph of *B. parva* previously known as *D. dominicana*) (Table 2; p71).

Isolates assigned to MG2 produces fluffy to appressed mycelium, becoming olivaceous grey (21''''') to olivaceous black (27''''m) (Fig. 3; p69) within three to four days of incubation. Aerial mycelium become appressed with culture maturity. The Petri dish reverse side became indigo blue (47''m) to black. The optimum temperature for growth was 25°C and the average colony growth rate is 4.7±1mm per day. Conidiomata were small, iron grey (24'''''k) and were rarely produced in culture. Immature conidia were hyaline, aseptate, smooth and fusiform, but became uni- to biseptate with darker pigmentation in some instances, prior to germination (Fig. 1, C-D; p65). Conidial apices were sub-obtuse and bases truncate to rounded. Width, taken at the widest part (middle to upper third), and length measurements of conidia are (17.5-)19.5 – 21(-24) x (5-)5.5 – 6.5(-7.1)µm [l/w = 3.4]. Based on these characteristics, isolates assigned to MG2, appeared to represent an unidentified *Fusicoccum* sp. (Table 2; p71).

Isolates assigned to MG3 produce white, sparse aerial mycelium clustered in concentric rings on PDA. A yellow [pale luteous (18f)] (Fig. 3; p69) colour pigment was produced in young cultures, diffusing into the medium. Mycelium became pale olivaceous grey (21''''d) to olivaceous grey (21''''') within five to seven days of incubation. The reverse side of Petri dishes displayed a grey olivaceous (21'''') to olivaceous grey (21''''') colour with a visible dendritic pattern. One isolate [BOT2421] remained light coloured at all times. The optimum temperature for growth of this group was 25°C and the average colony growth rate was 7±1mm per day. Small conidiomata were produced sparingly in concentric rings. Conidia were hyaline, aseptate, smooth and cylindrical to bacilliform (Fig. 1, E-F; p65). Conidia rarely became uniseptate at maturity. Conidial measurements of MG3 isolates were (18.8-)20.8 – 23(-24.9) x (3.7-)4.1 – 5.2(-5.7)µm [l/w = 4.6]. Isolates

assigned to MG3 resembled the unnamed *Fusicoccum* sp. that has previously been referred to as *Dothiorella* 'long' (Johnson *et al.*, 1991; Johnson, 1992) (Table 2; p71).

Isolates residing in MG4 were typical of *L. theobromae*. This identification is based on the following characteristics. Isolates produce very fluffy and white aerial mycelium that rapidly covered the surface of Petri dishes within two days of incubation. The optimum temperature for growth is 30°C and the average growth rate was 19±1mm per day. White mycelium rapidly became pale olivacoeus grey (33''''e) to iron grey (24'''''k) (Fig. 3; p69) and submerged mycelium gave rise to an olivaceous grey (21''''') to iron grey (24'''''k) colour viewed from the underside of the Petri dishes. Conidiomata occured scattered in mycelial mat and at the edges of the colonies. Conidiomata were covered with smooth hyphae. Mature conidia oozed from ostioles of conidiomata within nine to fifteen days incubation at 30°C. Immature conidia were hyaline, aseptate, ovoid to rounded (Fig. 1, G-H; p65). They became uniseptate, thick walled, light brown pigmented with longitudinal striations when mature. Averaged width of conidia were taken at the widest part (middle) and the average length and width was (8-)10 – 18(-20) x 4 – 5.2(-6)µm [l/w = 3.6].

Molecular characterisation

DNA amplification and sequence analysis

ITS and ß-tubulin gene regions were highly conserved in all species examined based on the size of the amplified PCR product fragments. Fragment sizes of approximately 550bp and 450bp in length for the ITS and ß-tubulin regions respectively, were obtained for all isolates used in this study. Approximately 515bp of the ITS sequence data were used in the phylogenetic analysis, amounting to 560 characters after alignment. Only 430bp for β-

tubulin were used in the phylogenetic analysis, amounting to 469 characters after alignment. The partition homogeneity test indicated that the datasets were combinable ($P<0.06$; g1 = -0.753). The total alignment of the combined data sets amounted to 1009 characters. Of the total combined data set after alignment for the ITS and β-tubulin regions, 725 characters were parsimony-uninformative and were, therefore, excluded from the heuristic searches. The variable and parsimony-informative characters amounted to 284. After heuristic searches in PAUP, 226 most parsimonious trees of 100 steps were retained (consistency index (CI) = 0.752; retention index (RI) = 0.918) (Fig. 2; p67).

After phylogenetic analyses, all isolates considered in this study could be grouped into ten clades (I – X) based on ITS and ß-tubulin sequence data (Fig. 3; p67). Clades I – VII represent *Botryosphaeria* spp. with hyaline *Fusicoccum*-like conidia, and clades VIII – X represent species with pigmented or darker *Diplodia*-like conidia. All the South African mango isolates grouped into one of four clades (clade I, IV, VI or VIII) and these corresponded to identifications based on morphology and the assignment of isolates to four morphological groups MG1 – MG4. Clade I [BOT2413, BOT2302, BOT2398, BOT2353, BOT2331, BOT2339, BOT2382, BOT2363, BOT2345, BOT2291, BOT2405, BOT7799, BOT7026, BOT7025, BOT2352] corresponded to *F. parvum* (MG1). Isolates in clade IV [BOT2351, BOT2355] did not group with any *Botryosphaeria* spp. currently known (MG2). Clade VI [BOT2417, BOT2421, CMW7802, CMW7022] is a separate clade which is represented by isolates assigned the informal name *Dothiorella* 'long' isolated from Australia and South Africa (MG3). The isolates from these countries very closely related, but the variation between them is supported by very strong bootstrap values. The fourth species, isolated from mango in South Africa, reside in clade VIII [BOT2399, BOT2376, BOT2422, BOT2430] and represents *L. theobromae* (*B. rhodina*) (MG4).

All isolates in clades II [CMW7801, CMW7024] and VII [CMW7803, CMW7020, CMW7027] were collected in Australia and represent the species *F. mangiferum* and *F. aesculi* respectively (Table 1; p63 - 64). Clade V [BOT945 (plum) and BOT931 (pear)] included *Botryosphaeria* isolates from other fruit trees in South Africa and represents the anamorph species, *F. luteum* (Slippers *et al.*, 2001; Phillips *et al.*, 2002). Clade III [BOT11, BOT32] represents *F. eucalyptorum* isolates from *Eucalyptus* trees in South Africa (Smith *et al.*, 2001). Clades VIII and IX represent sequence data for *B. obtusa* and *Sphaeropsis sapinea* isolates obtained from Genbank.

TAXONOMY

Results of morphological comparisons and DNA sequence comparisons have clearly shown that two undescribed species of *Fusicoccum* occur in South Africa. One is equivalent to the fungus previously known as *Dothiorella* 'long' in Australia and the other has not been isolated previously. These fungi are, therefore, described as new species in *Fusicoccum* as follows:

These preliminary descriptions are presented only for the purpose of this thesis and formal descriptions will be published in the mycological literature.

Anamorph. ***Fusicoccum indigoticum*** R. Jacobs, B. Slippers et M.J. Wingf. sp. nov.

(Fig. 1C-D)

Colonies initially white with appressed to fluffy mycelium, becoming olivaceous grey (21''''') to olivaceous black (27''''m) within three to four days after inoculation, and

mycelium on reverse side of petri dish indigo blue (47''m) to black. Quick growing on PDA at 25°C, little to no growth below 15°C or above 30°C.

Conidiomata small, eustromatic, immersed in host sub-epidermally, iron grey (24'''''k), covered with thick, dark hyphae, rarely produced in culture on PDA. Conidiomata are multilocular, locules totally embedded in some instances without distinct ostioles. Locule walls consist of dark *textura angularis*, becoming thinner and hyaline towards conidiophores and conidiogenous cells.

Conidiophores hyaline, smooth, cylindrical, aseptate, unbranched, 9.2 – 18.5 x 0.5 – 1.0, formed from cells of locule wall.

Conidiogenous cells hyaline, cylindrical, granulate, produce the first formed conidia holoblastically, subsequent conidia formed enteroblastically, proliferating precurrently with two to three precurrent proliferations and formation of annellations, (10.3-)10.5 – 13.6(-15.9) x (0.8-)1.3 – 1.7(2.4).

Conidia hyaline, ovoid to slightly ellipsoid, straight, granulate, thin walled, immature conidia aseptate. Conidia are evenly tapered at both ends with a bluntly rounded to obtuse base and truncate apex, widest part at the middle or upper third of conidia. In most instances, conidia become light brown and uni- to biseptate at maturity prior to germination. Long, sparingly branched germ tubes grown from one or more of the individual cells of the conidia. Conidia (17.5-)19.5 – 21(-24) x (5-)5.5 – 6.5(-7.1)µm [l/w = 3.4].

Teleomorph. Unknown Botryosphaeria sp. (not seen in this study)

Etymology. Name refers to the indigo-black colour of the reverse side of colonies on PDA.

Host. Mangiferae indica Linn.

Distribution. Mpumalanga, South Africa.

Specimens examined. Hoedspruit and Letsetele Valley, Mpumalanga, South Africa, 2000-2001, R. Jacobs.

Holotype: PREM 57316 (BOT 2355), isolated from canker lesion on mango leaf.

Paratype: PREM 57317 (BOT 2351), isolated from soft brown rot lesion on mango fruit.

Anamorph. ***Fusicoccum bacilliforme*** R. Jacobs, B. Slippers et M.J. Wingf. sp. nov.

(Fig. 1E-F)

Colonies initially white with sparse aerial mycelium clustered in concentric circles, becoming pale olivaceous grey (21''''d) to olivaceous grey (21''''') within five to seven days after inoculation. Mycelium colour on reverse side of petri dish grey olivaceous (21'''') to olivaceous grey (21'''''), dendritic pattern visible. Colonies quick growing on PDA at 25°C, with little to no growth below 15°C or above 30°C. A pale luteus (18f) pigment is produced in young cultures, which readily diffuses into the medium.

Conidiomata small, eustromatic, immersed in host sub-epidermally, covered with thick, pale white to smoke grey (21''''f) hyphae at all times, produced in concentric circles in culture on PDA. Conidiomata are multilocular, locules totally embedded in some instances, with ostioles. Locule walls consist of dark *textura angularis*, becoming thinner and hyaline towards conidiophores and conidiogenous cells.

Conidiophores hyaline, smooth, cylindrical, aseptate to uniseptate, unbranched, formed from cells of locule wall, 13.4 – 21.8(-22) x 0.4 – 0.9.

Conidiogenous cells hyaline, clavate to cylindrical, granulate, produce the first formed conidia holoblastically, subsequent conidia formed enteroblastically, proliferating precurrently with two to three precurrent proliferations, 18.7 – 20.8(-25.2).

Conidia hyaline, bacilliform to cylindrical, straight to slightly curved, smooth, thin walled, aseptate. Conidia are evenly rounded at both ends with a bluntly rounded to obtuse

base and truncate apex. Conidia not dark or septate prior to germination. Conidia (18.8-)20.8 – 23(-25.5) x (3.7-)4.1 – 5.2(-5.7)µm [l/w = 4.6].

Teleomorph. Unknown Botryosphaeria sp. (not seen in this study)

Etymology. The name referes to the distinctive bacilliform conidia in this fungus.

Host. Mangiferae indica Linn.

Distribution. Mpumalanga, South Africa.

Specimens examined. Malelaan, Mpumalanga, South Africa, 2001, R. Jacobs.

Holotype: PREM 57318 (BOT 2417), isolated from canker lesion on mango stem.

Paratype: PREM 57319 (BOT 2421), isolated from canker lesion on mango stem.

DISCUSSION

Results of this study show clearly that four *Botryosphaeria* spp. occur on mango in South Africa. This is the first time that the taxonomy of these fungi on mango has been studied in South Africa and results will facilitate more effective management of the various diseases associated with mango. In the past, at least three of the fungi found in this study, have been indiscriminately assigned to species. From a taxonomic point of view, names used in previous South African publications should be viewed with a level of discression. The four species of *Botryosphaeria* occurring on mango in South Africa can be identified relatively easily based on morphological characteristics, especially those pertaining to conidia. These morphological species could also consistently be separated based on ITS and ß-tubulin gene sequences. They represent *F. parvum*, *L. theobromae* (teleomorph *B. rhodina*) and two undescribed *Fusicoccum* spp., for which names are provided here.

The majority of isolates collected in this study reside in clade I, which represents *B. parva* (Slippers *et al.*, 2001). *Fusicoccum parvum*, the *Fusicoccum* anamorph of this species is the form most frequently encountered in nature. Conidia of this *Fusicoccum* sp. assigned to MG1 typically become uni- to bisepate and darker with maturity. In this sense, the conidia are similar than those of morphological group two (MG2). However, those of *B. parva* are more tapered at the ends with more truncate bases than those of MG2. The fluffy cultural morphology is also very distinctive for this species. On mango, this fungus has commonly been treated under the name *D. dominicana* (Johnson, 1992), but has been shown to be *F. parvum* by Slippers *et al.* (2001).

*Botryosphaeria parva (*previously known as *B. ribis)* is a well-known pathogen of many woody plants world-wide (Von Arx, 1987; Punithalingham, 1980). It is also recognised as existing in healthy plants as latent pathogens. Our isolates were from both healthy and symptomatic tissues, confirming the endophytic nature of this fungus on mango. *Fusicoccum parvum* has been isolated regularly from mango in various countries and is considered the primary causal agent of pre- and postharvest disease (Darvas, 1991; Ramos *et al.*, 1991; Johnson, 1992). Although pathogenicity tests are required, the frequency of collection of this fungus in the present study, tends to support results of previous pathological studies.

A unique *Fusicoccum* sp. was isolated from fruit and leaves of mango from South Africa, as part of this study. Both molecular and morphological data confirmed that the fungi represents a previously undescribed taxon and it was thus assigned the name *F. indigoticum*. The closest related species, based on ITS and ß-tubulin sequence data is *F. luteum* (clade V). The conidial morphology of this new species resembles that of *F.*

parvum, but it remains distinct in cultural morphology. Colonies are darker and more appressed than seen for other species. Mature conidia of *F. indigoticum* also tend not to become pigmented prior to germination, although this characteristic is not consistently useful. Conidial morphology alone may be confusing in defining this species and we recommend combining molecular and morphological data for identification.

Two isolates obtained in this study resided in a discrete clade (clade VI). The two isolates in this clade were recognised by Johnson *et al.* (1991) as an unknown species, from a mango SER pathogen survey in Australia. The fungus was not formally described, but referred to as *Dothiorella* 'long' (Johnson *et al.*, 1991; Johnson, 1992). *Dothiorella* 'long' has, however, been shown to belong to the genus *Fusicoccum* (Slippers *et al.*, 2001). In our study, clade VI isolates made up morphological group MG3, in which conidia are cylindrical to bacilliform and a yellow pigment is produced in the growth medium. Mycelial clumps are also produced in concentric rings, which is very different to any of the other *Botryosphaeria* spp. studied here. Sequence data separated the pairs of isolates from Australia and South Africa in clade VI. These groups were, however, not treated as distinct species due to the limited number of isolates and the lack of further distinction between them. We have, therefore, provided the name *Fusicoccum bacilliforme* for all isolates in clade VI. This species is made up of morphologically similar isolates from Australia and South Africa.

The only *Botryosphaeria* spp. with thick walled, dark conidia collected in this study was *L. theobromae* (*B. rhodina*). Isolates of this fungus were easily identified based on morphological characters and identifications were confirmed using DNA sequence data. *Lasiodiplodia theobromae* is known to cause SER of various fruit crops (Punithalingam,

1980) and infections are thought to occur both pre- and postharvest on mango. Althou this species is commonly isolated together with *Botryosphaeria* spp. having hyal conidia (eg. *B. ribis*), it tends to dominate the same niche only in warm, tropical regi (Brown & Britton, 1986; Johnson, 1992).

Results of this study lead us to conclude that four *Botryosphaeria* spp. occur on mango South Africa. Two are new species of *Fusicoccum* of which one occur on mango in So Africa and the other on mango in South Africa and Australia. Other *Botryosphaeria* s have, however, also been implicated as causal agents of diseases on mango in ot countries, such as *F. mangiferum* (known as *D. mangiferae*) and *F. aesculi* (known as *aromatica*) (Table 2; p71), which are commonly collected in Australia (Johnson, 19 Slippers *et al.*, 2001). These species are endophytes and care should be taken not introduce them on vegetative growing material, which is often transported betwe countries.

REFERENCES

Bruns, T.D., White, T.J. & Taylor, J.W. 1991. Fungal molecular systematics. *Annual Review of Ecological Systems* 22: 525-564.

Brown, E.A. & Britton, K.O. 1986. *Botryosphaeria diseases* of apple and peach in the Southern United States. *Plant Disease* 70: 480-484.

Carbone, I. & Kohn, L.M. 1993. Ribosomal DNA sequence divergence within internal transcribed spacer 1 of the Sclerotiniaceae. *Mycologia* 85: 415-427.

Crous, P.W. & Palm, M.E. 1999. Reassessment of the anamorph genera *Botriodiplodia, Dothiorella* and *Fusicoccum*. *Sydowia* 52: 167-175.

Darvas, J.M. 1991. *Dothiorella dominicana*, a new mango pathogen in South Africa. *Phytophylactica* 23: 2950-298.

Denman, S., Crous, P.W., Taylor, J.E., Kang, J., Pascoe, I. & Wingfield, M.J. 2000. An overview of the taxonomic history of *Botryosphaeria*, and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. *Studies in Mycology* 45: 129-140.

Felsenstein, J. 1985. Confidence intervals on phylogenetics: an approach using bootstrap. *Evolution* 39: 783-791.

Glass, N.L. & Donaldson, G.C. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61: 1323-1330.

Hillis, D.M. & Huelsenbeck, J.P. 1992. Signal, noise and reliability in molecular phylogenetic analysis. *Journal of Heredity* 83: 189-195.

Heulsenbeck, J.P., Bull, J.J. & Cunnigham, C.W. 1996. Combining data in phylogenetic analysis. *Tree* 11: 152-158.

Jacobs, K.A. & Rehner, S.A. 1998. Comparison of cultural and morphological characters and ITS sequencing in anamorphs of *Botryosphaeria* and related taxa. *Mycologia* 90: 601–610.

Johnson, G.I. 1992. Stem end rot pathogens of mango in Australia. PhD Thesis. University of Queensland, Australia.

Johnson, G.I., Cooke, A.W., Mead, A.J. & Wells, I.A. 1991. Stem-end rot of mangoes in Australia: Causes and control. *Acta Horticulturae* 291: 288-295.

Maniatis, T., Fritsch, E.F. & Sambrook, J. 1982. Molecular cloning: A laboratory manual. Cold Spring Harbour Laboratory, Cold Spring Harbour, New York.

McPartland, J.M. & Schoeneweiss, D.F. 1984. Hyphal morphology of *Botryosphaeria dothidea* in vessels of unstressed and drought-stressed stems of *Betula alba*. *Phytopathology* 74: 358-362.

Mitchell, J.I., Roberts, P.J. & Moss, S.T. 1995. Sequence or structure? A short review on the application of nucleic acid sequence information to fungal taxonomy. *Mycologist* 9: 67-75.

O'Donnell, K. 1992. Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete *Fusarium sambucinum* (*Gibberella pulicaris*). *Current Genetics* 22: 213-220.

O'Donnell, K. & Cigelnik, L. 1995. Multiple gene phylogenies reveal ITS 2 polymorphisms predate speciation within the '*Giberrella fujikoroi* complex' of *Fusarium*. *Inoculum* 46: 32.

Pennycook, S.R. & Samuels, G.J. 1985. *Botryosphaeria* and *Fusicoccum* species associated with ripe fruit rot of *Actinidia deliciosa* (kiwifruit) in New Zealand. *Mycotaxon* 24: 445–458.

Phillips, A.J.L., Fonesca, F., Povoa, V., Castilho, R. & Nolasco, G. 2002. A reassessment of the anamorphic fungus *Fusicoccum luteum* and description of its teleomorph *Botryosphaeria lutea* sp. nov. Sydowia (in press).

Ploetz, R.C. 1994. Part III. Mango: Stem-end rot. In: Compendium of tropical fruit diseases. Ploetz, R.C., Zentmyer, G.A., Nishijima, W.T., Rohrbach, K.G. & Ohr, H.D. (Eds.). pp. 36-37. APS Press. St Paul, Minnesota.

Punithalingam, E. 1980. Plant diseases attributed to *Botryodiplodia theobromae* Pat., pp. 42-43. J. Cramer. Germany. 2,

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

Ramos, L.J., Lam, S.P., McMillan, R.J. & Narayanan, K.R. 1991. Tip die-back of mango (*Mangiferae indica*) caused by *Botryosphaeria ribis*. *Plant Disease* 75: 315–318.

Rayner, R.W. 1970. A mycological colour chart. Commonwealth Mycological Institute, Kew, Surrey and British Mycological Society.

Slippers, B., Johnson, G.I., Cooke, A.W., Crous, P.W., Coutinho, T.A., Wingfield, B.D. & Wingfield, M.J. 2001. Taxonomy of *Botryosphaeria* spp. causing stem end rot of mango in Australia. In: Proceedings of the 13th American Plant Pathological Society. 25-29 August. Cairns, Australia.

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

Smith, H., Crous, P.W., Wingfield, M.J., Coutinho, T.A. & Wingfield, B.D. 2001. B*otryosphaeria eucalyptorum* sp. nov., a new species in the *B. dothidea*-complex on *Eucalyptus* in South Africa. *Mycologia* 93: 277-284.

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

Sutton, B.C. 1980. The *Coelomycetes*. Fungi Imperfecti with pycnidia, acervuli and stroma. pp 385. CMI, Kew, Surrey, England.

Swofford, D.L. 1998. PAUP*. Phylogenetic analysis using parsimony (* and other methods). Version4. Sunderland, Massachusetts: Sinauer Associates.

Taylor, J.W., Jacobson, D.J., Kroken, S., Kasuga, T., Geiser, D.M. & Hibbett, D.S. 2000. Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology* 31: 21-32.

Von Arx, J.A. 1987. Plant-pathogenic fungi. pp. 203-204. J. Cramer, Berlin, Germany.

White, T.J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols: A Guide to Methods and Applications*. M.A. Innis, D.H. Gelfand, J.J. Sninsky & T.J. White (Eds.). pp. 315-322. Academic Press: San Diego, U.S.A.

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

Table 1 Isolates used in the phylogenetic and morphological study of *Botryosphaeria* spp. from mango in South Africa

Culture nr.[a]	Identity[b]	Morphological group	Host	Location	Isolator	Original isolate nr.
BOT 2413	*Fusicoccum parvum*	MG1	*Mangiferae indica (mango)*	Mpumalanga, SA	R. Jacobs	***
BOT 2302	*F. parvum*	MG1	*M. indica*	Mpumalanga, SA	R. Jacobs	***
BOT2398	*F. parvum*	MG1	*M. indica*	Mpumalanga, SA	R. Jacobs	***
BOT 2353	*F. parvum*	MG1	*M. indica*	Mpumalanga, SA	R. Jacobs	***
BOT 2331	*F. parvum*	MG1	*M. indica*	Mpumalanga, SA	R. Jacobs	***
BOT2352	*F. parvum*	MG1	*M. indica*	Mpumalanga, SA	R. Jacobs	***
B OT 2339	*F. parvum*	MG1	*M. indica*	Mpumalanga, SA	R. Jacobs	***
BOT2382	*F. parvum*	MG1	*M. indica*	Mpumalanga, SA	R. Jacobs	***
BOT2363	*F. parvum*	MG1	*M. indica*	Mpumalanga, SA	R. Jacobs	***
BOT 2345	*F. parvum*	MG1	*M. indica*	Mpumalanga, SA	R. Jacobs	***
BOT2291	*F. parvum*	MG1	*M. indica*	Mpumalanga, SA	R. Jacobs	***
BOT2405	*F. parvum*	MG1	*M. indica*	Mpumalanga, SA	R. Jacobs	***
CMW 7799	*F. parvum*	***	*Persea americana*	Australia	G.I. Johnson	BRIP23300
CMW 7026	*F. parvum*	***	*M. indica*	Australia	G.I. Johnson	BRIP19684
CMW 7025	*F. parvum*	***	*M. indica*	Australia	G.I. Johnson	BRIP24083
BOT 2351	*F. indigoticum*	MG2	*M. indica*	Mpumalanga, SA	R. Jacobs	***
BOT 2355	*F. indigoticum*	MG2	*M. indica*	Mpumalanga, SA	R. Jacobs	***
BOT 2417	*F. bacilliforme*	MG3	*M. indica*	Mpumalanga, SA	R. Jacobs	***
BOT 2421	*F. bacilliforme*	MG3	*M. indica*	Mpumalanga, SA	R. Jacobs	***
CMW 7802	*F. bacilliforme*	MG3	*M. indica*	Phillipenes	G.I. Johnson	BRIP23491
CMW 7022	*F. bacilliforme*	MG3	*M. indica*	Phillipenes	G.I. Johnson	BRIP19782
BOT 2376	*Botryosphaeria rhodina*	MG4	*M. indica*	Mpumalanga, SA	R. Jacobs	***
BOT 2422	*B. rhodina*	MG4	*M. indica*	Mpumalanga, SA	R. Jacobs	***
BOT 2430	*B. rhodina*	MG4	*M. indica*	Mpumalanga, SA	R. Jacobs	***
BOT 2399	*B. rhodina*	MG4	*M. indica*	Mpumalanga, SA	R. Jacobs	***
CMW 7801	*F. mangiferum*	***	*M. indica*	Australia	G.I. Johnson	BRIP23396
CMW 7024	*F. mangiferum*	***	*M. indica*	Australia	G.I. Johnson	BRIP24101
CMW 7803	*F. aesculi*	***	*M. indica*	Australia	G.I. Johnson	BRIP23750
CMW 7020	*F. aesculi*	***	*M. indica*	Australia	G.I. Johnson	BRIP24286
CMW 7027	*F. aesculi*	***	*M. indica*	Australia	G.I. Johnson	BRIP24172
BOT 11	*B. eucaliptorum*	***	*Eucalyptus grandis*	Mpumalanga, SA	H. Smith	AF283684
BOT 32	*B. eucaliptorum*	***	*E. grandis*	Mpumalanga, SA	H. Smith	AF283685
BOT 931	*F. luteum*	***	*Pyrus communis* (pear)	Hermanus, SA	W.A. Smit	***

Table 1 Continued

Culture nr.[a]	Identity[b]		Host	Location	Isolator	Original isolate nr.
BOT 945	*F. luteum*	***	*Prunus* sp. (plum)	Pickstons, Klapmuts, SA	W.A. Smit	***
KJ93.52	*F. luteum*	***	*Actinidia deliciosa*	New Zealand	G.J. Sameuls	AF027745
KJ93.27	*B. rhodina*	***	*Quercus* sp.	California, USA	E. Hecht-Pointer	AF027761
KJ93.41	*B. rhodina*	***	*Pistacia* sp. (pastachio)	California, USA	T.J. Michailides	AF027762
KJ93.29	*Diplodia quercina*	***	*Quercus* sp.	California, USA	E. Hecht-Pointer	AF027753
KJ93.42	*B. dothidea*	***	*Malus* sp. (apple)	Washington D.C., USA	K.A. Jacobs	AF027741
KJ93.56	*B. obtusa*	***	Hardwood shrub	New York, USA	G.J. Sameuls	AF027759
KJ94.07	*Sphaeropsis sapinea*	***	*Pinus resinosa* (pine)	Wisconsin, USA	M. Palmer	AF027758
KJ94.09	*B. ribis*	***	*Melaleuca quinquenervia*	Florida, USA	M.B. Rayachhetry	AF027743
KJ94.26	*B. dothidea*	***	*P. persica* (peach)	Japan	P.L. Pusey	AF027749
KJ93.35	*B. stevensii*	***	*Q. suber*	Spain	K.A. Jacobs	AF027754
CMW 7063	*Guignardia philoprina*	***	*Taxus baccata*	Netherlands	H.A. van der Aa	***

[a]Culture collections where isolates are kept: BOT and CMW = Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria; BO = ARC Infruitec-Nietvoorbij; KJ = Jacobs & Rehner (1998); BPIR = Queensland Plant Pathology Herbarium.

[b]Identities as determined in this study

Figure 1. Morphological structures associated with *Botryosphaeria* spp. from mango in South Africa. *Fusicoccum parvum* showing (A) conidiophores with attached conidiogenous cells and (B) immature, aseptate conidia; *F. indigoticum* showing (C) Conidiophores with conidiogenous cell initials and conidiogenous cells. The formation of annelations with precurrent proliferation (arrow) is also evident and (D) immature, aseptate conidia; *F. bacilliforme* showing (E) conidiophores and conidiogenous cells and (F) aseptate, bacilliform conidia; *Lasiodiplodia theobromae* showing (G) conidiogenous cells attached to conidiophores with a dark, uniseptate mature conidium and (H) a mature, striate, dark, septate conidium and an immature, hyaline, aseptate conidia.

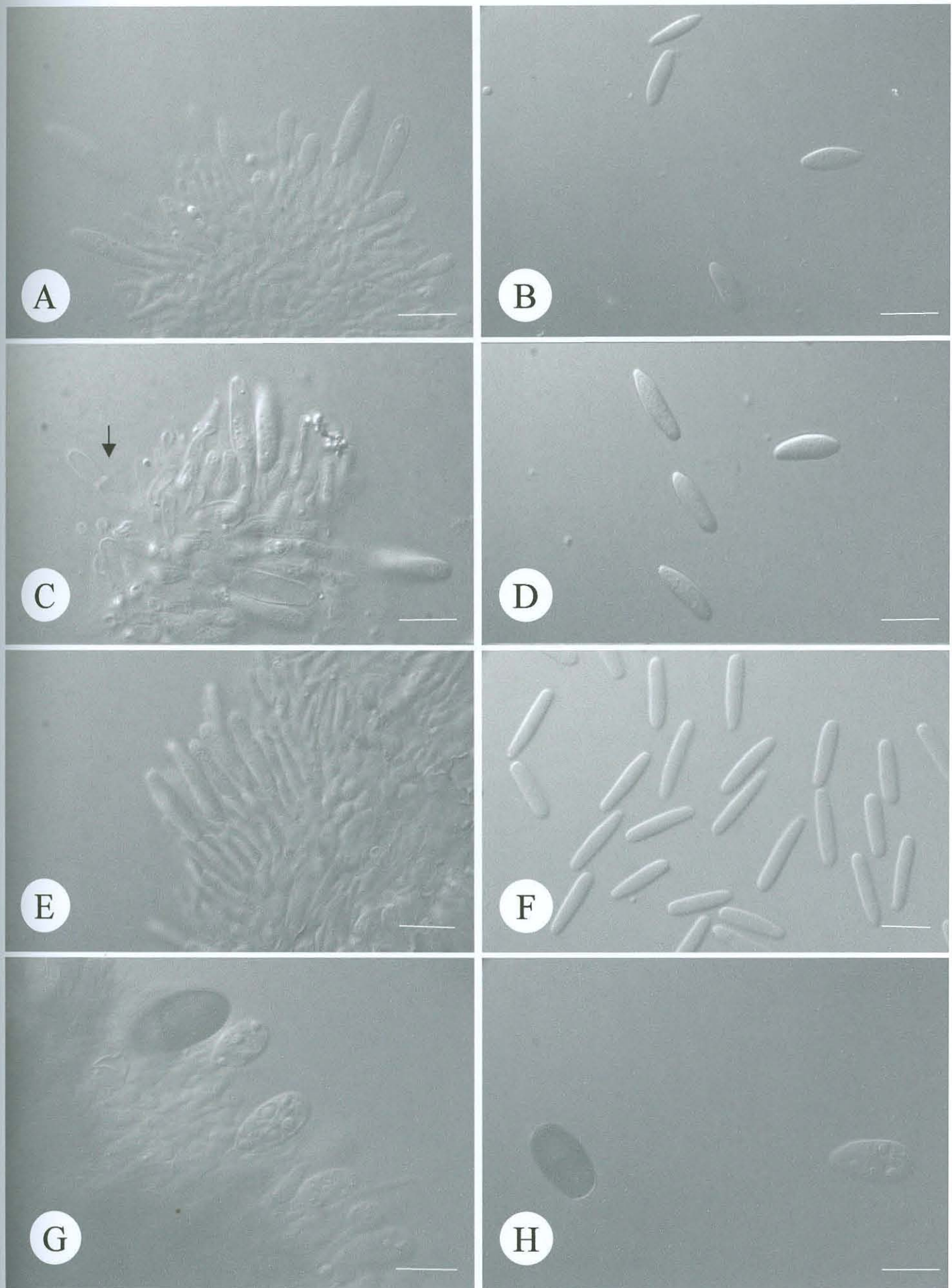


Figure 2. Cultural characteristics of *Botryosphaeria* species associated with mango diseases in South Africa. (A) *Fusicoccum indigoticum,* morphological group MG2; (B) *F. bacilliforme,* morphological group MG3; (C) *F. parvum,* morphological group MG1; (D) *Lasiodiplodia theobromae,* morphological group MG4. All isolates are on potato dextrose agar plates that are arranged to display three-day-old (top), nine-day-old (middle) and 14 day-old (bottom) cultures of each species.

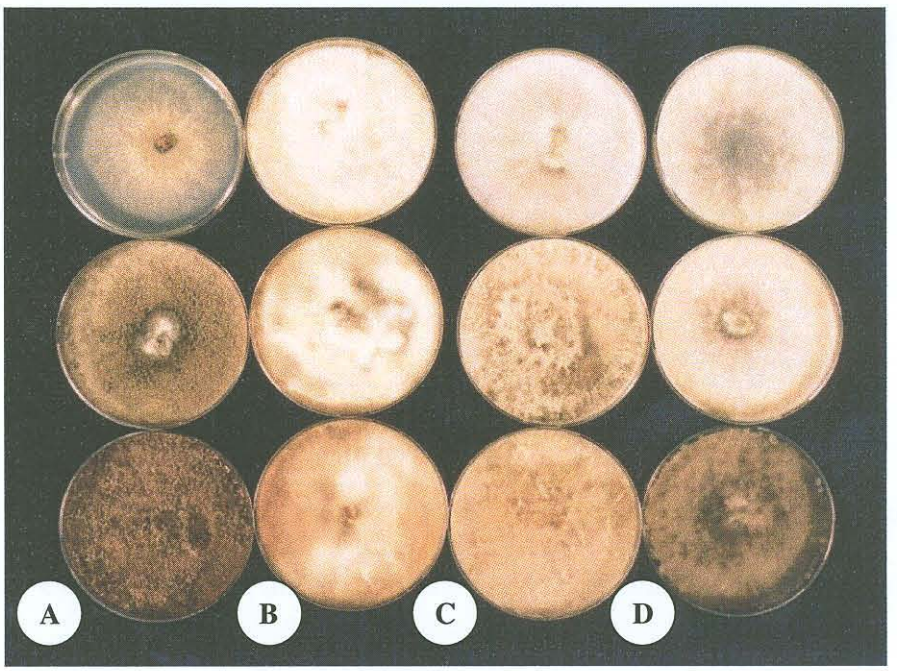


Figure 3. A phylogenetic tree generated after a heuristic search of the ITS 1, 5.8S and ITS 2 sequence data combined with the β-tubulin sequence data sets of *Botryosphaeria* spp. used in this study. Names of *Botryosphaeria* sp. And *Fusicoccum* anamorphs are given with each clade. Bootstrap values are indicated above each branch. The identity of each isolate is indicated by the culture number. Clades I, IV, VI and VIII contains mango isolates from South Africa, which were isolated during this study.

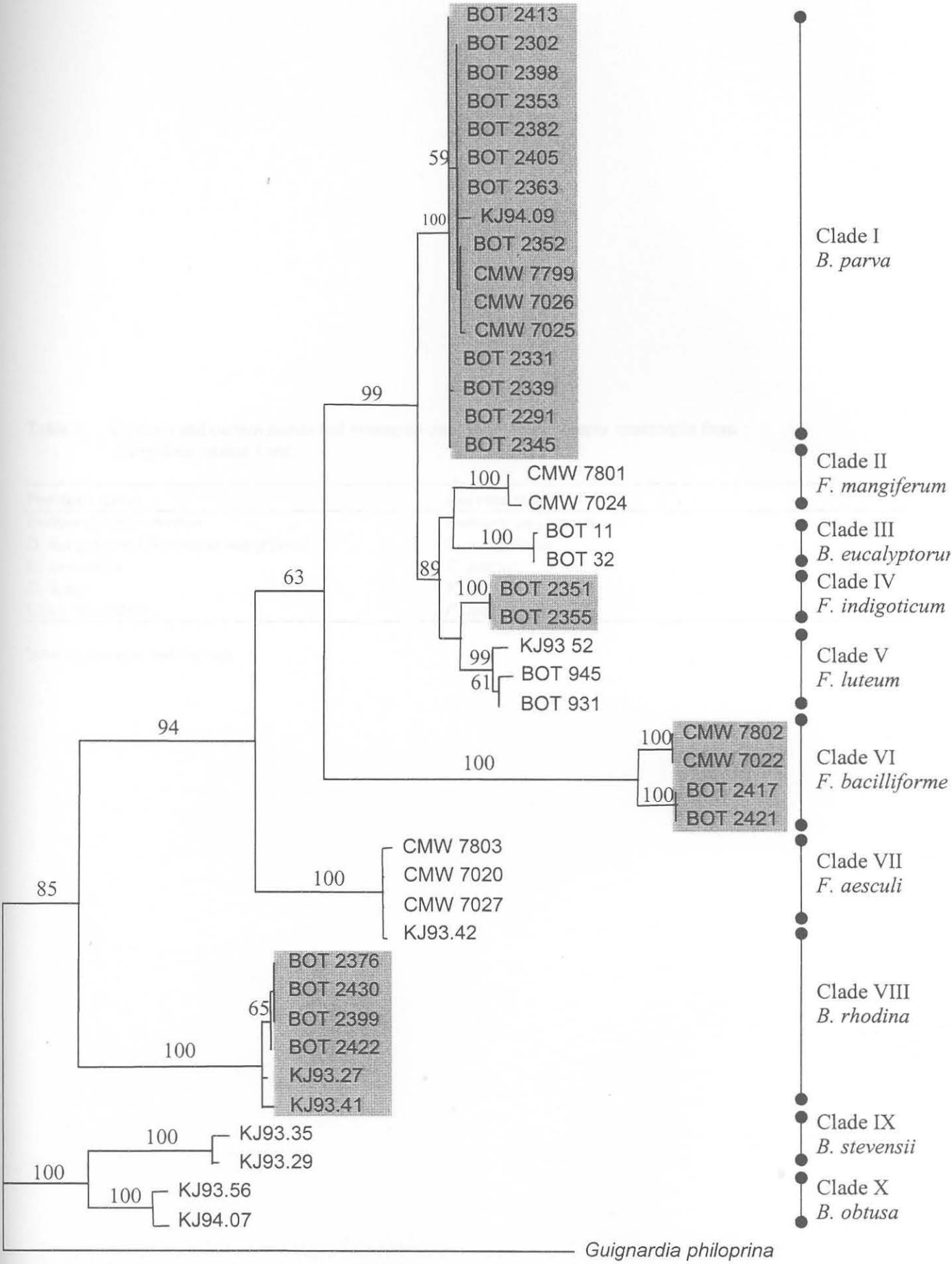


Table 2 Previous and current names and synonyms used for *Botryosphaeria* anamorphs from *Mangiferae indica* Linn.

Previous name	**Current name**[1]
Dothiorella dominicana	*Fusicoccum parvum*
D. mangiferae / Nattrassia mangiferae	*F. mangiferum*
D. aromatica	*F. aesculi*
D. 'long'	*F. bacilliforme*
Unknown species	*F. indigoticum*

[1]After Slippers *et al.* and this study

APPENDIX

Raw sequence data of the ITS1, 5.8S and ITS2 regions (characters 1-559) of the rDNA operon combined with the β-tubulin 2 gene region (characters 560-1009) of Botryosphaeria spp. used during this study. Unknown characters are indicated with a 'N', while gaps inserted in the sequence data are indicated with'-'.

APPENDIX

```
                                10         20         30         40         50
                                .          .          .          .          .
BOT 2413                GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
BOT 2302                GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
BOT 2398                GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
BOT 2353                GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
BOT 2331                GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
BOT 2339                GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
BOT 2382                GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
BOT 2405                GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
BOT 2291                GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
BOT 2345                GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
BOT 2363                GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
KJ94.09                 GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
BOT 2352                GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
CMW 7799                GGAAG-ATCA TTACCGAGTT GATTCGAGCT CCGGGTCGA- ----CTC-TC
CMW 7026                GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
CMW 7025                GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
CMW 7801                GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
CMW 7024                GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
BOT 2351                GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
BOT 2355                GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
KJ93.52                 GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
BOT 945                 GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
BOT 931                 GGAAGGATCA TTACCGAGTT GATTCGAGCT CCGGCTCGA- ----CTC-TC
BOT 11                  GGAAGGATCA TTACCGAGTT GACTCGAGTT CCGGCTCGA- ----CTC-TC
BOT 32                  GGAAGGATCA TTACCGAGTT GACTCGAGCT CCGGCTCGA- ----CTC-TC
CMW 7803                GGAAGGATCA TTACCGAGTT GATTCGGGCT CCGGCCCGA- -----TCCTC
CMW 7020                NNAAGGATCA TTACCGAGTT GATTCGGGCT CCGGCCCGA- -----TCCTC
CMW 7027                NNNAGGATCA TTACCGAGTT GATTCGGGCT CCGGCCCGA- -----TCCTC
KJ93.42                 GGAAGGATCA TTACCGAGTT GATTCGGGCT CCGGCCCGA- -----TCCTC
CMW 7802                GGAAGGATCA TTACCGAGTT TTGGGTCTCT TCACC--GAG CCCGCTC-TC
CMW 7022                GGAAGGATCA TTACCGAGTT TTGGGTCTCT TCACC--GAG CCCGCTC-TC
BOT 2417                GGAAGGATCA TTACCGAGTT TTGGGTCTCT TCACC--GAG CCCACTC-TC
BOT 2421                GGAAGGATCA TTACCGAGTT TTGGGTCTCT TCACC--GAG CCCACTC-TC
KJ93.35                 GGAAGGATCA TTACCGAGTG C-TACGAGCG AGAGCTCGTT A-----CCTC
KJ93.29                 GGAAGGATCA TTACCGAGTG C-TACGAGCG AGAGCTCGTT A-----CCTC
KJ93.56                 GGAAGGATCA TTACCGAGTT C-T-CGGGCT TCGGCTCGAA -----TC-TC
KJ94.07                 GGAAGGATCA TTACCGAGTT C-T-CGGGCT TCGGCTCGAA -----TC-TC
BOT 2376                GGAAGGATCA TTACCGAGTT --TTCGAGCT CCGGCTCGA- ----CTC-TC
BOT 2422                GGAAGGATCA TTACCGAGTT --TTCGAGCT CCGGCTCGA- ----CTC-TC
BOT 2430                GGAAGGATCA TTACCGAGTT --TTCGAGCT CCGGCTCGA- ----CTC-TC
BOT 2399                GGAAGGATCA TTACCGAGTT --TTCGAGCT CCGGCTCGA- ----CTC-TC
KJ93.27                 GGAAGGATCA TTACCGAGTT --TTCGGGCT TCGGCTCGA- ----CTC-TC
KJ93.41                 GGAAGGATCA TTACCGAGTT --TTCGGGCT TCGGCTCGA- ----CTC-TC
Guignardia philoprina   GGAAGGATCA TTACCGAGTT ---------- ---------T ACAACTC--C
```

```
                                  60         70         80         90         100
                                   .          .          .          .          .
BOT 2413                  CCACCCAATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
BOT 2302                  CCACCCAATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
BOT 2398                  CCACCCAATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
BOT 2353                  CCACCCAATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
BOT 2331                  CCACCCAATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
BOT 2339                  CCACCCAATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
BOT 2382                  CCACCCAATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
BOT 2405                  CCACCCAATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
BOT 2291                  CCACCCAATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
BOT 2345                  CCACCCAATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
BOT 2363                  CCACCCAATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
KJ94.09                   CCACCCAATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
BOT 2352                  CCACCCTATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
CMW 7799                  CCACCCTATG TGTACCT-AC TTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
CMW 7026                  CCACCCTATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
CMW 7025                  CCACCCTATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
CMW 7801                  CCACCCTATG TGTACCT-AC CTC-CGTTGC TTTGGCGGGC CGCGGTCCT-
CMW 7024                  CCACCCTATG TGTACCTTAC CTC-CGTTGC TTTGGCGGGC CGCGGTCCT-
BOT 2351                  CCACCCTATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
BOT 2355                  CCACCCTATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
KJ93.52                   CCACCCCATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
BOT 945                   CCACCCCATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
BOT 931                   CCACCCCATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
BOT 11                    CCACCCTATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
BOT 32                    CCACCCTATG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
CMW 7803                  CCACCCTTTG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGACCT-
CMW 7020                  CCACCCTTTG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
CMW 7027                  CCACCCTTTG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
KJ93.42                   CCACCCTTTG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTCCT-
CMW 7802                  CAACCCTTTG TGTACCT-AC CTC-TGTTGC TTTG-CGGGC CGCGGTTCT-
CMW 7022                  CAACCCTTTG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTTCT-
BOT 2417                  CAACCCTTTG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTTCT-
BOT 2421                  CAACCCTTTG TGTACCT-AC CTC-TGTTGC TTTGGCGGGC CGCGGTTCT-
KJ93.35                   CCACCCTTTG TGAACAT-AC CTC-TGTTGC TTTGGCGG-C -----TCTC-
KJ93.29                   CCACCCTTTG TGAACAT-AC CTC-TGTTGC TTTGGCGG-C -----TCTC-
KJ93.56                   CCACCCTTTG TGAACAT-AC CTC-TGTTGC TTTGGCGG-C -----TCTTT
KJ94.07                   CCACCCTTTG TGAACAT-AC CTC-TGTTGC TTTGGCGG-C -----TCTTT
BOT 2376                  CCACCCTTTG TGAACGT-AC CTC-TGTTGC TTTGGCGG-C -----TCCG-
BOT 2422                  CCACCCTTTG TGAACGT-AC CTC-TGTTGC TTTGGCGG-C -----TCCG-
BOT 2430                  CCACCCTTTG TGAACGT-AC CTC-TGTTGC TTTGGCGG-C -----TCCG-
BOT 2399                  CCACCCTTTG TGAACGT-AC CTC-TGTTGC TTTGGCGG-C -----TCCG-
KJ93.27                   CCACCCTTTG TGAACGT-AC CTC-TGTTGC TTTGGCGG-C -----TTCG-
KJ93.41                   CCACCCTTTG TGAACGT-AC CTC-TGTTGC TTTGGCGG-C -----TCCG-
Guignardia philoprina     CAAACCCATG TGAACAT-AC CTATTGTTGC TTCGGCGGG- ------ATT-
```

```
                                110         120         130         140         150
                                .           .           .           .           .

BOT 2413                -CCGC-ACCG GC-GCCCTT- --CG-GGGGG -CTGGCCA-- GCGC------
BOT 2302                -CCGC-ACCG GC-GCCCTT- --CG-GGGGG -CTGGCCA-- GCGC------
BOT 2398                -CCGC-ACCG GC-GCCCTT- --CG-GGGGG -CTGGCCA-- GCGC------
BOT 2353                -CCGC-ACCG GC-GCCCTT- --CG-GGGGG -CTGGCCA-- GCGC------
BOT 2331                -CCGC-ACCG GC-GCCCTT- --CG-GGGGG -CTGGCCA-- GCGC------
BOT 2339                -CCGC-ACCG GC-GCCCTT- --CG-GGGGG -CTGGCCA-- GCGC------
BOT 2382                -CCGC-ACCG GC-GCCCTT- --CG-GGGGG -CTGGCCA-- GCGC------
BOT 2405                -CCGC-ACCG GC-GCCCTT- --CG-GGGGG -CTGGCCA-- GCGC------
BOT 2291                -CCGC-ACCG GC-GCCCTT- --CG-GGGGG -CTGGCCA-- GCGC------
BOT 2345                -CCGC-ACCG GC-GCCCTT- --CG-GGGGG -CTGGCCA-- GCGC------
BOT 2363                -CCGC-ACCG GC-GCCCTT- --CG-GGGGG -CTGGCCA-- GCGC------
KJ94.09                 -CCGC-ACCG GC-GCCCTT- --CG-GGGGG GCTGGCCA-- GCGC------
BOT 2352                -CCGC-ACCG GC-GCCCTT- --CG-GGGGG -CTGGCCA-- GCGC------
CMW 7799                -CCGC-ACCG GC-GCCCTT- --CG-GGGGG -CTGGCCA-- GCGC------
CMW 7026                -CCGC-ACCG GC-GCCCTT- --CG-GGGGG -CTGGCCA-- GCGC------
CMW 7025                -CCGC-ACCG GC-GCCCTT- --CG-GGGGG -CTGGCCA-- GCGC------
CMW 7801                -CCGC-ACCG GCTCCCC-T- --CG-AGGGG GCTGGCCA-- GCGC------
CMW 7024                -CCGC-ACCG GCTCCCC-T- --CG-AGGGG GCTGGCCA-- GCGC------
BOT 2351                -CCGC-ACCG GCCCCCCTT- --CG--GGGG -CTGGCCA-- GCGC------
BOT 2355                -CCGC-ACCG GCCCCCCTT- --CG--GGGG -CTGGCCA-- GCGC------
KJ93.52                 -CCGC-ACCG ACCCCCGTT- --CG--GGGG GCCGGCCA-- GCGC------
BOT 945                 -CCGC-ACCG ACCCCCGTT- --CG--GGGG -CCGGCCA-- GCGC------
BOT 931                 -CCGC-ACCG ACCCCCGTT- --CG--GGGG -CCGGCCA-- GCGC------
BOT 11                  -CCGC-ACCG GCTCCC-TTT ---G--GGGG -CTGGCCA-- GCGT------
BOT 32                  -CCGC-ACCG GCTCCC-TTT ---G--GGGG -CTGGCCA-- GCGT------
CMW 7803                -CCGCGGCCG --CCCCCCTC CCCG-GGGGG G-TGGCCA-- GCGC------
CMW 7020                -CCGCGGCCG --CCCCCCTC CCCG-GGGGG G-TGGCCA-- GCGC------
CMW 7027                -CCGCGGCCG --CCCCCCTC CCCG-GGGGG G-TGGCCA-- GCGC------
KJ93.42                 -CCGCGGNCG --CCCCCCTC CCCG-GGGGG G-TGGCCA-- GCGC------
CMW 7802                -CCGCGGCCG GCCCCC--TA GCCG-GGG-- -CTGGCC-T- GCGC------
CMW 7022                -CCGCGGCCG GCCCCC--TA GCCG-GGG-- -CTGGCC-T- GCGC------
BOT 2417                -CCGCGGCCG GCCCC--TTA ACCG-GGG-- -CTGGCC-T- GCGC------
BOT 2421                -CCGCGGCCG GCCCC--TTA ACCG-GGG-- -CTGGCC-T- GCGC------
KJ93.35                 GCCGCGAGGG GAGGCCC-TG AAAA-GGGCC CGCCCCCCTC GCGC-GCCCT
KJ93.29                 GCCGCGAGGG GAGGCCC-TG AAAA-GGGCC CGCCCCCCTC GCGC-GCCCT
KJ93.56                 CCCGCGAGG- -AGGCCC-T- --CGCGGGCC --C-CCCC-- -GCGCGCTTT
KJ94.07                 GCCGCGAGG- -AGGCCC-T- --CGCGGGCC --C-CCCC-- -GCGCGCTTT
BOT 2376                GCCGC------ ---------- ---------- ---------- ----------
BOT 2422                GCCGC------ ---------- ---------- ---------- ----------
BOT 2430                GCCGC------ ---------- ---------- ---------- ----------
BOT 2399                GCCGC------ ---------- ---------- ---------- ----------
KJ93.27                 GCCGC------ ---------- ---------- ---------- ----------
KJ93.41                 GCCGC------ ---------- ---------- ---------- ----------
Guignardia philoprina   GCCCCGGGC- ---GCC--T- --CGTGTGC- ----CCCGGA TCAGGCGCCC
```

```
                                  160         170         180         190         200
                                   .           .           .           .           .

BOT 2413                  CCGCCAGAGG ACCAT-AAAA CTCCAGTCAG TGAACTTCGC AGTCTGAAAA
BOT 2302                  CCGCCAGAGG ACCAT-AAAA CTCCAGTCAG TGAACTTCGC AGTCTGAAAA
BOT 2398                  CCGCCAGAGG ACCAT-AAAA CTCCAGTCAG TGAACTTCGC AGTCTGAAAA
BOT 2353                  CCGCCAGAGG ACCAT-AAAA CTCCAGTCAG TGAACTTCGC AGTCTGAAAA
BOT 2331                  CCGCCAGAGG ACCAT-AAAA CTCCAGTCAG TGAACTTCGC AGTCTGAAAA
BOT 2339                  CCGCCAGAGG ACCAT-AAAA CTCCAGTCAG TGAACTTCGC AGTCTGAAAA
BOT 2382                  CCGCCAGAGG ACCAT-AAAA CTCCAGTCAG TGAACTTCGC AGTCTGAAAA
BOT 2405                  CCGCCAGAGG ACCAT-AAAA CTCCAGTCAG TGAACTTCGC AGTCTGAAAA
BOT 2291                  CCGCCAGAGG ACCAT-AAAA CTCCAGTCAG TGAACTTCGC AGTCTGAAAA
BOT 2345                  CCGCCAGAGG ACCAT-AAAA CTCCAGTCAG TGAACTTCGC AGTCTGAAAA
BOT 2363                  CCGCCAGAGG ACCAT-AAAA CTCCAGTCAG TGAACTTCGC AGTCTGAAAA
KJ94.09                   CCGCCAGAGG ACCAT-AAAA CTCCAGTCAG TGAACTTCGC AGTCTGAAAA
BOT 2352                  CCGCCAGAGG ACCAT-AAAA CTCCAGTCAG TGAACTTCGC AGTCTGAAAA
CMW 7799                  CCGCCAGAGG ACCAT-AAAA CTCCAGTCAG TGAACTTCGC AGTCTGAAAA
CMW 7026                  CCGCCAGAGG ACCAT-AAAA CTCCAGTCAG TGAACTTCGC AGTCTGAAAA
CMW 7025                  CCGCCAGAGG ACCAT-AAAA CTCCAGTCAG TGAACTTCGC AGTCTGAAAA
CMW 7801                  CCGCCAGAGG ACCA-CAAAA CTCCAGTCAG TGAACGTTGC AGCCTGAAAA
CMW 7024                  CCGCCAGAGG ACCA-CAAAA CTCCAGTCAG TGAACGTTGC AGCCTGAAAA
BOT 2351                  CCGCCAGAGG ACCA-CAAAA CTCCAGTCAG TGAACGTCGC AGTCTGAAAA
BOT 2355                  CCGCCAGAGG ACCA-CAAAA CTCCAGTCAG TGAACGTCGC AGTCTGAAAA
KJ93.52                   CCGCCAGAGG ACCA-CAAAA CTCCAGTCAG TAAACGTCGC AGTCTGAGAA
BOT 945                   CCGCCAGAGG ACCA-CAAAA CTCCAGTCAG TAAACGTCGC AGTCTGAGAA
BOT 931                   CCGCCAGAGG ACCA-CAAAA CTCCAGTCAG TAAACGTCGC AGTCTGAGAA
BOT 11                    CCGCCAGAGG ACCA-CAAAA CTCCAGTCAG TAAACGTTGC AGTCTGAAAA
BOT 32                    CCGCCAGAGG ACCA-CAAAA CTCCAGTCAG TAAACGTTGC AGTCTGAAAA
CMW 7803                  CCGCCAGAGG ACCATCAAA- CTGCA-TCAG TAAACGATGC AGTCTGAAAA
CMW 7020                  CCGCCAGAGG ACCATCAAA- CTCCAGTCAG TAAACGATGC AGTCTGAAAA
CMW 7027                  CCGCCAGAGG ACCATCAAA- CTCCAGTCAG TAAACGATGC AGTCTGAAAA
KJ93.42                   CCGCCAGAGG ACCATCAAA- CTCCAGTCAG TAAACGATGC AGTCTGAAAA
CMW 7802                  CCGCCAGAGG ACCA-CAAAA CTCCAGTCAG TGAACTTTGC TGTCTGATAT
CMW 7022                  CCGCCAGAGG ACCA-CAAAA CTCCAGTCAG TGAACTTTGC TGTCTGATAT
BOT 2417                  CCGCCAGAGG ACCA-CAAAA CTCCAGTCAG TGAACTTTGC TGTCTGATAT
BOT 2421                  CCGCCAGAGG ACCA-CAAAA CTCCAGTCAG TGAACTTTGC TGTCTGATAT
KJ93.35                   CCGCCAGAGG ACCTTCAAA- CTCCAGTCAG TAAACGTCGA CGTCTGATAC
KJ93.29                   CCGCCAGAGG ACCTTCAAA- CTCCAGTCAG TAAACGTCGA CGTCTGATAC
KJ93.56                   CCGCCAGAGG ACCTTCAAA- CTCCAGTCAG TAAACGTCGA CGTCTGATAA
KJ94.07                   CCGCCAGAGG ACCTTCAAA- CTCCAGTCAG TAAACGTCGA CGTCTGATAA
BOT 2376                  ----CAAAGG ACCTTCAAA- CTCCAGTCAG TAAACGCAGA CGTCTGATAA
BOT 2422                  ----CAAAGG ACCTTCAAA- CTCCAGTCAG TAAACGCAGA CGTCTGATAA
BOT 2430                  ----CAAAGG ACCTTCAAA- CTCCAGTCAG TAAACGCAGA CGTCTGATAA
BOT 2399                  ----CAAAGG ACCTTCAAA- CTCCAGTCAG TAAACGCAGA CGTCTGATAA
KJ93.27                   ----CAAAGG ACCTTCAAA- CTCCAGTCAG TAAACGCAGA CGTCTGATAA
KJ93.41                   ----CAAAGG ACCTCCAAA- CTCCGGTCAG TAAACGCAGA CGTCTGATAA
Guignardia philoprina     GCCTAGGAAA --CTT--AA- CTCTTGTTTT ATTTTGGAAT CTTCTGAGTA
```

```
                                210        220        230        240        250
                                 .          .          .          .          .

BOT 2413                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 2302                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 2398                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 2353                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 2331                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 2339                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 2382                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 2405                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 2291                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 2345                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 2363                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
KJ94.09                 ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 2352                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
CMW 7799                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
CMW 7026                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
CMW 7025                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
CMW 7801                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
CMW 7024                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 2351                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 2355                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
KJ93.52                 ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 945                 ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 931                 ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 11                  ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 32                  ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
CMW 7803                ACA-TTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
CMW 7020                ACA-TTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
CMW 7027                ACA-TTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
KJ93.42                 ACATTTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
CMW 7802                A-AA-TT-C- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
CMW 7022                A-AA-TT-C- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 2417                A-AA-TT-C- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 2421                A-AA-TT-C- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
KJ93.35                 ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
KJ93.29                 ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
KJ93.56                 ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
KJ94.07                 ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 2376                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 2422                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 2430                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
BOT 2399                ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
KJ93.27                 ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
KJ93.41                 ACAAGTT--- AATAAACTAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
Guignardia philoprina   GTTT-TTACA AATAAATAAA AACTTTCAAC AACGGATCTC TTGGTTCTGG
```

```
                                260       270       280       290       300
                                .         .         .         .         .
BOT 2413                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 2302                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 2398                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 2353                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 2331                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 2339                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 2382                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 2405                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 2291                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 2345                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 2363                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
KJ94.09                 CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 2352                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
CMW 7799                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
CMW 7026                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
CMW 7025                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
CMW 7801                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
CMW 7024                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 2351                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 2355                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
KJ93.52                 CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 945                 CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 931                 CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 11                  CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 32                  CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
CMW 7803                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
CMW 7020                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
CMW 7027                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
KJ93.42                 CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
CMW 7802                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
CMW 7022                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 2417                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 2421                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
KJ93.35                 CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
KJ93.29                 CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
KJ93.56                 CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
KJ94.07                 CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 2376                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 2422                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 2430                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
BOT 2399                CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
KJ93.27                 CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
KJ93.41                 CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
Guignaqrdia philoprina  CATCGATGAA GAACGCAGCG AAATGCGATA AGTAATGTGA ATTGCAGAAT
```

```
                                310       320       330       340       350
                                .         .         .         .         .
BOT 2413                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
BOT 2302                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
BOT 2398                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
BOT 2353                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
BOT 2331                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
BOT 2339                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
BOT 2382                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
BOT 2405                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
BOT 2291                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
BOT 2345                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
BOT 2363                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
KJ94.09                 TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
BOT 2352                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
CMW 7799                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
CMW 7026                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
CMW 7025                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
CMW 7801                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
CMW 7024                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
BOT 2351                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
BOT 2355                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
KJ93.52                 TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
BOT 945                 TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
BOT 931                 TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
BOT 11                  TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
BOT 32                  TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
CMW 7803                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCTT TGGTATTCCG
CMW 7020                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCTT TGGTATTCCG
CMW 7027                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCTT TGGTATTCCG
KJ93.42                 TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCTT TGGTATTCCG
CMW 7802                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCTT TGGTATTCCG
CMW 7022                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCTT TGGTATTCCG
BOT 2417                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCTT TGGTATTCCG
BOT 2421                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCTT TGGTATTCCG
KJ93.35                 TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGCATTCCG
KJ93.29                 TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGCATTCCG
KJ93.56                 TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCC TGGCATTCCG
KJ94.07                 TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGCATTCCG
BOT 2376                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
BOT 2422                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
BOT 2430                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
BOT 2399                TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
KJ93.27                 TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
KJ93.41                 TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCCT TGGTATTCCG
Guignardia philoprina   TCAGTGAATC ATCGAATCTT TGAACGCACA TTGCGCCCGC CAGTATTCTG
```

```
                                360       370       380       390       400
                                .         .         .         .         .

BOT 2413                AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
BOT 2302                AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
BOT 2398                AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
BOT 2353                AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
BOT 2331                AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
BOT 2339                AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
BOT 2382                AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
BOT 2405                AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
BOT 2291                AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
BOT 2345                AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
BOT 2363                AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
KJ94.09                 AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
BOT 2352                AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
CMW 7799                AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
CMW 7026                AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
CMW 7025                AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
CMW 7801                AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
CMW 7024                AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
BOT 2351                AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
BOT 2355                AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
KJ93.52                 AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
BOT 945                 AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
BOT 931                 AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT CTGCTTGGTA
BOT 11                  AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT TTGCTTGGTA
BOT 32                  AGGGGCATGC CTGTTCGAGC GTCATTTCAA CCCTCAAGCT TTGCTTGGTA
CMW 7803                AAGGGCATGC CTGTTCGAGC GTCATTACAA CCCTCAAGCT CTGCTTGGTA
CMW 7020                AAGGGCATGC CTGTTCGAGC GTCATTACAA CCCTCAAGCT CTGCTTGGTA
CMW 7027                AAGGGCATGC CTGTTCGAGC GTCATTACAA CCCTCAAGCT CTGCTTGGTA
KJ93.42                 AAGGGCATGC CTGTTCGAGC GTCATTACAA CCCTCAAGCT CTGCTTGGTA
CMW 7802                AAGGGCATGC CTGTTCGAGC GTCATTACAC CCCTCAAGCT CTGCTTGGTG
CMW 7022                AAGGGCATGC CTGTTCGAGC GTCATTACAC CCCTCAAGCT CTGCTTGGTG
BOT 2417                AAGGGCATGC CTGTTCGAGC GTCATTACAC CCTTCAAGCT CTGCTTGGTA
BOT 2421                AAGGGCATGC CTGTTCGAGC GTCATTACAC CCTTCAAGCT CTGCTTGGTA
KJ93.35                 AGGGGCATGC CTGTTCGAGC GTCATTACAA CCCTCAAGCT CTGCTTGGTA
KJ93.29                 AGGGGCATGC CTGTTCGAGC GTCATTACAA CCCTCAAGCT CTGCTTGGTA
KJ93.56                 GGGGGCATGC CTGTTCGAGC GTCATTACAA CCCTCAAGCT CTGCTTGGTA
KJ94.07                 AGGGGCATGC CTGTTCGAGC GTCATTACAA CCCTCAAGCT CTGCTTGGTA
BOT 2376                GGGGGCATGC CTGTTCGAGC GTCATTACAA CCCTCAAGCT CTGCTTGGAA
BOT 2422                GGGGGCATGC CTGTTCGAGC GTCATTACAA CCCTCAAGCT CTGCTTGGAA
BOT 2430                GGGGGCATGC CTGTTCGAGC GTCATTACAA CCCTCAAGCT CTGCTTGGAA
BOT 2399                GGGGGCATGC CTGTTCGAGC GTCATTACAA CCCTCAAGCT CTGCTTGGAA
KJ93.27                 GGGGGCATGC CTGTTCGAGC GTCATTACAA CCCTCAAGCT CTGCTTGGAA
KJ93.41                 GGGGGCATGC CTGTTCGAGC GTCATTACAA CCCTCAAGCT CTGCTTGGAA
Guignardia philoprina   GCGGGCATGC CTGTCTGAGC GTCATTTCAA CCCTCATGCC CCTAGGGCGT
```

```
                                410        420        430        440        450
                                 .          .          .          .          .
BOT 2413                TTGGGCCCCG TCCTC-C-AC GGACGCGCCT TAAAGACCTC GGCGGTGGCG
BOT 2302                TTGGGCCCCG TCCTC-C-AC GGACGCGCCT TAAAGACCTC GGCGGTGGCG
BOT 2398                TTGGGCCCCG TCCTC-C-AC GGACGCGCCT TAAAGACCTC GGCGGTGGCG
BOT 2353                TTGGGCCCCG TCCTC-C-AC GGACGCGCCT TAAAGACCTC GGCGGTGGCG
BOT 2331                TTGGGCCCCG TCCTC-C-AC GGACGCGCCT TAAAGACCTC GGCGGTGGCG
BOT 2339                TTGGGCCCCG TCCTC-C-AC GGACGCGCCT TAAAGACCTC GGCGGTGGCG
BOT 2382                TTGGGCCCCG TCCTC-C-AC GGACGCGCCT TAAAGACCTC GGCGGTGGCG
BOT 2405                TTGGGCCCCG TCCTC-C-AC GGACGCGCCT TAAAGACCTC GGCGGTGGCG
BOT 2291                TTGGGCCCCG TCCTC-C-AC GGACGCGCCT TAAAGACCTC GGCGGTGGCG
BOT 2345                TTGGGCCCCG TCCTC-C-AC GGACGCGCCT TAAAGACCTC GGCGGTGGCG
BOT 2363                TTGGGCCCCG TCCTC-C-AC GGACGCGCCT TAAAGACCTC GGCGGTGGCG
KJ94.09                 TTGGGCCCCG TCCTC-C-AC GGACGCGCCT TAAAGACCTC GGCGGTGGCG
BOT 2352                TTGGGCCCCG TCCTC-C-AC GGACGCGCCT TAAAGACCTC GGCGGTGGCG
CMW 7799                TTGGGCCCCG TCCTC-C-AC GGACGCGCCT TAAAGACCTC GGCGGTGGCG
CMW 7026                TTGGGCCCCG TCCTC-C-AC GGACGCGCCT TAAAGACCTC GGCGGTGGCG
CMW 7025                TTGGGCCCCG TCCTC-C-AC GGACGCGCCT TAAAGACCTC GGCGGTGGCG
CMW 7801                TTGGGCTCCG TCCTC-C-GC GGACGCGCCT CAAAGACCTC GGCGGTGGCG
CMW 7024                TTGGGCTCCG TCCTC-C-GC GGACGCGCCT CAAAGACCTC GGCGGTGGCG
BOT 2351                TTGGGCTCCG TCCTC-C-AC GGACGCGCCT CAAAGACCTC GGCGGTGGCC
BOT 2355                TTGGGCTCCG TCCTC-C-AC GGACGCGCCT CAAAGACCTC GGCGGTGGCC
KJ93.52                 TTGGGCTCCG TCCTCT--GT GGACGCGCCT CGAAGACCTC GGCGGTGGCG
BOT 945                 TTGGGCTCCG TCCTC-C-GC GGACGCGCCT CGAAGACCTC GGCGGTGGCG
BOT 931                 TTGGGCTCCG TCCTC-C-GC GGACGCGCCT CGAAGACCTC GGCGGTGGCG
BOT 11                  TTGGGCCCCG TCCTCT--GT GGACGCGCCT CAAAGACCTC GGCGGTGGCG
BOT 32                  TTGGGCCCCG TCCTCT--GT GGACGCGCCT CAAAGACCTC GGCGGTGGCG
CMW 7803                TTGGGCACCG TCCT-T-TGC GGGCGCGCCT CAAAGACCTC GGCGGTGGCG
CMW 7020                TTGGGCACCG TCCT-T-TGC GGGCGCGCCT CAAAGACCTC GGCGGTGGCG
CMW 7027                TTGGGCACCG TCCT-T-TGC GGGCGCGCCT CAAAGACCTC GGCGGTGGCG
KJ93.42                 TTGGGCACCG GCCT-T-TGC GGGCGCGCCT CAAAGACCTC GGCGGTGGCG
CMW 7802                TTGGGCAGCG TCCTCTC--- GGACGCGCCT CAAAGACCTC GGCGGTGGCG
CMW 7022                TTGGGCAGCG TCCTCTC--- GGACGCGCCT CAAAGACCTC GGCGGTGGCG
BOT 2417                TTGGGCAGCG TCCTTTC--- GGACGCGCCT CAAAGACCTC GGCGGTGGCG
BOT 2421                TTGGGCAGCG TCCTTTC--- GGACGCGCCT CAAAGACCTC GGCGGTGGCG
KJ93.35                 TTGGGCGCCG TCCTCTCTGC GGACGCGCCT CAAAGACCTC GGCGGTGGC-
KJ93.29                 TTGGGCGCCG TCCTCTCTGC GGACGCGCCT CAAAGACCTC GGCGGTGGC-
KJ93.56                 TTGGGCGCCG TCCTCTCTGC GGACGCGCCT TAAAGACCTC GGCGGTGGC-
KJ94.07                 TTGGGCGCCG TCCTCTCTGC GGACGCGCCT TAAAGACCTC GGCGGTGGC-
BOT 2376                TTGGGCACCG TCCTCACTGC GGACGCGCCT CAAAGACCTC GGCGGTGGC-
BOT 2422                TTGGGCACCG TCCTCACTGC GGACGCGCCT CAAAGACCTC GGCGGTGGC-
BOT 2430                TTGGGCACCG TCCTCACTGC GGACGCGCCT CAAAGACCTC GGCGGTGGC-
BOT 2399                TTGGGCACCG TCCTCACTGC GGACGCGCCT CAAAGACCTC GGCGGTGGC-
KJ93.27                 TTGGGCACCG TCCTCACTGC GGACGCGCCT CAAAGACCTC GGCGGTGGC-
KJ93.41                 TTGGGCACCG TCCTCACTGC GGACGCGCCT CAAAGACCTC GGCGGTGGC-
Guignardia philoprina   GGTGTTGGGG ATCGGCCAAA GCCCGCGAGG GACGGCCGGC CCCTAAATCT
```

```
                                460       470       480       490       500
                                .         .         .         .         .

BOT 2413                TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGCGCAC
BOT 2302                TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGCGCAC
BOT 2398                TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGCGCAC
BOT 2353                TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGCGCAC
BOT 2331                TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGCGCAC
BOT 2339                TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGCGCAC
BOT 2382                TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGCGCAC
BOT 2405                TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGCGCAC
BOT 2291                TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGCGCAC
BOT 2345                TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGCGCAC
BOT 2363                TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGCGCAC
KJ94.09                 TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGCGCAC
BOT 2352                TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGCGCAC
CMW 7799                TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGCGCAC
CMW 7026                TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGCGCAC
CMW 7025                TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGCGCAC
CMW 7801                TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGCGCAC
CMW 7024                TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGCGCAC
BOT 2351                TCTT--GCC- TCAAGCGTAG TAAAAAA-CA C--CTCGCTT TGGAGCGCAC
BOT 2355                TCTT--GCC- TCAAGCGTAG TAAAAAA-CA C--CTCGCTT TGGAGCGCAC
KJ93.52                 TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGCGCAC
BOT 945                 TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGCGCAT
BOT 931                 TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGCGCAT
BOT 11                  TCTT--GCC- TCAAGCGTAG TAGAAA-TCA C--CTCGCTT TGGAGCGCAT
BOT 32                  TCTT--GCC- TCAAGCGTAG TAGAAA-TCA C--CTCGCTT TGGAGCGCAT
CMW 7803                TCTT--GCC- TCAAGCGTAG TAGAACAT-A CATCTCGCTT CGGAGCGCAG
CMW 7020                TCTT--GCC- TCAAGCGTAG TAGAACAT-A CATCTCGCTT CGGAGCGCAG
CMW 7027                TCTT--GCC- TCAAGCGTAG TAGAACAT-A CATCTCGCTT CGGAGCGCAG
KJ93.42                 TCTT--GCC- TCAAGCGTAG TAGAACAT-A CATCTCGCTT CGGAGCGCAG
CMW 7802                TCTT--GCC- TCNAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGGACGG
CMW 7022                TCTT--GC-- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGGACGG
BOT 2417                TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGGACGG
BOT 2421                TCTT--GCC- TCAAGCGTAG TAGAAAA-CA C--CTCGCTT TGGAGGACGG
KJ93.35                 TGTCCAGCCC TCAAGCGTAG TAGAATA-CA C--CTCGCTT TGGAGCGGCT
KJ93.29                 TGTCCAGCCC TCAAGCGTAG TAGAATA-CA C--CTCGCTT TGGAGCGGCT
KJ93.56                 TGTTCAGCCC TCAAGCGTAG TAGAATA-CA C--CTCGCTT TGGAGCGGTT
KJ94.07                 TGTTCAGCCC TCAAGCGTAG TAGAATA-CA C--CTCGCTT TGGAGCGGTT
BOT 2376                TGTTCAGCCC TCAAGCGTAG TAGAATA-CA C--CTCGCTT TGGAGCGGTT
BOT 2422                TGTTCAGCCC TCAAGCGTAG TAGAATA-CA C--CTCGCTT TGGAGCGGTT
BOT 2430                TGTTCAGCCC TCAAGCGTAG TAGAATA-CA C--CTCGCTT TGGAGCGGTT
BOT 2399                TGTTCAGCCC TCAAGCGTAG TAGAATA-CA C--CTCGCTT TGGAGCGGTT
KJ93.27                 TGTTCAGCCC TCAAGCGTAG TAGAATA-CA C--CTCGCTT TGGAGCGGTT
KJ93.41                 TGTTCAGCCC TCAAGCGTAG TAGAATA-CA C--CTCGCTT TGGAGCGGTT
Guignardia philoprina   AGTGGCGGAC CCGTCGTGGC CTCCTCTGCG AAGTAGTGAT ATTCCGCATC
```

```
                                  510         520         530         540         550
                                   .           .           .           .           .
BOT 2413                GGCGTCG-CC CGCCGGACGA ACCTT-TGAA TT-ATTT--- CTCAAGGTTG
BOT 2302                GGCGTCG-CC CGCCGGACGA ACCTT-TGAA TT-ATTT--- CTCAAGGTTG
BOT 2398                GGCGTCG-CC CGCCGGACGA ACCTT-TGAA TT-ATTT--- CTCAAGGTTG
BOT 2353                GGCGTCG-CC CGCCGGACGA ACCTT-TGAA TT-ATTT--- CTCAAGGTTG
BOT 2331                GGCGTCG-CC CGCCGGACGA ACCTT-TGAA TT-ATTT--- CTCAAGGTTG
BOT 2339                GGCGTCG-CC CGCCGGACGA ACCTT-TGAA TT-ATTT--- CTCAAGGTTG
BOT 2382                GGCGTCG-CC CGCCGGACGA ACCTT-TGAA TT-ATTT--- CTCAAGGTTG
BOT 2405                GGCGTCG-CC CGCCGGACGA ACCTT-TGAA TT-ATTT--- CTCAAGGTTG
BOT 2291                GGCGTCG-CC CGCCGGACGA ACCTT-TGAA TT-ATTT--- CTCAAGGTTG
BOT 2345                GGCGTCG-CC CGCCGGACGA ACCTT-TGAA TT-ATTT--- CTCAAGGTTG
BOT 2363                GGCGTCG-CC CGCCGGACGA ACCTT-TGAA TT-ATTT--- CTCAAGGTTG
KJ94.09                 GGCGTCG-CC CGCCGGACGA ACCTT-TGAA TT-ATTT--- CTCAAGGTTG
BOT 2352                GGCGTCG-CC CGCCGGACGA ACCTT-TGAA TT-ATTT--- CTCAAGGTTG
CMW 7799                GGCGTCG-CC CGCCGGACGA ACCTT-TGAA TT-ATTT--- CTCAAGGTTG
CMW 7026                GGCGTCG-CC CGCCGGACGA ACCTT-TGAA TT-ATTT--- CTCAAGGTTG
CMW 7025                GGCGTCG-CC CGCCGGACGA ACCTT-TGAA TT-ATTT--- CTCAAGGTTG
CMW 7801                GGCGTCG-CC CGCCGGACGA ACCTT-TGAA T--ATTTT-- CTCAAGGTTG
CMW 7024                GGCGTCG-CC CGCCGGACGA ACCTT-TGAA T--ATTTTTT CTCAAGGTTG
BOT 2351                GGCGTCC-CC CGCCGGACGA ACCTT-TGAA TT--TTT--- CTCAAGGTTG
BOT 2355                GGCGTCC-CC CGCCGGACGA ACCTT-TGAA TT--TTT--- CTCAAGGTTG
KJ93.52                 GGCGTCG-CC CGCCGGACGA ACCTT-TGAA TT--TTT--- CTCAAGGTTG
BOT 945                 GGCGTCG-CC CGCCGGACGA ACCTT-TGAA TT--TTT--- CTCAAGGTTG
BOT 931                 GGCGTCG-CC CGCCGGACGA ACCTT-TGAA TT--TTT--- CTCAAGGTTG
BOT 11                  GGCGTCG-CC CGCCGGACGA AC-TT-TGAA TT--TTT--- CTCAAGGTTG
BOT 32                  GGCGTCG-CC CGCCGGACGA ACCTT-TGAA TT--TTT--- CTCAAGGTTG
CMW 7803                GGCGTCG-CC CGCCGGACGA ACCTTCTGAA CT--TTT--- CTCAAGGTTG
CMW 7020                GGCGTCG-CC CGCCGGACGA ACCTTCTGAA CT--TTT--- CTCAAGGTTG
CMW 7027                GGCGTCG-CC CGCCGGACGA ACCTTCTGAA CT--TTT--- CTCAAGGTTG
KJ93.42                 GGCGTCG-CC CGCCGGACGA ACCTTCTGAA CT--TTT--- CTCAAGGTTG
CMW 7802                GACGACCGCT CGCCGGACGA ACCTT-TGAA TTCATTTTC- CT--AGGTTG
CMW 7022                GACGACCGCT CGCCGGACGA ACCTT-TGAA TTCATTTTC- CT--AGGTTG
BOT 2417                GACGACCGCT CGCCGGACGA ACCTC-TGAA TTTATTTTC- CT--AGGTTG
BOT 2421                GACGACCGCT CGCCGGACGA ACCTC-TGAA TTTATTTTC- CT--AGGTTG
KJ93.35                 GGCGTCG-CC CGCCGGACGA ACCTTCTGAA CT--TTT--- CTCAAGGTTG
KJ93.29                 GGCGTCG-CC CGCCGGACGA ACCTTCTGAA CT--TTT--- CTCAAGGTTG
KJ93.56                 GGCGTCG-CC CGCCGGACGA ACCTTCTGAA CT--TTT--- CTCAAGGTTG
KJ94.07                 GGCGTCG-CC CGCCGGACGA ACCTTCTGAA CT--TTT--- CTCAAGGTTG
BOT 2376                GGCGTCG-CC CGCCGGACGA ACCTTCTGAA CT--TTT--- CTCAAGGTTG
BOT 2422                GGCGTCG-CC CGCCGGACGA ACCTTCTGAA CT--TTT--- CTCAAGGTTG
BOT 2430                GGCGTCG-CC CGCCGGACGA ACCTTCTGAA CT--TTT--- CTCAAGGTTG
BOT 2399                GGCGTCG-CC CGCCGGACGA ACCTTCTGAA CT--TTT--- CTCAAGGTTG
KJ93.27                 GGCGTCG-CC CGCCGGACGA ACCTTCTGAA CT--TTT--- CTCAAGGTTG
KJ93.41                 GGCGTCG-CC CGCCGGACGA ACCTTCTGAA CT--TTT--- CTCAAGGTTG
Guignardia philoprina   GGAGAGCGAC GAGCCCCTGC CGTTAAACCC CCAACTTT-- C-CAAGGTTG
```

```
                                560        570        580        590        600
                                 .          .          .          .          .

BOT 2413                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
BOT 2302                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
BOT 2398                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
BOT 2353                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
BOT 2331                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
BOT 2339                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
BOT 2382                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
BOT 2405                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
BOT 2291                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
BOT 2345                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
BOT 2363                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
KJ94.09                 ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
BOT 2352                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
CMW 7799                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
CMW 7026                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
CMW 7025                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAA-CACTC
CMW 7801                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
CMW 7024                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
BOT 2351                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTG
BOT 2355                ACCTCGGATA CCAAATCGGG GATGCTCTCT GGTTTGTTGC CAAAACACTG
KJ93.52                 ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTG
BOT 945                 ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACCG
BOT 931                 ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTG
BOT 11                  ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
BOT 32                  ACCTCGGATN NNNNATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
CMW 7803                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACAC--
CMW 7020                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACAC--
CMW 7027                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACAC--
KJ93.42                 ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACAC--
CMW 7802                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAA-CACGC
CMW 7022                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAA-CACGC
BOT 2417                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAA-CACGC
BOT 2421                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAA-CACGC
KJ93.35                 ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
KJ93.29                 ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
KJ93.56                 ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
KJ94.07                 ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
BOT 2376                ACCTCGGATA CCAAATCGGT GCTGCTTTTT GGTTTGTTGC CAAAACACTC
BOT 2422                ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
BOT 2430                ACCTCGGATA CCAAATCGGT GCTGCTTTTT GGTTTGTTGC CAAAACACTC
BOT 2399                ACCTCGGATA CCAAATCGGT GCTGCTTTTT GGTTTGTTGC CAAAACACTC
KJ93.27                 ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
KJ93.41                 ACCTCGGATA CCAAATCGGT GCTGCTTTCT GGTTTGTTGC CAAAACACTC
Guignardia philoprina   ACCTCAGATA CCAAATCGGT GCTGCTTTCT GG-------- ----------
```

```
                                610        620        630        640        650
                                 .          .          .          .          .
BOT 2413                CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC ACAGGCAGAC
BOT 2302                CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC ACAGGCAGAC
BOT 2398                CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC ACAGGCAGAC
BOT 2353                CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC ACAGGCAGAC
BOT 2331                CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC ACAGGCAGAC
BOT 2339                CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC ACAGGCAGAC
BOT 2382                CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC ACAGGCAGAC
BOT 2405                CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC ACAGGCAGAC
BOT 2291                CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC ACAGGCAGAC
BOT 2345                CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC ACAGGCAGAC
BOT 2363                CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC ACAGGCAGAC
KJ94.09                 CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC ACAGGCAGAC
BOT 2352                CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC ACAGGCAGAC
CMW 7799                CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC ACAGGCAGAC
CMW 7026                CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC ACAGGCAGAC
CMW 7025                CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC ACAGGCAGAC
CMW 7801                CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC ACAGGCAGAC
CMW 7024                CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC ACAGGCAGAC
BOT 2351                CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC ACAGGCAAAC
BOT 2355                CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC ACAGGCAGAC
KJ93.52                 CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC GCAGGCAGAC
BOT 945                 CCGCTCCCGC GCT-CCC--G CTGACGCGAA TC--GACACC GCAGGCAGAC
BOT 931                 CCGCTCCCGC GC-CCCC--G CTGACGCGAA TC--GACACC GCAGGCAGAC
BOT 11                  TCGCTCCTGC GC-CCCC--G CTGACGCGAA TC--GACACC ATAGGCAGAC
BOT 32                  TCGCTCCTGC GC-CCCC--G CTGACGCGAA TC--GACACC ACAGGCAGAC
CMW 7803                CCGCTCCCGC GC-CCCC--G CTAACGCTTT CTGGGACACC ACAGGCAGAC
CMW 7020                CCGCTCCCGC GC-CCCC--G CTAACGCGAA TC--GACACC ACAGGCAGAC
CMW 7027                CCGCTCCCGC GC-CCCC--G CTAACGCGAA TC--GACACC ACAGGCAGAC
KJ93.42                 CCGCTCCCGC GC-CCCC--G CTAACGCGAA TC--GACACC ACAGGCAGAC
CMW 7802                CCGCCCCCGC GCT-CCTG-G CTGACGCGAA TC--GACACC ACAGGCAGAC
CMW 7022                CCGCCCCCGC GCT-CCTG-G CTGACGCGAA TC--GACACC ACAGGCAGAC
BOT 2417                CCGCCCCCGC GCT-CCTG-G CTGACGCGAA TC--GACACC ACAGGCAGAC
BOT 2421                CCGCCCCCGC GCT-CCTG-G CTGACGCGAA TC--GACACC ACAGGCAGAC
KJ93.35                 CCGCAGCCGC GC-CCCCCCG CTGACCCCAA TC--GACAC- ACAGGCAGAC
KJ93.29                 CCGCAGCCGC GC-CCCCCCG CTGACCCCAA TC--GACAC- ACAGGCAGAC
KJ93.56                 CCGCTGCCGC GC-CCCCC-G CTGACGCCAA TC--GACACC ACAGGCAGAC
KJ94.07                 CCGCTGCCGC GC-CCCCC-G CTGACGCCAA TC--GACACC ACAGGCAGAC
BOT 2376                CTGCTCCTGC GC-CCCCC-G CTGACGG-AA GC--GACACC ATAGGCAGAC
BOT 2422                CTGCTCCTGC GC-CCCCC-G CTGACGG-AA GC--GACACC ATAGGCAGAC
BOT 2430                CTGCTCCTGC GC-CCCCC-G CTGACGG-AA GC--GACACC ATAGGCAGAC
BOT 2399                CTGCTCCTGC GC-CCCCC-G CTGACGG-AA GC--GACACC ATAGGCAGAC
KJ93.27                 CTGCTCCTGC GC-CCCCC-G CTGACGG-AA GC--GACACC ATAGGCAGAC
KJ93.41                 CTGCTCCTGC GC-CCCCC-G CTGACGG-AA GC--GACACC ATAGGCAGAC
Guignardia philoprina   ---------- ---------- ---------- ---------- ---GGCAGAC
```

```
                                660       670       680       690       700
                                .         .         .         .         .

BOT 2413                CATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
BOT 2302                CATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
BOT 2398                CATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
BOT 2353                CATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
BOT 2331                CATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
BOT 2339                CATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
BOT 2382                CATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
BOT 2405                CATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
BOT 2291                CATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
BOT 2345                CATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
BOT 2363                CATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
KJ94.09                 CATTTCCGGC GAGCACGGCC TGGACGGCTC TGGCGTGTGA GTCTGCGCCG
BOT 2352                CATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
CMW 7799                CATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
CMW 7026                CATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
CMW 7025                CATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
CMW 7801                TATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTTTGCGCCG
CMW 7024                TATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTTTGCGCCG
BOT 2351                CATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
BOT 2355                CATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
KJ93.52                 CATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
BOT 945                 CATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
BOT 931                 CATTTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
BOT 11                  CATTTCTGGT GAACACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
BOT 32                  CATTTCTGGT GAACACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCGCCG
CMW 7803                CATCTCCGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCATCA
CMW 7020                CATCTCCGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCATCA
CMW 7027                CATCTCCGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCATCA
KJ93.42                 CATCTCCGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTCTGCATCA
CMW 7802                CATCTCTGGC GAGCACGGCC TGGATGGCTC CGGCGTGTGA GTTTGCGCGC
CMW 7022                CATCTCTGGC GAGCACGGCC TGGATGGCTC CGGCGTGTGA GTTTGCGCGC
BOT 2417                CATCTCTGGC GAGCACGGCC TGGACGGCTC CGGCGTGTGA GTTTGCGCGC
BOT 2421                CATCTCTGGC GAGCACGGCC TGGACGGCTC CGGCGTGTGA GTTTGCGCGC
KJ93.35                 CATCTCTGGC GAGCACGGCC TGGACGGCTC CGGCGTGTAA GTGTGCGCTG
KJ93.29                 CATCTCTGGC GAGCACGGCC TGGACGGCTC TGGCGTGTAA GTGTGCGCTG
KJ93.56                 TATCTCTGGC GAGCACGGCC TGGACGGCTC CGGCGTGTAA GTTTGCGCTG
KJ94.07                 TATCTCTGGC GAGCACGGCC TGGACGGCTC GGGCGTGTAA GTTTGCGCTG
BOT 2376                CATCTCCGGC GAGCACGGCC TGGATGGCTC CGGTGTGTAA GTGTGCGCCT
BOT 2422                CATCTCCGGC GAGCACGGCC TGGATGGCTC CGGTGTGTAA GTGTGCGCCT
BOT 2430                CATCTCCGGC GAGCACGGCC TGGATGGCTC CGGTGTGTAA GTGTGCGCCT
BOT 2399                CATCTCTGGC GAGCACGGCC TGGATGGCTC CGGTGTGTAA GTGTGCGCCT
KJ93.27                 CATCTCCGGC GAGCACGGCC TGGATGGCTC CGGTGTGTAA GTGTGCGCCT
KJ93.41                 CATCTCCGGC GAGCACGGCC TGGATGGCTC CGGTGTGTAA GTGTGCGCCT
Guignardia philoprina   CATCTCTGGC GAGCACGGCC TCGACAGCAA TGGTGTCTAC A---GC----
```

```
                                710        720        730        740        750
                                .          .          .          .          .

BOT 2413                TTTCCC---- ---GCGCGAA ---TGGCAAT GGCTGACCC- G--CAGCAGC
BOT 2302                TTTCCC---- ---GCGCGAA ---TGGCAAT GGCTGACCC- G--CAGCAGC
BOT 2398                TTTCCC---- ---GCGCGAA ---TGGCAAT GGCTGACCC- G--CAGCAGC
BOT 2353                TTTCCC---- ---GCGCGAA ---TGGCAAT GGCTGACCC- G--CAGCAGC
BOT 2331                TTTCCC---- ---GCGCGAA ---TGGCAAT GGCTGACCC- G--CAGCAGC
BOT 2339                TTTCCC---- ---GCGCGAA ---TGGCAAT GGCTGACCC- G--CAGCAGC
BOT 2382                TTTCCC---- ---GCGCGAA ---TGGCAAT GGCTGACCC- G--CAGCAGC
BOT 2405                TTTCCC---- ---GCGCGAA ---TGGCAAT GGCTGACCC- G--CAGCAGC
BOT 2291                TTTCCC---- ---GCGCGAA ---TGGCAAT GGCTGACCC- G--CAGCAGC
BOT 2345                TTTCCC---- ---GCGCGAA ---TGGCAAT GGCTGACCC- G--CAGCAGC
BOT 2363                TTTCCC---- ---GCGCGAA ---TGGCAAT GGCTGACCC- G--CAGCAGC
KJ94.09                 TTTCCC---- ---GCGCGAA ---TGGCAAT GGCTGACCC- G--TAGCAGC
BOT 2352                TTTCCC---- ---GCGCGAA ---TGGCAAT GGCTGACCC- G--CAGCAGC
CMW 7799                TTTCCC---- ---GCGCGAA ---TGGCAAT GGCTGACCC- G--CAGCAGC
CMW 7026                TTTCCC---- ---GCGCGAA ---TGGCAAT GGCTGACCC- G--CAGCAGC
CMW 7025                TTTCCC---- ---GCGCGAA ---TGGCAAT GGCTGACCC- G--CAGCAGC
CMW 7801                TTTTCC---- ---GCGCGAA ---TGGCAAT GGCTGACC-T G--CAACAGC
CMW 7024                TTTTCC---- ---GCGCGAA -----GCAAT GGCTGACC-T G--CAACAGC
BOT 2351                TTTC------ -TTGCGCGAA ---TGGCAAT GGCTGACCC- G--CAGCAGC
BOT 2355                TTTC------ -TTGCGCGAA ---TGGCAAT GGCTGACCC- G--CAGCAGC
KJ93.52                 TTTC------ -TTGCGCGAA ---TGGCAAT GGCTGACCC- G--CAGCAGC
BOT 945                 TTTC------ -TTGCGCGAA ---TGGCAAT GGCTGACCC- G--CAGCAGC
BOT 931                 TTTC------ -TTGCGCGAA ---TGGCAAT GGCTGACCC- G--CAGCAGC
BOT 11                  TTTCCC---- ---GCTCGAA ---TGGCAAT GGCTGACCC- G--CAACAGC
BOT 32                  TTTCCC---- ---GCTCGAA ---TGGCAAT GGCTGACCC- G--CAACAGC
CMW 7803                TT-CTCAGCG -T-GGGAGAA C-AT--CAAT GACTAAAC-T G--TAGCAGC
CMW 7020                TT-CTCAGCG -T-GGGAGAA C-AT--CAAT GACTAAAC-T G--TAGCAGC
CMW 7027                TT-CTCAGCG -T-GGGAGAA C-AT--CAAT GACTAAAC-T G--TAGCAGC
KJ93.42                 TT-CTCAGCG -T-GGGAGAA C-AT--CAAT GACTAAAC-T G--TAGCAGC
CMW 7802                T--CCCCGCA CTAGGGCGCA CCTT-GCAAT G-CTAA---T GCACAACAGC
CMW 7022                T--CCCCGCA CTAGGGCGCA CCTT-GCAAT G-CTAA---T GCACAACAGC
BOT 2417                TT-CCC-GCA CTTTGGCGCA TCTT-GCAAT G-CTAA---T GCACAGCAGC
BOT 2421                TT-CCC-GCA CTTTGGCGCA TCTT-GCAAT G-CTAA---T GCACAGCAGC
KJ93.35                 T--CT----- -TT-GCCGCG CTTT-GCAAT CGCTGACTCT ---CGGCAGC
KJ93.29                 T--CT----- -TT-GCCGCG CTGT-GCAAT CGCTGACTCT ---CGGCAGC
KJ93.56                 T--CT----- -TT-GCCGCG CTCT-GCAAT CGCTGACCCT ---TGGCAGC
KJ94.07                 T--CT----- -TT-GCCGCG CTCT-GCAAT CGCTGACCCT ---TTGCAGC
BOT 2376                T--CTCC--- ----GCCGCG C-ATGGCAAT CGCTGACC-T G--TAGCAGC
BOT 2422                T--CTCC--- ----GCCGCG C-ATGGCAAT CGCTGACC-T G--TAGCAGC
BOT 2430                T--CTCC--- ----GCCGCG C-ATGGCAAT CGCTGACC-T G--TAGCAGC
BOT 2399                T--CTCC--- ----GCCGCG C-ATGGCAAT CGCTGACC-T G--TAGCAGC
KJ93.27                 T--CTCC--- ----GCCGCG C-ATGGCAAT CGCTGACC-T G--TAGCAGC
KJ93.41                 T--CTCC--- ----GCCGCG C-ATGGCAAT CGCTGACC-T G--TAGCAGC
Guignardia philoprina   ---------- ---------- ---------- ---------- ----------
```

```
                                760        770        780        790        800
                                .          .          .          .          .

BOT 2413                TACAATGGCA CCTCCGACCT GCAGCTCGAG CGCATGAACG TCTACTTCAA
BOT 2302                TACAATGGCA CCTCCGACCT GCAGCTCGAG CGCATGAACG TCTACTTCAA
BOT 2398                TACAATGGCA CCTCCGACCT GCAGCTCGAG CGCATGAACG TCTACTTCAA
BOT 2353                TACAATGGCA CCTCCGACCT GCAGCTCGAG CGCATGAACG TCTACTTCAA
BOT 2331                TACAATGGCA CCTCCGACCT GCAGCTCGAG CGCATGAACG TCTACTTCAA
BOT 2339                TACAATGGCA CCTCCGACCT GCAGCTCGAG CGCATGAACG TCTACTTCAA
BOT 2382                TACAATGGCA CCTCCGACCT GCAGCTCGAG CGCATGAACG TCTACTTCAA
BOT 2405                TACAATGGCA CCTCCGACCT GCAGCTCGAG CGCATGAACG TCTACTTCAA
BOT 2291                TACAATGGCA CCTCCGACCT GCAGCTCGAG CGCATGAACG TCTACTTCAA
BOT 2345                TACAATGGCA CCTCCGACCT GCAGCTCGAG CGCATGAACG TCTACTTCAA
BOT 2363                TACAATGGCA CCTCCGACCT GCAGCTCGAG CGCATGAACG TCTACTTCAA
KJ94.09                 TACAATGGCA CCTCCGACCT GCAGCTCGAG CGCATGAACG TCTACTTCAA
BOT 2352                TACAATGGCA CCTCCGACCT GCAGCTCGAG CGCATGAACG TCTACTTCAA
CMW 7799                TACAATGGCA CCTCCGACCT GCAGCTCGAG CGCATGAACG TCTACTTCAA
CMW 7026                TACAATGGCA CCTCCGACCT GCAGCTCGAG CGCATGAACG TCTACTTCAA
CMW 7025                TACAATGGCA CCTCCGACCT GCAGCTCGAG CGCATGAACG TCTACTTCAA
CMW 7801                TACAATGGCA CCTCCGACCT CCAGCTCGAG CGCATGAACG TCTACTTCAA
CMW 7024                TACAATGGCA CCTCCGACCT CCAGCTCGAG CGCATGAACG TCTACTTCAA
BOT 2351                TACAATGGCA CCTCCGACCT CCAGCTCGAG CGCATGAACG TCTACTTCAA
BOT 2355                TACAATGGCA CCTCCGACCT CCAGCTCGAG CGCATGAACG TCTACTTCAA
KJ93.52                 TACAATGGCA CCTCCGACCT CCAGCTCGAG CGCATGAACG TCTACTTCAA
BOT 945                 TACAATGGCA CCTCCGACCT CCAGCTCGAG CGCATGAACG TCTACTTCAA
BOT 931                 TACAATGGCA CCTCCGACCT CCAGCTCGAG CGCATGAACG TCTACTTCAA
BOT 11                  TACAATGGCA CCTCCGACCT CCAGCTCGAG CGCATGAACG TCTACTTCAA
BOT 32                  TACAATGGCA CCTCCGACCT CCAGCTCGAG CGCATGAACG TCTACTTCAA
CMW 7803                TACAATGGCA CCTCGGACCT TCAGCTCGAG CGCATGAACG TCTATTTCAA
CMW 7020                TACAATGGCA CCTCGGACCT TCAGCTCGAG CGCATGAACG TCTATTTCAA
CMW 7027                TACAATGGCA CCTCGGACCT TCAGCTCGAG CGCATGAACG TCTATTTCAA
KJ93.42                 TACAATGGCA CCTCGGACCT TCAGCTCGAG CGCATGAACG TCTATTTCAA
CMW 7802                TACAACGGCA CCTCGGACCT CCAGCTTGAG CGCATGAATG TCTACTTCAA
CMW 7022                TACAACGGCA CCTCGGACCT CCAGCTTGAG CGCATGAATG TCTACTTCAA
BOT 2417                TACAACGGCA CTTCGGACCT CCAGCTTGAG CGCATGAACG TCTACTTCAA
BOT 2421                TACAACGGCA CTTCGGACCT CCAGCTTGAG CGCATGAACG TCTACTTCAA
KJ93.35                 TACAATGGCA CCTCCGACCT CCAGCTGGAG CGCATGAACG TCTACTTCAA
KJ93.29                 TACAATGGCA CCTCCGACCT CCAACTGGAG CGCATGAACG TCTACTTCAA
KJ93.56                 TACAATGGCA CCTCCGACCT CCAGCTGGAG CGCATGAACG TCTACTTCAA
KJ94.07                 TACAATGGCA CCTCCGACCT CCAGCTGGAG CGCATGAACG TCTACTTCAA
BOT 2376                TACAATGGCA CTTCGGACCT CCAACTGGAG CGCATGAACG TCTACTTCAA
BOT 2422                TACAATGGCA CTTCGGACCT CCAACTGGAG CGCATGAACG TCTACTTCAA
BOT 2430                TACAATGGCA CTTCGGACCT CCAACTGGAG CGCATGAACG TCTACTTCAA
BOT 2399                TACAATGGCA CTTCGGACCT CCAACTGGAG CGCATGAACG TCTACTTCAA
KJ93.27                 TACAATGGCA CTTCGGACCT CCAACTGGAG CGCATGAACG TCTACTTCAA
KJ93.41                 TACAATGGCA CTTCGGACCT CCAACTGGAG CGCATGAACG TCTACTTCAA
Guignardia philoprina   ---------- ---------- ---------- ---------- ----------
```

```
                                  810        820        830        840        850
                                   .          .          .          .          .

BOT 2413                  CGAGGTACTC TCTCACTAAT TGCACAAACA CGTAAAGTAT GGCAATCTTC
BOT 2302                  CGAGGTACTC TCTCACTAAT TGCACAAACA CGTAAAGTAT GGCAATCTTC
BOT 2398                  CGAGGTACTC TCTCACTAAT TGCACAAACA CGTAAAGTAT GGCAATCTTC
BOT 2353                  CGAGGTACTC TCTCACTAAT TGCACAAACA CGTAAAGTAT GGCAATCTTC
BOT 2331                  CGAGGTACTC TCTCACTAAT TGCACAAACA CGTAAAGTAT GGCAATCTTC
BOT 2339                  CGAGGTACTC TCTCACTAAT TGCACAAACA CGTAAAGTAT GGCAATCTTC
BOT 2382                  CGAGGTACTC TCTCACTAAT TGCACAAACA CGTAAAGTAT GGCAATCTTC
BOT 2405                  CGAGGTACTC TCTCACTAAT TGCACAAACA CGTAAAGTAT GGCAATCTTC
BOT 2291                  CGAGGTACTC TCTCACTAAT TGCACAAACA CGTAAAGTAT GGCAATCTTC
BOT 2345                  CGAGGTACTC TCTCACTAAT TGCACAAACA CGTAAAGTAT GGCAATCTTC
BOT 2363                  CGAGGTACTC TCTCACTAAT TGCACAAACA CGTAAAGTAT GGCAATCTTC
KJ94.09                   CGAGGTACTC TCTCACTAAT TGCACAAACA CGTAAAGTAT GGCAATCTTC
BOT 2352                  CGAGGTACTC TCTCACTAAT TGCACAAACA CGTAAAGTAT GGCAATCTTC
CMW 7799                  CGAGGTACTC TCTCACTAAT TGCACAAACA CGTAAAGTAT GGCAATCTTC
CMW 7026                  CGAGGTACTC TCTCACTAAT TGCACAAACA CGTAAAGTAT GGCAATCTTC
CMW 7025                  CGAGGTACTC TCTCACTAAT TGCACAAACA CGTAAAGTAT GGCAATCTTC
CMW 7801                  CGAGGTACTC TCTCACTAAT CACACAAACA CGTAAAGTAT GGCAATCTTC
CMW 7024                  CGAGGTACTC TCTCACTAAT CACACAAACA CGTAAAGTAT GGCAATCTTC
BOT 2351                  CGAGGTACTC TCTCACTAAT TGCACGCACA CGTAAAGTAT GGCAATCTTC
BOT 2355                  CGAGGTACTC TCTCACTAAT TGCACGCACA CGTAAAGTAT GGCAATCTTC
KJ93.52                   CGAGGTACTC TCTCACTAAT CGCACGAACA CGTAAAGTAT GGCAATCTTC
BOT 945                   CGAGGTACTC TCTCACTAAT CGCACAAACA CGTAAAGTAT GGCAATCTTC
BOT 931                   CGAGGTACTC TCTCACTAAT CGCACGAACA CGTAAAGTAT GGCAATCTTC
BOT 11                    CGAGGTACTC TCTCACTAAT CACACAAACA CATAAAGTAT GGCAATCTTC
BOT 32                    CGAGGTACTC TCTCACTAAT CACACAAACA CATAAAGTAT GGCAATCTTC
CMW 7803                  CGAGGTACTC TCTCACTAAT TAGACAAACA CGTAAAGTAT GGCAATCTTC
CMW 7020                  CGAGGTACTC TCTCACTAAT TAGACAAACA CGTAAAGTAT GGCAATCTTC
CMW 7027                  CGAGGTACTC TCTCACTAAT TAGACAAACA CGTAAAGTAT GGCAATCTTC
KJ93.42                   CGAGGTACTC TCTCACTAAT TAGACAAACA CGTAAAGTAT GGCAATCTTC
CMW 7802                  CGAGGTACTC TCTCACTAAT TA-ACTCACC CGTAAAGTAT GGCAATCTTC
CMW 7022                  CGAGGTACTC TCTCACTAAT TA-ACTCACC CGTAAAGTAT GGCAATCTTC
BOT 2417                  CGAGGTACTC TCTCACTAAT TA-ACTCACC CGTAAAGTAT GGCAATCTTC
BOT 2421                  CGAGGTACTC TCTCACTAAT TA-ACTCACC CGTAAAGTAT GGCAATCTTC
KJ93.35                   CGAGGTACTC TCTCACTAAT TAGACAAACA CGTAAAGTAT GGCAATCTTC
KJ93.29                   CGAGGTACTC TCTTGCTAAT TAGACAAACA CGTAAAGTAT GGCAATCTTC
KJ93.56                   CGAGGTACTC TCT-ACTAGT TAGACAAACA CGTAAAGTAT GGCAATCTTC
KJ94.07                   CGAGGTACTC TCT-ACTAGT TAGACAAACA CGCAAAGTAT GGCAATCTTC
BOT 2376                  CGAGGTACTC TCTCCATAAT TAGACAAACA CGTAAAGTAT GGCAATCTTC
BOT 2422                  CGAGGTACTC TCTCCATAAT TAGACAAACA CGTAAAGTAT GGCAATCTTC
BOT 2430                  CGAGGTACTC TCTCCATAAT TAGACAAACA CGTAAAGTAT GGCAATCTTC
BOT 2399                  CGAGGTACTC TCTCCATAAT TAGACAAACA CGTAAAGTAT GGCAATCTTC
KJ93.27                   CGAGGTACTC TCTCCATAAT TAGACAAACA CGTAAAGTAT GGCAATCTTC
KJ93.41                   CGAGGTACTC TCTCCATAAT TAGACAAACA CGTAAAGTAT GGCAATCTTC
Guignardia philoprina     ---GGTAC-- -CTCCGAGCT CCAGCTCGAG CGCATGAACG TCTA--CTTC
```

```
                                860       870       880       890       900
                                .         .         .         .         .

BOT 2413                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
BOT 2302                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
BOT 2398                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
BOT 2353                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
BOT 2331                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
BOT 2339                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
BOT 2382                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
BOT 2405                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
BOT 2291                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
BOT 2345                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
BOT 2363                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
KJ94.09                 TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
BOT 2352                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
CMW 7799                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
CMW 7026                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
CMW 7025                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
CMW 7801                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTT
CMW 7024                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTT
BOT 2351                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTT
BOT 2355                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTT
KJ93.52                 TGAACGCGCA GCAGGCGTCG AACAACAAGT ACGTTCCTCG TGCCGTCCTC
BOT 945                 TGAACGCGCA GCAGGCGTCG AACAACAAGT ACGTTCCTCG TGCCGTCCTC
BOT 931                 TGAACGCGCA GCAGGCGTCG AACAACAAGT ACGTTCCTCG TGCCGTCCTC
BOT 11                  TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
BOT 32                  TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
CMW 7803                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
CMW 7020                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
CMW 7027                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
KJ93.42                 TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCCGTCCTC
CMW 7802                TGAACGCGCA GCAGGCGTCA AACAACAAGT ATGTGCCCCG TGCCGTCCTT
CMW 7022                TGAACGCGCA GCAGGCGTCA AACAACAAGT ATGTGCCCCG TGCCGTCCTT
BOT 2417                TGAACGCGCA GCAGGCGTCA AACAACAAGT ATGTGCCCCG TGCCGTCCTT
BOT 2421                TGAACGCGCA GCAGGCGTCA AACAACAAGT ATGTGCCCCG TGCCGTCCTT
KJ93.35                 TGAACGCGCA GCAGGCTTCC AACAACAAGT ACGTTCCTCG TGCTGTCCTC
KJ93.29                 TGAACGCGCA GCAGGCTTCC AACAACAAGT ACGTTCCTCG TGCTGTCCTC
KJ93.56                 TGAACGCGCA GCAGGCATCC AACAATAAGT ACGTTCCTCG TGCTGTCCTC
KJ94.07                 TGAACGCGCA GCAGGCTTCG AACAATAAGT ACGTTCCTCG TGCTGTCCTC
BOT 2376                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCTGTCCTC
BOT 2422                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCTGTCCTC
BOT 2430                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCTGTCCTC
BOT 2399                TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCTGTCCTC
KJ93.27                 TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCTGTCCTC
KJ93.41                 TGAACGCGCA GCAGGCGTCC AACAACAAGT ACGTTCCTCG TGCTGTCCTC
Guignardia philoprina   --AACGAGGT AT----GTCC AGACCGAGCT TCACATATTC TGGTGATTTT
```

```
                                910         920         930         940         950
                                .           .           .           .           .

BOT 2413                GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
BOT 2302                GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
BOT 2398                GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
BOT 2353                GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
BOT 2331                GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
BOT 2339                GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
BOT 2382                GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
BOT 2405                GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
BOT 2291                GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
BOT 2345                GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
BOT 2363                GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
KJ94.09                 GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
BOT 2352                GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
CMW 7799                GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
CMW 7026                GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
CMW 7025                GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
CMW 7801                GTCGACCTCG AGCCTGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
CMW 7024                GTCGACCTCG AGCCTGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
BOT 2351                GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
BOT 2355                GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
KJ93.52                 GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
BOT 945                 GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
BOT 931                 GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
BOT 11                  GTTGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
BOT 32                  GTTGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
CMW 7803                GTCGACCTCG AGCCCGGCAC GATGGATGCC GTCCGCGCCG GCCCCTTCGG
CMW 7020                GTCGACCTCG AGCCCGGCAC GATGGATGCC GTCCGCGCCG GCCCCTTCGG
CMW 7027                GTCGACCTCG AGCCCGGCAC GATGGATGCC GTCCGCGCCG GCCCCTTCGG
KJ93.42                 GTCGACCTCG AGCCCGGCAC GATGGATGCC GTCCGCGCCG GCCCCTTCGG
CMW 7802                GTCGACCTGG AGCCCGGCAC CATGGACGCT GTCCGCGCCG GCCCCTTCGG
CMW 7022                GTCGACCTGG AGCCCGGCAC CATGGACGCT GTCCGCGCCG GCCCCTTCGG
BOT 2417                GTCGACCTGG AGCCCGGCAC CATGGACGCT GTCCGCGCCG GTCCCTTCGG
BOT 2421                GTCGACCTGG AGCCCGGCAC CATGGACGCT GTCCGCGCCG GTCCCTTCGG
KJ93.35                 GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
KJ93.29                 GTTGACCTCG AGCCCGGTAC CATGGATGCC GTCCGTGCCG GCCCCTTCGG
KJ93.56                 GTTGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
KJ94.07                 GTTGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
BOT 2376                GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
BOT 2422                GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
BOT 2430                GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
BOT 2399                GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
KJ93.27                 GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
KJ93.41                 GTCGACCTCG AGCCCGGCAC CATGGATGCC GTCCGCGCCG GCCCCTTCGG
Guignardia philoprina   CATCTTCTGA CCGAGATTTG GGTATAGGCC -TCCGGCAAC AAGTATGTTC
```

```
                                960       970       980       990       1000
                                .         .         .         .         .
BOT 2413                CCAGCTCTTC CGCCCCGACA ACTTCGTCTT CGGCCAGTCT GGCGCCGGTA
BOT 2302                CCAGCTCTTC CGCCCTGACA ACTTCGTCTT CGGTCAGTCT GGCGCCGGTA
BOT 2398                CCAGCTCTTC CGCCCTGACA ACTTCGTCTT CGGTCAGTCT GGCGCCGGTA
BOT 2353                CCAGCTCTTC CGCCCTGACA ACTTCGTCTT CGGTCAGTCT GGCGCCGGTA
BOT 2331                CCAGCTCTTC CGCCCCGACA ACTTCGTCTT CGGCCAGTCT GGCGCCGGTA
BOT 2339                CCAGCTCTTC CGCCCCGACA ACTTCGTCTT CGG-CAGTCT GGCGCCGGTA
BOT 2382                CCAGCTCTTC CGCCCTGACA ACTTCGTCTT CGGTCAGTCT GGCGCCGGTA
BOT 2405                CCAGCTCTTC CGCCCTGACA ACTTCGTCTT CGGTCAGTCT GGCGCCGGTA
BOT 2291                CCAGCTCTTC CGCCCCGACA ACTTCGTCTT CGGCCAGTCT GGCGCCGGTA
BOT 2345                CCAGCTCTTC CGCCCCGACA ACTTCGTCTT CGGCCAGTCT GGCGCCGGTA
BOT 2363                CCAGCTCTTC CGCCCTGACA ACTTCGTCTT CGGTCAGTCT GGCGCCGGTA
KJ94.09                 CCAGCTCTTC CGCCCTGACA ACTTCGTCTT CGGTCAGTCT GGCGCCGGTA
BOT 2352                CCAGCTCTTC CGCCCTGACA ACTTCGTCTT CGGTCAGTCT GGCGCCGGTA
CMW 7799                CCAGCTCTTC CGCCCTGACA ACTTCGTCTT CGGTCAGTCT GGCGCCGGTA
CMW 7026                CCAGCTCTTC CGCCCTGACA ACTTCGTCTT CGGTCAGTCT GGCGCCGGTA
CMW 7025                CCAGCTCTTC CGCCCTGACA ACTTCGTCTT CGGTCAGTCT GGCGCCGGTA
CMW 7801                CCAGCTCTTC CGCCCCGACA ACTTTGTCTT CGGCCAGTCT GGTGCCGGTA
CMW 7024                CCAGCTCTTC CGCCCCGACA ACTTTGTCTT CGGCCAGTCT GGTGCCGGTA
BOT 2351                CCAGCTCTTC CGCCCCGACA ACTTTGTCTT CGGCCAGTCT GGTGCTGGTA
BOT 2355                CCAGCTCTTC CGCCCCGACA ACTTTGTCTT CGGCCAGTCT GGTGCTGGTA
KJ93.52                 CCAGCTCTTC CGCCCCGACA ACTTTGTCTT CGGCCAGTCT GGTGCCGGTA
BOT 945                 CCAGCTCTTC CGCCCCGACA ACTTTGTCTT CGGCCAGTCT GGTGCCGGTA
BOT 931                 CCAGCTCTTC CGCCCCGACA ACTTTGTCTT CGGCCAGTCT GGTGCCGGTA
BOT 11                  CCAGCTCTTC CGCCCCGACA ACTTTGTCTT CGGCCAGTCT GGTGCCGGTA
BOT 32                  CCAGCTCTTC CGCCCCGACA ACTTTGTCTT CGGCCAGTCT GGTGCCGGTA
CMW 7803                CCAGCTTTTC CGCCCCGACA ACTTCGTCTT CGGTCAGTCC GGTGNCGGTA
CMW 7020                CCAGCTTTTC CGCCCCGACA ACTTCGTCTT CGGTCAGTCC GGTGCCGGTA
CMW 7027                CCAGCTTTTC CGCCCCGACA ACTTCGTCTT CGGTCAGTCC GGTGCCGGTA
KJ93.42                 CCAGCTTTTC CGCCCCGACA ACTTCGTCTT CGGTCAGTCC GGTGCCGGTA
CMW 7802                CCAGCTCTTC CGCCCCGACA ACTTCGTCTT CGGCCAGTCT GGTGCCGGTA
CMW 7022                CCAGCTCTTC CGCCCCGACA ACTTCGTCTT CGGCCAGTCT GGTGCCGGTA
BOT 2417                CCAGCTCTTC CGCCCCGACA ACTTCGTCTT CGGCCAGTCT GGTGCCGGTA
BOT 2421                CCAGCTCTTC CGCCCCGACA ACTTCGTCTT CGGCCAGTCT GGTGCCGGTA
KJ93.35                 CCAGCTCTTC CGTCCCGACA ACTTCGTTTT CGGCCAGTCT GGTGCCGGTA
KJ93.29                 CCAGCTCTTC CGTCCCGACA ACTTCGTTTT CGGCCAGTCT GGTGCCGGTA
KJ93.56                 CCAGCTCTTC CGTCCCGACA ACTTCGTTTT CGGCCAGTCT GGTGCCGGTA
KJ94.07                 CCAGCTCTTC CGTCCCGACA ACTTCGTTTT CGGCCAGTCT GGTGCCGGTA
BOT 2376                CCAGCTCTTC CGCCCCGACA ACTTCGTCTT CGGCCAGTCT GGTGCCGGTA
BOT 2422                CCAGCTCTTC CGCCCCGACA ACTTCGTCTT CGGCCAGTCT GGTGCCGGTA
BOT 2430                CCAGCTCTTC CGCCCCGACA ACTTCGTCTT CGGCCAGTCT GGTGCCGGTA
BOT 2399                CCAGCTCTTC CGCCCCGACA ACTTCGTCTT CGGCCAGTCT GGTGCCGGTA
KJ93.27                 CCAGCTCTTC CGCCCCGACA ACTTCGTCTT CGGCCAGTCT GGTGCCGGTA
KJ93.41                 CCAGCTCTTC CGCCCCGACA ACTTCGTCTT CGGCCAGTCT GGTGCCGGTA
Guignardia philoprina   CTCGCGCTGT CCTCGTCG-A TCTTGAGCCC GGTACCATGG A-TGCCG-TC
```

1009

BOT 2413	ACAACTGGG
BOT 2302	ACAACTGGG
BOT 2398	ACAACTGGG
BOT 2353	ACAACTGGG
BOT 2331	ACAACTGGG
BOT 2339	ACAACTGGG
BOT 2382	ACAACTGGG
BOT 2405	ACAACTGGG
BOT 2291	ACAACTGGG
BOT 2345	ACAACTGGG
BOT 2363	ACAACTGGG
KJ94.09	ACAACTGGG
BOT 2352	ACAACTGGG
CMW 7799	ACAACTGGG
CMW 7026	ACAACTGGG
CMW 7025	ACAACTGGG
CMW 7801	ACAACTGGG
CMW 7024	ACAACTGGG
BOT 2351	ACAACTGGG
BOT 2355	ACAACTGGG
KJ93.52	ACAACTGGG
BOT 945	ACAACTGGG
BOT 931	ACAACTGGG
BOT 11	ACAATTGGG
BOT 32	ACAATTGGG
CMW 7803	ACAACTGGG
CMW 7020	ACAACTGGG
CMW 7027	ACAACTGGG
KJ93.42	ACAACTGGG
CMW 7802	ACAACTGGG
CMW 7022	ACAACTGGG
BOT 2417	ACAACTGGG
BOT 2421	ACAACTGGG
KJ93.35	ACAACTGGG
KJ93.29	ACAACTGGG
KJ93.56	ACAACTGGG
KJ94.07	ACAACTGGG
BOT 2376	ACAACTGGG
BOT 2422	ACAACTGGG
BOT 2430	ACAACTGGG
BOT 2399	ACAACTGGG
KJ93.27	ACAACTGGG
KJ93.41	ACAACTGGG
Guignardia philoprina	CGTGCTGGA

CHAPTER 3

PATHOGENICITY OF *BOTRYOSPHAERIA* SPECIES ON TWO MANGO CULTIVARS IN SOUTH AFRICA

ABSTRACT

Botryosphaeria spp. cause a wide range of disease symptoms on mango trees and fruit in South Africa. There are limited options available for control of these diseases and this is partly attributed to a lack of understanding of the etiology of these fungi. The purpose of this study was, therefore, to evaluate the pathogenicity of four *Botryosphaeria* spp. from mango in South Africa and to evaluate the susceptibility of two commercial cultivars, Keitt and Tommy Atkins. Isolates obtained from mango in South Africa were grown on potato dextrose agar, and screened for pathogenicity using an apple-based screening procedure. Most pathogenic isolates were then inoculated into the stems of potted mango trees representing both cultivars. All isolates were pathogenic to mango trees, but they varied in the extent of lesion development. The most pathogenic species on both cultivars were *B. parva*, *B. rhodina* and *Fusicoccum indigoticum*. *Fusicoccum bacilliforme* isolates were least pathogenic on both mango cultivars. Results of this study represent the first inoculations with the newly described *Botryosphaeria* spp., *F. indigoticum* and *F. bacilliforme*. They also provide the first clear indication of the relative importance of the four *Botryosphaeria* spp. now known to occur on mango in South Africa.

INTRODUCTION

The South African mango (*Mangiferae indica* Linn.) industry is relatively small in export value (59 000 tons for the 2000 season) compared to other fruit exports, but it remains an important source of foreign exchange for the country (Finnemore, 2000). Locally, this is one of the most important sub-tropical crops, earning in excess of R 98 million annually. The area planted to mango in South Africa has increased rapidly during the last decade (1990 – 2000), however, fruit production did not increase by the same factor during this period (Finnemore, 2000). This is partly due to increased levels of tree and fruit diseases, and in particular fungal diseases such as those caused by *Botryosphaeria* spp. Similarly, these fungal pathogens are threatening mango industries world-wide (Donkin & Oosthuyse, 1996; Finnemore, 2000).

Botryosphaeria spp. cause various disease symptoms on mango throughout the tropical and sub-tropical regions of the world (Ramos *et al.*, 1991; Johnson, 1992). These fungi infect through natural or mechanical wounds and directly through natural plant openings such as stomata (Britton & Hendrix, 1986; Johnson, 1992; Lonsdale, 1993). In South Africa, die-back, stem cankers, blossom blight, stem end rot (SER) and soft brown rot (SBR) are all associated with *Botryosphaeria* spp. infecting mango (Lonsdale, 1993). Stress weakens host defence responses and predisposes trees to infection by *Botryosphaeria* spp. (McPartland & Schoeneweiss, 1984). Infection, disease incidence and symptom expression may, therefore, vary due to seasonal and environmental factors (Singh, 1960; Britton & Hendrix, 1986; Darvas, 1991; Johnson, 1992; Nakasone & Paul, 1998).

Botryosphaeria spp. are recognised as endophytes and latent pathogens of mango (Johnson, 1992). When trees are weakened by stress, the quiescent state of these endophytic fungi ends, and disease symptoms develop (Schoeneweiss, 1979; Wene & Schoeneweiss, 1980; Johnson, 1992). Predisposition of mango trees, is mainly due to mineral deficiencies (iron, zinc, manganese) and environmental factors such as sunscald and hail damage (Schaffer *et al.*, 1988; Ramos *et al.*, 1991). Water stress is known to predispose young vascular tissue near the cambium to attack by *Botryosphaeria* spp. (Schoeneweiss, 1979; Pusey, 1989; Ramos *et al.*, 1991). Trees can, however, outgrow infection during periods of vigorous growth, which in turn reduces disease impact (Brown & Britton, 1986; Britton & Hendrix, 1986; Johnson, 1992).

Botryosphaeria spp. systemically colonise the vascular system of their hosts (Maas & Uecker, 1984; McPartland & Schoeneweiss, 1984). Infection of young mango trees results in a light reddish brown discoloration of the xylem vessels, while light to dark brown discoloration of the phloem becomes visible later (Herbert & Grech, 1987; Shearer *et al.*, 1987). Xylem and phloem vessels become clogged with tyloses and mycelium and later become necrotic, as the pathogen spreads through the trees. This necrosis may lead to branch and stem dieback, canker formation and eventually tree death (Maas & Uecker, 1984; Shearer *et al.*, 1987; Ramos *et al.*, 1991).

Controlling *Botryosphaeria* diseases on various economically important fruit crops has had limited success (Johnson *et al.*, 1991; Peterson *et al.*, 1991; Sanchote, 1991; Johnson, 1992; Johnson & Sanchote, 1994). In mango, fruit infections by *Botryosphaeria* spp. from external sources can be minimised by spraying with copper oxychloride, but this fails to control endophytic infections (Peterson *et al.*, 1991; Johnson, 1992; Johnson & Sanchote,

1994; Sanchote, 1993). Therefore, in order to manage *Botryosphaeria* diseases, an integrated strategy that combines orchard management, reduction of inoculum through pre- and postharvest practices and the use of timely field spray or postharvest application of chemicals, is used. There is, however, a growing demand for new cultivars with improved resistance to *Botryosphaeria* and other mango pathogens (Johnson & Sanchote, 1994; Finnemore, 2000).

Until recently, little has been known regarding the taxonomy of *Botryosphaeria* spp. on mango in South Africa. Names of fungi belonging to this group have been used interchangeably and arbitrarily. In a recent study (Chapter 2), we have shown that four *Botryosphaeria* spp. occur on mango in South Africa. They include *F. parvum* Penycook & Sameuls, *B. rhodina* (Pat.) Griff. et Maubl., *F. indigoticum* Jacobs, Slippers & Wingf. and *F. bacilliforme* Jacobs, Slippers & Wingf. Virtually nothing is known regarding the pathogenicity of these fungi on mango. Although some pathogenicity tests have been done in the past (Ramos *et al.*, 1991; Johnson, 1992), the isolates were from other continents and the taxa of the fungi involved has received considerable attention since then. The aim of this study was to test the pathogenicity of the four species occurring on mango in South Africa. Two commercially important mango cultivars grown in South Africa were also evaluated for their resistance to the four *Botryosphaeria* spp. under glasshouse conditions.

Isolates used

Fourty eight monoconidial *Botryosphaeria* isolates, representing all four *Botryosphaeria* species that have been isolated from mango in South Africa (Chapter 2), were used in a preliminary apple-based screening trial. Based on the results of this preliminary screening, nine isolates representing the four *Botryosphaeria* spp. from mango in South Africa, were chosen for inoculation of mango trees (Table 1; p111). All isolates were grown on potato dextrose agar (PDA) (Biolab) for seven days prior to inoculations. Preservation and maintenance of all cultures was the same as that used in a previous study (Chapter 2).

Apple fruit assay

Granny Smith apples were used for an initial assessment of pathogenicity of all 48 isolates. This assay was chosen because it has been shown to provide an indication of pathogenicity in other fungi (Enebak *et al.*, 1994; De Lange *et al.*, 1996; Steenkamp *et al.*, 1996) and because mango fruit were not available. Healthy fruit were selected for uniform size and ripeness. Fruit were surface disinfested by dipping them for two minutes in a 2% sodium hypochlorite (NaHOCl) solution, followed by a distilled water rinse and 70% ethanol (EtOH) dip for two minutes. Fruit were left to air-dry for approximately five minutes.

Inoculation wounds were made in the apples by cutting a single three to four mm deep well in the apple body with a cork borer (5mm diam.). Mycelial plugs (5mm diam.) were cut from the edge of actively growing cultures, with a cork borer. Three fruit were inoculated once for each isolate to be screened. Six fruit were used as controls with half of these being either wounded and not inoculated or inoculated with a sterile PDA plug. All wounds were

covered with strips of parafilm for the duration of the trial, to prevent desiccation and secondary infection.

Fruit were incubated at 25°C for approximately eight days, until they were completely rotten. Lesion lengths and widths were measured every two-days from the second day after inoculation. Average sizes of lesions including the initial wound were computed and data were analysed.

Inoculation of mango trees

Isolates for the inoculation on mango trees were selected based on the apple screening assay. One of the most and least pathogenic isolates of all four species were selected to be inoculated in mango trees. For *B. parva*, an intermediately pathogenic isolate was also included. The isolates chosen for tree inoculations were *B. rhodina* [BOT2399, BOT2376], *B. parva* [BOT2413, BOT2302, BOT2353], *F. indigoticum* [BOT2351, BOT2355] and *F. bacilliforme* [BOT2421, BOT2417].

A total of 120 one-year-old trees (60 of each of the cultivars Keitt and Tommy Atkins) were obtained from Westvalia Estates, Tzaneen. These cultivars are respectively reported to be tolerant and susceptible to *Botryosphaeria* spp. in the orchard (Lonsdale, personal communication). Trees were maintained in a glasshouse at 20°C – 28°C for six weeks, prior to inoculation. Inoculations were conducted on two completely randomised blocks of trees, during July - September (mid-winter to early spring) 2001. In each trial, three trees of each cultivar were inoculated per isolate or sterile PDA plugs, which served as controls. Stems were surface disinfested by wiping them with 70% EtOH prior to inoculation. Wounds were made with a cork borer (5mm diam.) between two nodes, situated above the

graft union, but underneath the first branch. Mycelium plugs (5mm diam.) were cut with a cork borer from the edges of actively growing colonies and inserted into the wounds. The inoculation wounds were covered with Parafilm to prevent desiccation and contamination. Lesion length measurements were taken six weeks after inoculation. The lesions were measured by calculating the maximum length of vascular discoloration below the bark of the tree (Britton *et al.*, 1990). For re-isolation of the inoculated fruit, small tissue pieces were cut from the edges of discoloured tissue and incubated on PDA at 25°C.

Statistical analyses

In this study, pathogenicity was defined based on the extent of lesion development arising from inoculation. All statistical analyses were performed using SAS statistical software (Version 7, SAS Institution, Cary, NC). For the apple fruit assay and potted tree inoculation, analysis of variance (ANOVA) was by the General Linear Model (GLM) procedure. Data were not corrected and all actual values are reported. Means were grouped by Duncan's multiple range test with $P = 0.05$.

RESULTS

Apple fruit assay

All isolates inoculated on apples produced typical fruit rots (Fig. 1; p113). Inoculations of fruit with the four *Botryosphaeria* spp. mostly produced a soft rotten circular area with a light tan colour, up to the lesion edges. Inoculations with *Fusicoccum indigoticum,* however, produced a firmer and darker brown rotten area. *Botryosphaeria rhodina* isolates showed very little variation in pathogenicity (Fig. 2; p115). Little variation was also seen for most *B. parva* isolates, other than for two isolates [BOT 2400, BOT 2350], which

produced significantly smaller lesions (Pr = 0.0936). One isolate of *F. indigoticum* [BOT 2315] produced similar sized lesions to isolates of *B. rhodina* and *B. parva*. The other isolate of this species [BOT 2355] produced lesions that were significantly smaller than the former isolate (Fig. 2; p115). One *F. bacilliforme* isolate produced lesions smaller than those of the other species, while the other isolate did not produce lesions. No lesions developed in any of the control inoculations (Fig. 2; p115).

Potted tree assay

All four species of *Botryosphaeria* were pathogenic and produced lesions on inoculated mango stems in both trials. In all cases, lesion lengths differed significantly from the controls (Fig. 3; p117). External symptom development was minimal with no dieback, cankering or bark cracks developing in any tree during the six week incubation period of both trials. Lateral movement of all *Botryosphaeria* isolates inoculated into mango stems was limited. All inoculated fungi could easily be re-isolated from lesions but not from control inoculations.

Pathogenicity of isolates did not vary greatly within species (Fig. 3; p117). Isolates of the *F. parvum* [BOT2413, BOT2302, BOT2353], *B. rhodina* [BOT2399, BOT2376] and *F. indigoticum* [BOT2351, BOT2355] were equally pathogenic (Fig. 3; p117). One isolate of *F. bacilliforme* [BOT2421] produced lesions of similar size to the other *Botryosphaeria* spp. inoculated (Fig. 3; p117). The second *F. bacilliforme* isolate [BOT2417] used in this study, however, produced significantly smaller lesions than all other isolates. These were not significantly larger than the lesions produced in the control inoculations (Fig. 3; p117). Control inoculations resulted in small lesions that were ascribed to a wound reaction.

Lesions on mango trees in the first trial were significantly (Pr = 0.0001) longer than those produced in the second trail for all isolates and species (Fig. 4; p119). The greatest variation in lesion size between the two trails was seen for *F. indigoticum* [BOT2355, BOT2351] and *B. rhodina* [BOT2399, BOT 2376] (Fig. 4; p119). These species produced approximately one third smaller lesion lengths in the second trial, compared to those associated with the same isolates in the first trial.

Both mango cultivars were susceptible to all *Botryosphaeria* spp. tested. Lesions were slightly larger on the Keitt compared to the Tommy Atkins cultivar, but this variation was not statistically significant (Fig. 3; p117). The relative pathogenicity of all isolates remained the same, regardless of the cultivar inoculated.

Isolates of *B. rhodina* [BOT 2399] and [BOT 2376] that were most and least pathogenic respectively in the apple inoculation trials (Fig 2; p115), were equally pathogenic during both potted tree trials (Fig. 4; p119). *Botryosphaeria parva* isolates [BOT 2302], [BOT 2353] and [BOT 2413] tree trials (Fig. 4; p119). *Botryosphaeria parva* misolates [BOT 2302], [BOT 2353] and [BOT 2413] were most, intermediately and least pathogenic respectively in the apple inoculation trials (Fig. 2; p115). BOT 2353 were, however, more pathogenic than BOT 2302 in the potted tree trials. The isolate, BOT 2413, was the least pathogenic isolate in both apple and tree trials (Fig. 4; p119). Significant differences were found in lesion sizes produced by isolates of *F. indigoticum* [BOT 2315, BOT 2355] when inoculated on apples (Fig. 2; p115), but lesion sizes between these isolates in inoculated trees did not differ significantly (Fig. 4; p119). *Fusicoccum bacilliforme* isolate BOT 2417 was significantly more pathogenic than BOT 2421 (Fig. 2; p115), but BOT 2421 seemed

significantly more pathogenic that isolate BOT 2417 during the tree inoculation trials (Fig. 4; p119).

DISCUSSION

Results of this study have shown that all four *Botryosphaeria* species recently recognised as occurring on mango in South Africa are pathogenic. These fungi were also found to be pathogenic on the two most commonly grown commercial cultivars in this country. The symptoms arising from inoculation also suggest that these species have the ability to colonise and spread rapidly within mango trees. Results are similar to those with other *Botryosphaeria* spp., which have been shown to spread through the vascular system by causing tissue discoloration and clogging vessels with tyloses and mycelium (Maas & Uecker, 1984; Ramos *et al.*, 1991).

The most and least pathogenic isolates for each of the four *Botryosphaeria* spp., identified using the apple assay, did not produce significantly different lesion sizes on inoculated mango stems. Our results are similar to those of Brown-Rytlewski & McManus (2000) who reported a lack of correlation between the pathogenicity of *Botryosphaeria* isolates inoculated on fruit and stems. This lack of correlation between apple and potted-tree assays was suggested to be attributable to variation in incubation temperature, fruit ripeness, fruit size and tree vigour (Sutton, 1983; Brown-Rytlewski & McManus, 2000). Clearly, lesions on apple fruit do not reflect relative pathogenicity on mango plants. Mango fruit have been used in pathogenicity and cultivar resistance trials (Johnson,1992; Sanchote, 1991), however, fruit are not readily available and fruit ripeness levels at inoculation and endophytic colonisation can confuse results. However, we chose not to use mango fruit in

this study because *Botryosphaeria* spp. are commonly isolated from mango fruit and these may have influenced our results.

Botryosphaeria parva, B. rhodina and *F. indigoticum* were equally pathogenic to mango trees in this study. *Botryosphaeria parva* is the most frequently encountered fungus in mango orchards and appears to be the most important *Botryosphaeria* spp. causing mango decline in South Africa. Both *B. parva* and *B. rhodina* are also common on mango fruit (Darvas, 1991; Chapter 2). *Fusicoccum indigoticum* is, however, very rarely found in mango orchards or on fruit (Chapter 2). The dominant occurrence, together with the pathogenicity of *B. parva* and *B. rhodina* in orchards and on fruit is thus important to consider when developing disease control strategies. The smaller lesions, lower virulence and low isolation frequency (Chapter 2) of *F. bacilliforme*, as well as the variation in pathogenicity of isolates, suggests that this species does not contribute significantly to mango diseases in South Africa under these conditions.

Botryosphaeria rhodina and *F. indigoticum* gave rise to significantly smaller lesions in the second inoculation trial on mango stems. It is possible that these differences were due to physiological changes in the mango trees (Britton & Hendrix, 1986; Johnson, 1992). The fact that *B. parva* and *F. bacilliforme* produced lesions of similar length in both trials, suggests that different *Botryosphaeria* spp. may respond differently to the environment and host. Pathogen reaction to seasonal variation should thus be considered before final conclusions are made regarding the role of different *Botryosphaeria* spp. in disease (Britton & Hendrix, 1986; Brown-Rytlewski & McManus, 2000).

There were no obvious differences in the extent of vascular discoloration resulting from the four *Botryosphaeria* spp. on the two mango cultivars used in this study. Keitt has, however, previously been noted as more tolerant to infection by *Botryosphaeria* spp. under field conditions (Lonsdale, personal communication). Our results may suggest that, in the absence of environmental stress, the cultivars express the same level of susceptibility to infection by *Botryosphaeria* spp. It is, however, possible that under field conditions, cultivar Keitt may tolerate environmental stress more effectively, and this may give rise to an impression of disease resistance. Variation in tolerance to *Botryosphaeria* infection of different cultivars under field conditions has been reported with other woody hosts (English & De Vay, 1975; Sutton, 1983).

The pathogenic ability of *Botryosphaeria* spp. on mango in South Africa suggests that most of these species have the ability to cause diseases. *Botryosphaeria parva* and *B. rhodina* are, however, the most important to consider when management strategies are implemented. Resistance of cultivars to these pathogens should be tested under field conditions, as greenhouse trials do not accurately reflect this in a cultivar. Currently, the most effective means of control of *Botryosphaeria* diseases can be achieved through increasing plant vigour by reducing stress. This can minimise disease incidence due to *Botryosphaeria* spp., which will possibly impact on mango quality and production both pre- and postharvest.

REFERENCES

Britton, K.O. & Hendrix, F.F. 1986. Population dynamics of *Botryosphaeria* spp. in Peach gummosis cankers. *Plant Disease* 70: 134-136.

Britton, K.O., Hendrix, F.F.; Pusey, P.L., Okie, W.R., Reilly, C.C. & Daniell, J.W. 1990. Evaluating the reaction of peach cultivars to infection by three *Botryosphaeria* species. *HortScience* 25: 468-470.

Brown, E.A. & Britton, K.O. 1986. *Botryosphaeria* diseases of apple and peach in the Southern United States. *Plant Disease* 70: 480-484.

Brown-Rytlewski, D.E. & McManus, P.S. 2000. Virulence of *Botryosphaeria dothidea* and *Botryosphaeria obtusa* on apple and management of stem cankers with fungicides. *Plant Disease* 84: 1031-1037.

Darvas, J.M. 1991. *Dothiorella dominicana*, a new mango pathogen in South Africa. *Phytophylactica* 23: 295-298.

De Lange, W.J. 1996. Isolation of dsRNA and screening for virulence in infected South African isolates of *Cryphonectria cubensis*. MSc thesis. University of Pretoria, Pretoria, South Africa.

Donkin, D.J. & Oosthuyse, S.A. 1996. Quality evaluations of sea-exported South African mangoes in Europe during the 1995/1996 season. *South African Mango Growers' Association Yearbook* 16: 1-5.

Enebak, S.A., Hillman, B.I. & Mac Donald, W.L. 1994. A hypovirulent isolate of *C. paracitica* with multiple, genetically unique dsRNA segments. *Molecular Plant-Microbe Interactions* 7: 590-595.

English, H., Davis, J.R. & DeVay, J.E. 1975. Relationship of *Botryosphaeria dothidea* and *Hendersonula toruloidea* to a canker disease of almond. *Phytopathology* 65: 114-122.

Finnemore, H.J. 2000. An overview of the South African mango industry (past and future). Pp 1 - 11 http://students.washington.edu/melliott/arbutus/natt.htm

Herbert, J.A. & Grech, N.M. 1985. Branch dieback of macadamias in South Africa induced by *Botryosphaeria ribis*. *Plant Disease* 69: 83.

Johnson, G.I. 1992. Biology and control of stem end rot pathogens of mango. Ph.D. thesis. University of Queensland, Queensland, Australia.

Johnson, G.I., Cooke, A.W., Mead, A.J. & Wells, I.A. 1991. Stem end rot of mango in Australia: Causes and control. *Acta Horticulturae* 291: 288-295.

Johnson, G.I. & Sangchote, S. 1994. Control of post-harvest diseases of tropical fruits: Challenges for the 21st century. In: Post-harvest handling of tropical fruit. Proceedings of an International Congress Chaing Mai. Thailand. Champ, B.R., Highley, E. and Johnson, G.I. (Eds.). pp 140-161. Australian Centre for International Agricultural Research, Canberra.

Lonsdale, J.H. 1993. Preliminary results on the mode of infection of *Nattrassia mangiferae* in mango. *South African Mango Growers' Association Yearbook* 13: 97-99.

Maas, J.L. & Uecker, F.A. 1984. *Botryosphaeria dothidea* cane canker of Thornless blackberry. *Plant Disease* 68: 720-726.

McPartland, J.M. & Schoeneweiss, D.F. 1984. Hyphal morphology of *Botryosphaeria dothidea* in vessels of unstressed and drought-stressed stems of *Betula alba*. *Phytopathology* 74: 358-362.

Nakasone, H.Y. & Paull, R.E. 1998. Mango. In: Tropical Fruits. pp. 208-237. CAB International, Wallingford.

Peterson, R.A., Johnson, G.I., Schipke, L.G. & Cooke, A.W. 1991. Chemical control of stem end rot in mango. *Acta Horticulturae* 291: 304-307.

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

Ramos, L. J., Lara, S.P., McMillan Jr, R.T. & Narayanan, K.R. 1991. Tip dieback of mango (*Mangiferae indica*) caused by *Botryosphaeria ribis. Plant Disease* 75: 315-318.

Sangchote, S. 1991. *Botryodiplodia* stem end rot of mango and its control. *Acta Horticulturae* 291: 296–304.

Sanchote, S. 1993. Effects of fruit bagging, fruit position, cultivar and post-harvest traetment on post-harvest diseases of mangoes. In: Post-harvest handling of tropical fruit. Proceedings of an International Congress Chaing Mai, Thailand. Champ, B.R., Highley, E. and Johnson, G.I. (Eds.). pp 63-66, Australian Centre for Internationsl Agricultural Research, Canberra.

Schaffer, B., Larson, K.D., Snyder, G.H. & Sanchez, C.A. 1988. Identification of mineral deficiencies associated with mango decline by DRIS. *HortScience* 23: 617-619.

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

Schoeneweiss, D.F. 1979. Protection against stress predisposition to *Botryosphaeria* canker in containerized *Cornus stolonifera* by soil infection with Benomyl. *Plant Disease Reporter* 63: 896-900.

Singh, L.B. 1960. Mango. In: The Mango. World Crop Series. pp 320-343. Interscience Publishers Inc., New York.

Steenkamp, E.T. 1996. A preliminary study of hypovirulence and dsRNA in *Sphaeropsis sapinea*. MSc thesis. University of Pretoria, Pretoria, South Africa.

Sutton, T.B. 1983. Inoculum availability and pathogenic variation in *Botryosphaeria dothidea* in apple production areas of North Carolina. *Plant Disease* 67: 503-506.

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

Table 1 Isolates used for potted-tree inoculations in this study

Culture nr.[1]	Identity[2]	Isolated from	Mango cultivar	Isolation area	Location	Isolator
BOT 2413	*Fusicoccum parvum*	Branch	Tommy Atkins	Hoedspruit	Mpumalanga, SA	R. Jacobs
BOT 2302	*F. parvum*	Side branch	Tommy Atkins	Hoedspruit	Mpumalanga, SA	R. Jacobs
BOT 2353	*F. parvum*	Fruit	Sensation	Letsetele Valley	Mpumalanga, SA	R. Jacobs
BOT 2351	*F. indigoticum*	Leaf stem	Tommy Atkins	Hoedspruit	Mpumalanga, SA	R. Jacobs
BOT 2355	*F. indigoticum*	Fruit	Sensation	Letsetele Valley	Mpumalanga, SA	R. Jacobs
BOT 2417	*F. bacilliforme*	Main stem	Heidi	Malelaan	Mpumalanga, SA	R. Jacobs
BOT 2421	*F. bacilliforme*	Side branch	Heidi	Malelaan	Mpumalanga, SA	R. Jacobs
BOT 2376	*Botryosphaeria rhodina*	Fruit	Sensation	Hoedspruit	Mpumalanga, SA	R. Jacobs
BOT 2399	*B. rhodina*	Side branch	Sensation	Mariepskop	Mpumalanga, SA	R. Jacobs

[1]Culture collections where isolates are kept: BOT = Forestry and Agricultiral Biotechnology Institute (FABI), University of Pretoria.

[2]Identities as determined in this study

Figure 1. Symptom development after apple fruit were inoculated with *Botryosphaeria parva* [BOT 2302]. (A) represents the inoculation wound with symptom development (B) after two days, (C) four days, (D) six days and (E) seven days incubation at 25°C.

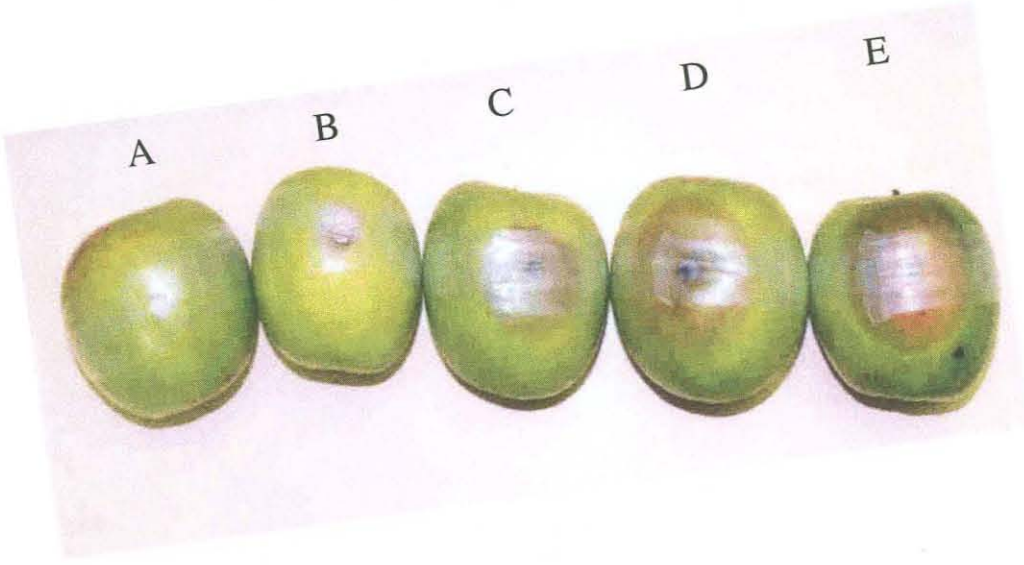


Figure 2. Graph illustrating variation in pathogenicity (y-axis) of *Botryosphaeria* isolates (x-axis) screened for pathogenicity on apple fruit (Pr = 0.0936). (A) represents isolates of *Botryosphaeria rhodina*, (B) represents *F. parvum*, (C) represent *F. indigoticum* and (D) represent isolates of *F. bacilliforme*.

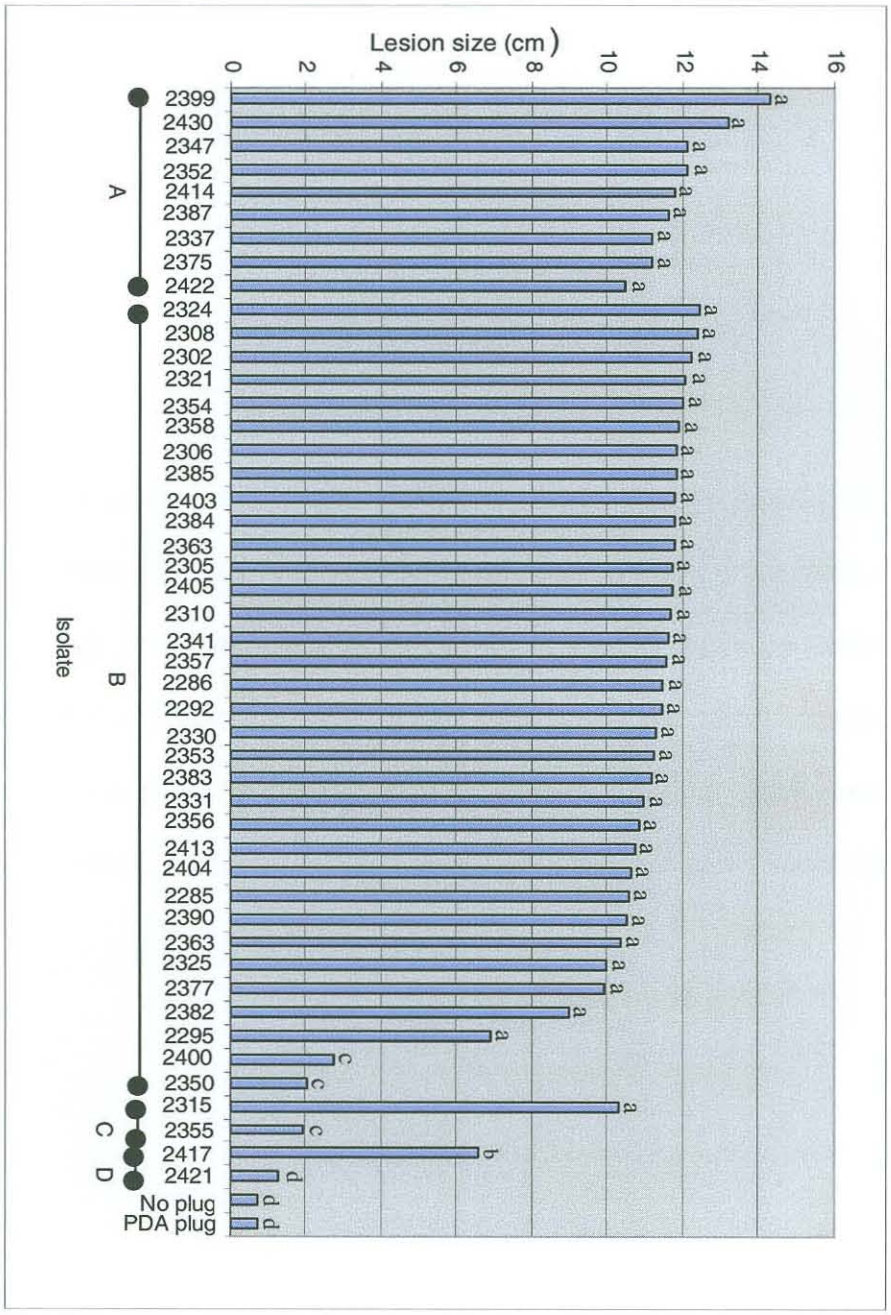

Lesion size (cm)
0
2
4
6
8
10
12
14
16
Isolate
A
B
C
D
2399
2430
2347
2352
2414
2387
2337
2375
2422
2324
2308
2302
2321
2354
2358
2306
2385
2403
2384
2363
2305
2405
2310
2341
2357
2286
2292
2330
2353
2383
2331
2356
2413
2404
2285
2390
2363
2325
2377
2382
2295
2400
2350
2315
2355
2417
2421
No plug
PDA plug

Figure 3. Lesions lengths (y-axis) associated with *Botryosphaeria* isolates inoculated on two mango cultivars Keitt and Tommy Atkins. Bars with the same letter do not differ significantly for each other. Bars bearing different letters differ significantly according to Duncan's multiple range test (Pr = 0.0001). (A) represents isolates of *Botryosphaeria rhodina*, (B) represents *F. parvum*, (C) represent *F. indigoticum* and (D) represent isolates of *F. bacilliforme*.

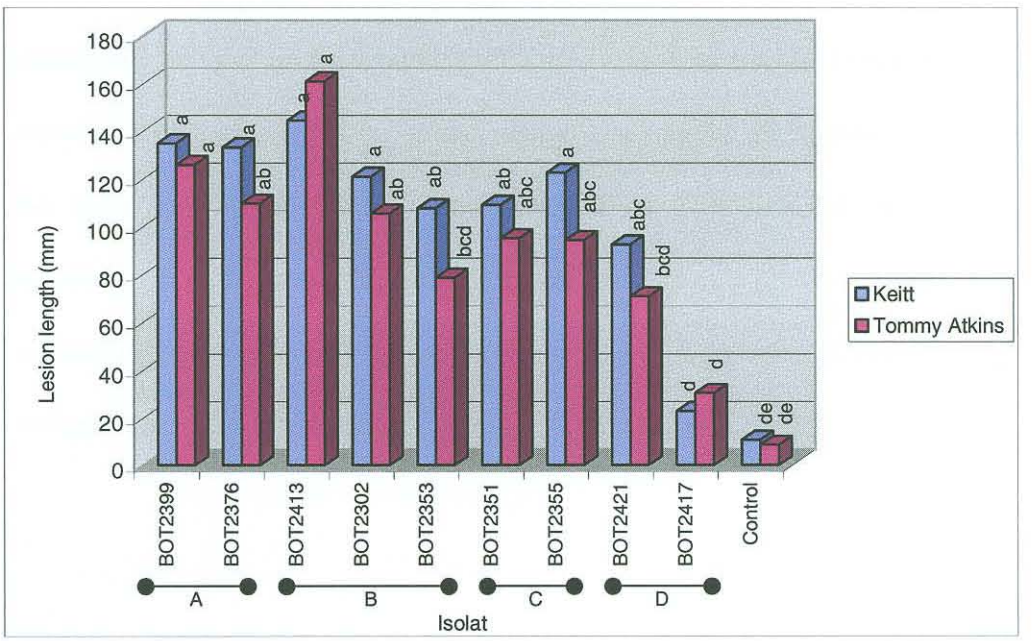
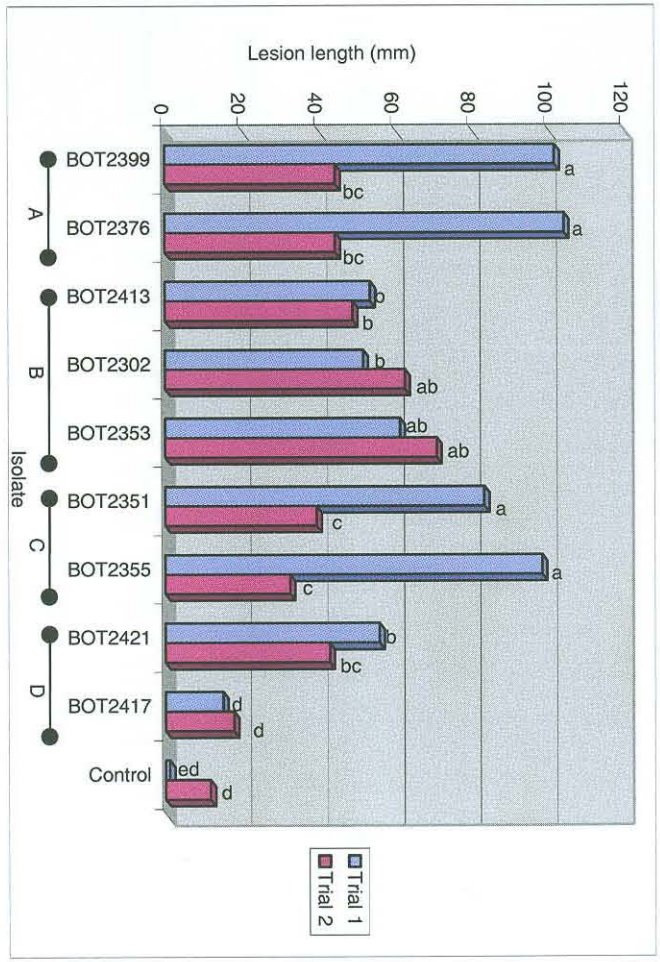


Figure 4. Lesion lengths (y-axis) associated with *Botryosphaeria* isolates (x-axis) in two separate inoculation trials on potted mango trees in the glasshouse. Bars with the same letter do not differ significantly. Bars with different letters differ significantly with Duncan's multiple range test (Pr = 0.0001). (A) represents isolates of *Botryosphaeria rhodina*, (B) represents *F. parvum*, (C) represent *F. indigoticum* and (D) represent isolates of *F. bacilliforme*.


Lesion length (mm)
0
20
40
60
80
100
120
BOT2399
BOT2376
BOT2413
BOT2302
BOT2353
BOT2351
BOT2355
BOT2421
BOT2417
Control
A
B
C
D
Isolate
a
bc
a
bc
b
b
b
ab
ab
ab
a
c
a
c
b
bc
d
d
ed
d
Trial 1
Trial 2

CHAPTER 4

DEVELOPMENT AND TESTING OF A PCR-RFLP IDENTIFICATION SYSTEM FOR *BOTRYOSPHAERIA* SPECIES FROM MANGO

ABSTRACT

Botryosphaeria spp. are the primary cause of many diseases of mango fruit and trees. The taxonomy of these species is currently in disarray, because traditional morphological characteristics do not sufficiently distinguish species. In recent years, DNA sequencing has been introduced to resolve identification problems with *Botryosphaeria* spp., but this approach is impractical and inordinately expensive for the rapid identification of large numbers of isolates. The aim of this study was, therefore, to develop a PCR-RFLP system for rapid and reliable identification of *Botryosphaeria* isolates from mango. This technique was then used to identify a large collection of *Botryosphaeria* isolates from the main mango producing regions and cultivars in South Africa. ITS-PCR amplicons were digested with *Cfo*I, *Alu*I or *Bst*71I restriction enzymes (RE) to obtain polymorphic banding patterns. All four *Botryosphaeria* spp. from mango in South Africa were distinguished with *Cfo*I digestion of the PCR products. *Alu*I and *Bst*71I could distinguish Australian isolates *Fusicoccum aesculi* and *F. mangiferum* from the South African isolates. *Botryosphaeria parva* was the most dominant species among the isolates from mango in South Africa, followed by *B. rhodina*.

INTRODUCTION

Botryosphaeria spp. regularly cause stem and branch cankers, twig die-back, blossom blight, leaf spot and fruit rot on various woody hosts, including subtropical fruit crops such as mango (Punithalingam, 1980; Sutton, 1980; Pennycook & Samuels, 1984; Johnson, 1992; Lonsdale, 1993). These disease symptoms, of which stem end rot (SER) and soft brown rot (SBR) are amongst the most serious, are responsible for great losses for mango producers globally (Ramos *et al.*, 1991; Johnson, 1992; Donkin & Oosthuyse, 1996). In South Africa, the seriousness of mango tree diseases has recently been emphasised by wide-spread outbreaks in orchards resulting in tree deaths and substantial economic losses for mango producers (Finnemore, 2000).

Various anamorphs of *Botryosphaeria* are regularly found on trees and tree debri in mango orchards. The species identified as endophytes and opportunistic pathogens of mango in South Africa are the *F. parvum* Pennecook & Samuels (previously known as *Dothiorella dominicana*) (Chapter 2, Table 2; p71), *F. bacilliforme* Jacobs, Slippers & Wingf. (known as *Dothiorella* 'long'*), F. indigoticum* Jacobs, Slippers & Wingf. and *B. rhodina* (Berk. & Curt.) von Arx (Chapter 2). The relative importance of these different species on mango in South Africa is currently not known. Other *Botryosphaeria* spp. that also affect mango in Australia have been identified as *F. aesculi* Corda (known as *D. aromaticum*) and *F. mangiferum* (known as *Nattrassia mangiferae* (Nattrass.) Sutton et Dyko) (Chapter 2, Table 2; p71)(Johnson *et al.*, 1991; Johnson, 1992; Slippers et al., 2001).

Identification of *Botryosphaeria* spp. is mostly based on morphological characteristics of the associated anamorphs. Morphological similarities between the anamorphs, especially

among the *Fusicoccum* spp. has, however, hindered the identification of these fungi in the past (Johnson, 1992). In recent years, the use of molecular techniques, together with morphological characteristics, has proven useful in identifying *Botryosphaeria* anamorphs (Jacobs & Rehner, 1998; Denman *et al.*, 2000; Zhou & Stanosz, 2001; Slippers *et al.*, 2001; Smith & Stanosz, 2001; Smith *et al.*, 2001; Chapter 2). These molecular analyses are, however, mostly based on sequence data, the generation of which can be time consuming and costly to obtain for large numbers of isolates.

Various DNA-based techniques, other than DNA sequencing have been applied to identify fungal genera and species. These include random amplified polymorphic DNA (RAPD) (Zimand *et al.*, 1994; Thompson & Latorre, 1999), protein profiles (Lattore *et al.*, 1995), amplified fragment length polymorphisms (AFLPs) (Janssen *et al.*, 1996; Rosendahl & Taylor, 1997) and simple sequence repeats (SSRs) (Tautz, 1989; Weber & May, 1989). These techniques, however, have various limiting factors, such as non-repeatability, high levels of technical difficulty and the need for careful optimisation (Weising *et al.*, 1995; Buscot *et al.*, 1996). A relatively simple, yet reliable technique for distinguishing between strains is restriction fragment length polymorphism (RFLP) fingerprinting of polymerase chain reaction (PCR) products. This technique has often been used for identifying fungi up to species level (Bruns *et al.*, 1991; Buscot *et al.*, 1996; Taylor *et al.*, 1999).

Information regarding the occurrence and relative importance of different *Botryosphaeria* spp. on mango is important when developing effective disease control strategies. The first aim of this study was, therefore, to develop a rapid and effective identification system for *Botryosphaeria* isolates from mango, using PCR-RFLPs. This technique was subsequently utilised in an orchard survey to identify the dominant *Botryosphaeria* spp. in the main

mango producing regions of South Africa. From these data, the relationship between dominant *Botryosphaeria* species, symptoms expressed and cultivars affected, was also considered.

MATERIALS AND METHODS

Development of a PCR-RFLP identification protocol

Sequence data of the internally transcribed spacer (ITS1, 5.8S and ITS 2) region of known *Botryosphaeria* spp. from mango in Australia and South Africa (Chapter 2), were used to identify polymorphic restriction enzyme (RE) sites. This was achieved visually and using the programme Webcutter (http://www.firstmarket.com/cgi-bin/cutter). Restriction enzymes *Cfo*I, *Alu*I and *Bst*71I were identified from sequence data as potentially useful and thus utilised to produce distinguishable polymorphic banding patterns for the different species.

A modified version of the method of Raeder and Broda (1985) was used for DNA isolation from all isolates obtained during this study as described in Chapter 2. A portion of the nuclear rDNA operon was amplified with the polymerase chain reaction (PCR) using primers ITS1 and ITS4 (MWG Biotech, Germany) (White et al., 1990) as described in Chapter 2. All PCR products were digested with the restriction enzymes described above, one per reaction, and visualised on a 3% horizontal agarose gel using a TAE buffer electrophoresis system (Maniatis *et al.*, 1982).

Survey using PCR-RFLP

Botryosphaeria isolates were obtained during an orchard survey of five regions in the Northern province and Mpumalanga, namely Constantia (20%), Hoedspruit (32%), Letsetele Valley (24%), Malelane (20%) and Mariepskop (6%) (Table 1; p135)(Fig. 1; p137). This is the primary mango producing areas of South Africa. To obtain the total number of samples, the commercial cultivars Sensation (26%), Tommy Atkins (37%), Keitt (11%), Kent (19%) and Heidi (6%) were sampled (Table 1; p135). Isolations were made from asymptomatic material (8%) or symptomatic tree trunks (8.5%), branches (29.5%), leaves (5%), blossoms (9%) and fruit (40%) from different orchards during August to September 1999 and 2000 (late winter to early spring) (Table 1; p135) (Fig. 2; p139).

Samples were disinfested twice with 70% (v/v) ethanol and air died for five minutes. Isolations from symptomatic material were made from disks cut at the lesion edge. Disks of asymptomatic plant tissue were cut from all plant parts. All isolates were cultured on potato dextrose agar (PDA) (Biolab) amended with chloramphenicol and were incubated at 25°C for seven to twelve days. Isolates were induced to sporulate on water agar (Biolab) amended with sterile pine needles, and single spore isolates were made, as described in Chapter 2. All single spore isolates were identified by using the PCR-RFLP identification system as described previously. All cultures are maintained at 4°C in the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria.

Development of PCR RFLP identification

All four *Botryosphaeria* spp. isolated from mango in South Africa could be differentiated by cleavage of the ITS PCR products with RE *Cfo*I and visualisation of the polymorphic banding patterns (Fig. 3; p141). *Cfo*I digestion of the PCR amplicons did not produce polymorphic banding patterns for *F. indigoticum* and two *Botryosphaeria* spp. isolated from mango in Australia, namely *F. mangiferum* and *F. aesculi* (Fig. 3; p141). Cleavage of the ITS PCR products with RE *Alu*I differentiated *F. aesculi* from *F. indigoticum* and *F. mangiferum* (Fig. 4; p143). *F. indigoticum* and *F. mangiferum* ITS PCR amplicons were then separated with the RE *Bst*71I (Fig. 5; p145). Sizes of the fragments of the ITS PCR products after cleavage with *Cfo*I, *Alu*I and *Bst*71I are indicated on a RE cleavage site map (Fig. 6; p147).

Survey using PCR-RFLP

A total of 156 *Botryosphaeria* isolates were obtained from mango tree trunks (5.1%), branches (35.3%), leafs (0.6%), blossoms (5.1%) and fruit (46.2%) (Table 1). These *Botryosphaeria* spp. were isolated from all five mango producing regions in the Northern province and Mpumalanga, namely Mariepskop (7.7%), Letsetele Valley (34.6%), Constantia (9.6%), Hoedspruit (35.6%) and Malelane (12.2%). The different mango cultivars namely, Sensation (34.6%), Tommy Atkins (49.4%), Kent (1.9%), Keitt (13.5%) and Heidi (1.3%), all yielded *Botryosphaeria* isolates.

All isolates obtained were identified to species level, using the PCR-RFLP technique described previously (Table 1; p135). Results of this survey showed that *B. parva*

represents 82.1% of isolates obtained in this study from diseased fruit, leaves, branches and tree trunk material, as well as asymptomatic tissue (Table 1; p135). This species was, however, isolated most frequently from tree branches and fruit (Table 1; p135). *Botryosphaeria rhodina* was isolated as the second most dominant species (16.0%), mostly from fruit and asymptomatic plant material (Table 1; p135). *Fusicoccum indigoticum* was isolated only from a fruit rot and *F. bacilliforme* from a cankered leaf and discoloured tree branch with a 1.3% isolation frequency each (Table 1; p135).

DISCUSSION

In this study, the four *Botryosphaeria* spp. found on mango in South Africa, namely *F. indigoticum*, *F. bacilliforme*, *B. parva* and *B. rhodina,* were easily identified using the PCR-RFLP technique that was developed. These species could also be distinguished from two species found in Australia, which have not yet been identified from mango in South Africa, namely *F. mangiferum* and *F. aesculi*. This technique overcomes the difficulties experienced using morphological characteristics to identify *Botryosphaeria* spp. from mango. It is simple and rapid and negates problems experienced when needing to sequence DNA from large numbers of isolates in order to confirm their identity.

Botryosphaeria parva was the dominant *Botryosphaeria* spp. found on mango in South Africa. It was isolated from symptomatic and asymptomatic mango tissue from four commercial cultivars in all five production regions surveyed. This species was more frequently obtained than any other *Botryosphaeria* spp., from all plant parts, and was most frequent on fruit and branches. *Botryosphaeria parva* is also the dominant *Botryosphaeria* sp. reported as a pathogen on other woody hosts in various countries, where it causes

diseases that contribute to substantial economic losses (Brown & Britton, 1986; Reckhaus, 1987; Shearer *et al.*, 1987; Darvas, 1991). Furthermore, *B. parva* has been shown to be one of the most pathogenic *Botryosphaeria* spp. on mango (Ramos *et al.*, 1991; Chapter 3). We, therefore, consider *B. parva* to be the main cause of *Botryosphaeria* diseases on mango trees in the orchards and on mango fruit in South Africa. Management practices should thus focus strongly on controlling this species.

In this survey, *B. rhodina* was the second most dominant species isolated. This species was mostly obtained from fruit rots and asymptomatic plant tissue. These findings are similar to those from previously published literature, where *B. rhodina* is well-documented as endophyte and the most common fruit rot pathogen of many fruit crops, including mango (Punitalingham, 1980; Sanchote, 1991; Johnson, 1992). The fact that this species was infrequent or absent from any symptomatic plant parts other than the fruit, suggests that it is probably insignificant in causing tree diseases. This is despite the fact that it has been shown to be able to cause significant lesions on inoculated trees (Chapter 3).

No isolates of *F. indigoticum* and *F. bacilliforme* were identified, other than those included for reference purposes from a previous study (Chapter 2). These species have recently been described from South Africa for the first time in Chapter 2, and have been shown to be pathogenic and weakly pathogenic, respectively (Chapter 3). *Fusicoccum indigoticum* was isolated from diseased fruit and leaves, while *F. bacilliforme* isolates were obtained only from diseased branches. Our survey suggests that these species are relatively unimportant in causing disease on mango in South Africa.

Botryosphaeria spp. were isolated from all mango producing regions of South Africa surveyed, but were isolated more commonly in certain regions. The highest frequency of *Botryosphaeria* spp. present was in the regions, Letsetele Valley, Hoedspruit and Mariepskop. These regions contributed to over 75% of all isolates obtained, although only 62% of the samples were collected from the areas. These results can be due to different environmental stress conditions on mangoes in the different regions (Johnson, 1992). Regions with higher rainfall (such as the Letsetele Valley) are usually more severely affected by *Botryosphaeria* spp. due to water stress (Johnson *et al.*, 1992).

Botryosphaeria spp. were isolated in varying frequency from the different commercial mango cultivars grown in South Africa. For example, *Botryosphaeria* spp. were isolated from Sensation and Tommy Atkins with a higher frequency (63% of total samples yielding 80% of total isolates) than was the case with Keitt and Kent (30% of total samples yielding 18% of total isolates). This correlates with the fact that Keitt and Kent are more disease tolerant under field conditions than Tommy Atkins and Sensation (Finnemore, 2000). Very few isolates were obtained from cultivar Heidi, but this might also be due to the fact that very few samples of this cultivar were available.

Botryosphaeria diseases symptoms were most common on mango tree branches and fruit. By far the highest number (80%) of all *Botryosphaeria* isolates obtained in this study were isolated from these symptoms, while 12% were obtained from diseased tree trunks, leaves and blossoms. A very small number (8%) of all isolates were from asymptomatic tissue, but these come from all different plant parts. These data correspond with findings of Johnson *et al.*, (1992), in which the endophytic colonisation of healthy mango tissue by *Botryosphaeria* spp. was evident in all mango plant parts. The fact that endophytic

colonisation is found in all healthy plant parts can be explained by movement of *Botryosphaeria* spp. between plant parts through the vascular system or by individual infections on the same tree (Johnson *et al.*, 1992; Ramos *et al.*, 1991; Johnson *et al.*, 1992; Lonsdale, 1993).

The PCR-RFLP identification system developed in this study was used successfully to identify a large number of *Botryosphaeria* isolates to species. In future, this technique could be used to identify *Botryosphaeria* pathogens responsible for disease outbreaks and will thus influence the information used to implement the appropriate control measures. This RFLP technique should also be useful in quarantine measures, enabling screening of samples to prevent introduction of mango pathogens, e.g. *F. aesculi* and *F. mangiferum* that currently do not occur in South Africa.

REFERENCES

Brown, E.A. & Britton, K.O. 1986. *Botryosphaeria* diseases of apple and peach in the Southern United States. *Plant Disease* 70: 480-484.

Bruns, T.D., White, T.J. & Taylor, J.W. 1991. Fungal molecular systematics. *Annual Review of Ecological Systematics*. 22: 525-561.

Buscot, F., Wipf, D., Di Battista, C., Munch, J., Botton, B. & Martin, F. 1996. DNA polymorphism in morels: PCR/RFLP analysis of the ribosomal DNA spacers and microsatellite-primed PCR. *Mycological Research* 100: 63-71.

Darvas, J.M. 1991. *Dothiorella dominicana*, a new mango pathogen in South Africa. *Phytophylactica* 23: 295-298.

Denman, S., Crous, P.W., Taylor, J.E., Kang, J., Pascoe, I. & Wingfield, M.J. 2000. An overview of the taxonomic history of *Botryosphaeria*, and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. *Studies in Mycology* 45: 129-140.

Donkin, D.J. & Oosthuyse, S.A. 1996. Quality evaluations of sea-exported South African mangoes in Europe during the 1995/1996 season. *South African Mango Growers' Association Yearbook* 16: 1-5.

Finnemore H J. 2000. An overview of the South African mango industry (past and future) 1-11 pp. http://students.washington.edu/melliott/arbutus/natt.htm

Jacobs, K.A. & Rehner, S.A. 1998. Comparison of cultural and morphological characters and ITS sequencing in anamorphs of *Botryosphaeria* and related taxa. *Mycologia* 90: 601–610.

Janssen, P., Coopman, R, Huys, G., Swings, J., Bleeker, Vos, P., Zabeau, M. & Kersters, K. 1996. Evaluation of the DNA fingerprinting method AFLP as a new tool in bacterial taxonomy. *Microbiology* 142: 1881-1893.

Johnson, G.I. 1992. Biology and control of stem end rot pathogens of mango. Ph.D. thesis. University of Queensland, Queensland, Australia.

Johnson, G.I., Cooke, A.W., Mead, A.J. & Wells, I.A. 1991. Stem-end rot of mangoes in Australia: Causes and control. *Acta Horticulturae* 291: 288-295.

Johnson, G.I., Mead, A.J., Cooke, A.W. & Dean, J.R. 1992. Mango stem end rot pathogens – Fruit infection by endophytic colonisation of the inflorescence and pedicel. *Annals of Applied Biology* 120: 225–234.

Latorre, B.A., Perez, G.F., Wilcox, W.F. & Torres, R. 1995. Comparative protein electrophoretic and isoenzymic patterns of *Phytophthora crytogea* isolates from Chilean kiwifruit and North American deciduous fruits. *Plant Disease* 79: 703-708.

Lonsdale, J.H. 1993. Preliminary results on the mode of infection of *Nattrassia mangiferae* in mango. *South African Mango Growers' Association Yearbook* 13: 97-99.

Maniatis, T., Fritsch, E.F. & Sambrook, J. 1982. Molecular cloning: A laboratory manual. Cold Spring Harbour Laboratory, Cold Spring Harbour, New York.

Pennycook, S.R. & Samuels, G.J. 1985. *Botryosphaeria* and *Fusicoccum* species associated with ripe fruit rot of *Actinidia deliciosa* (kiwifruit) in New Zealand. *Mycotaxon* 24: 445–458.

Punithalingam, E. 1980. Plant diseases attributed to *Botryodiplodia theobromae* Pat., J. Cramer. Germany. 2, 42-43 pp.

Ramos, L.J., Lam, S.P., McMillan, R.J. & Narayanan, K.R. 1991. Tip dieback of mango (*Mangiferae indica*) caused by *Botryosphaeria ribis*. *Plant Disease* 75: 315–318.

Reckhaus, P. 1987. *Hendersonula* dieback of mango in Niger. *Plant Disease* 71: 1045.

Rosendahl, S. & Taylor, J.W. 1997. Development of multiple genetic markers for studies of genetic variation in arbuscular mycorrhizal fungi using AFLP. *Molecular Ecology* 6: 821-829.

Sangchote, S. 1991. *Botryodiplodia* stem end rot of mango and its control. *Acta Horticulturae* 291: 296–304.

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

Slippers, B., Johnson, G.I., Cooke, A.W., Crous, P.W., Coutinho, T.A., Wingfield, B. D. & Wingfield, M.J. 2001. Taxonomy of *Botryosphaeria* spp. causing stem end rot of mango in Australia. In: Proceedings of the 13th American Plant Pathological Society. 25-29 August. Cairns, Australia.

Smith, H., Crous, P.W., Wingfield, M.J., Coutinho, T.A. & Wingfield, D.B. 2001. *Botryosphaeria eucaliptorum* sp. nov., a new species in the *B. dothidea*-complex on *Eucalyptus* in South Africa. *Mycologia* 93: 277-284.

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

Sutton, B.C. 1980. The *Coelomycetes*. Fungi Imperfecti with pycnidia, acervuli and stroma. CMI, Kew, Surrey, England.

Tautz, D. 1989. Hypervariability of simple sequences as a general source of polymorphic DNA markers. *Nucleic Acid Research* 17: 6463-6471.

Taylor, J.W., Geiser, D.M., Burt, A. & Koufopanou, V. 1999. The evolutionary biology and population genetics underlying fungal strain typing. *Clinical Microbiological Reviews* 12: 126-146.

Thompson, J.R. & Latorre, B.A. 1999. Characterisation of *Botrytis cinerea* from Table grapes in Chile using RAPD-PCR. *Plant Disease* 83: 1090-1094.

Weber, J. & May, P.E. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *American Journal of Human Genetics* 44: 388-396.

Weising, K., Nybom, H., Wolff, K. & Meyer, W. 1995. Genetic variation at the DNA level. In: DNA fingerprinting in plants and fungi. pp 3-23. CRC Press, Florida, U.S.A.

White, T.B., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: A Guide to Methods and Applications. pp. 315-322. M.A. Innis, D.H. Gelfand, J.J. Sninsky & T.J. White. Academic Press, San Diego, U.S.A.

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

Zimand, G., Valinsky, Y.E., Chet, I. & Manulis, S. 1994. Use of the RAPD procedure for the identification of *Trichoderma* strains. *Mycological Research* 98: 531-534.

Table 1 Distribution of four *Botryosphaeria* spp. from mango obtained in this study

Species	Area	Total samples	Total isolates	*B. ribis*	*F. indigoticum*	*F. bacilliforme*	*B. rhodina*
Isolates		**250**	**156**	**128**	**2**	**2**	**25**
Region	Mariepskop	15	12	7	***	***	5
	Letsetele Valley	59	54	52	1	***	1
	Constantia	48	15	15	***	***	***
	Hoedspruit	80	56	38	1	***	18
	Malelane	48	19	16	***	2	1
Cultivar	Sensation	65	54	45	1	***	8
	Tommy Atkins	92	77	60	1	***	16
	Kent	49	3	3	***	***	***
	Keitt	29	21	20	***	***	1
	Heidi	15	2	***	***	2	***
Plant part	Tree trunks	21	8	8	***	***	***
	Branches	74	55	49	***	2	3
	Leafs and leaf stems	14	1	1	1	***	***
	Blossoms	22	8	8	***	***	***
	Fruit	99	72	54	1	***	17
	Asymptomatic tissue	20	13	8	***	***	5

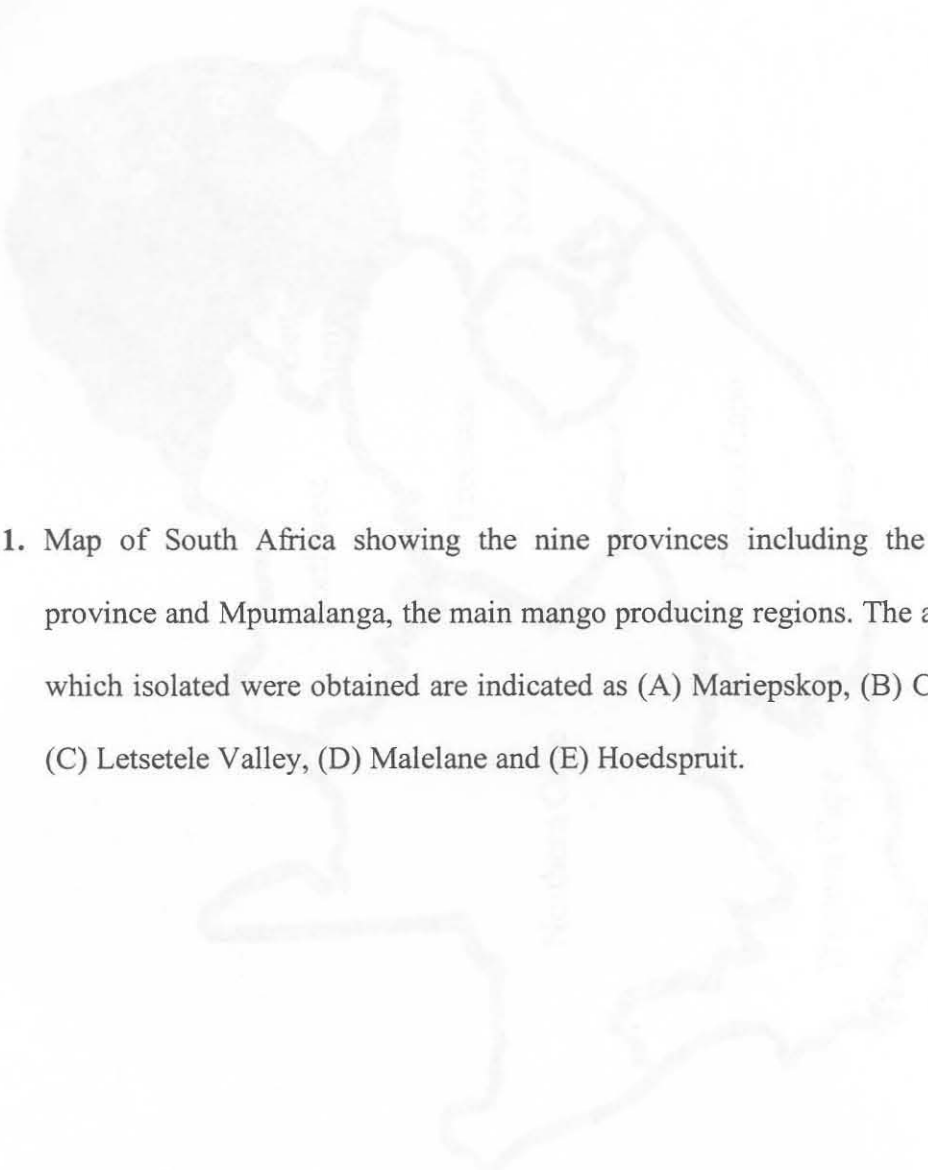
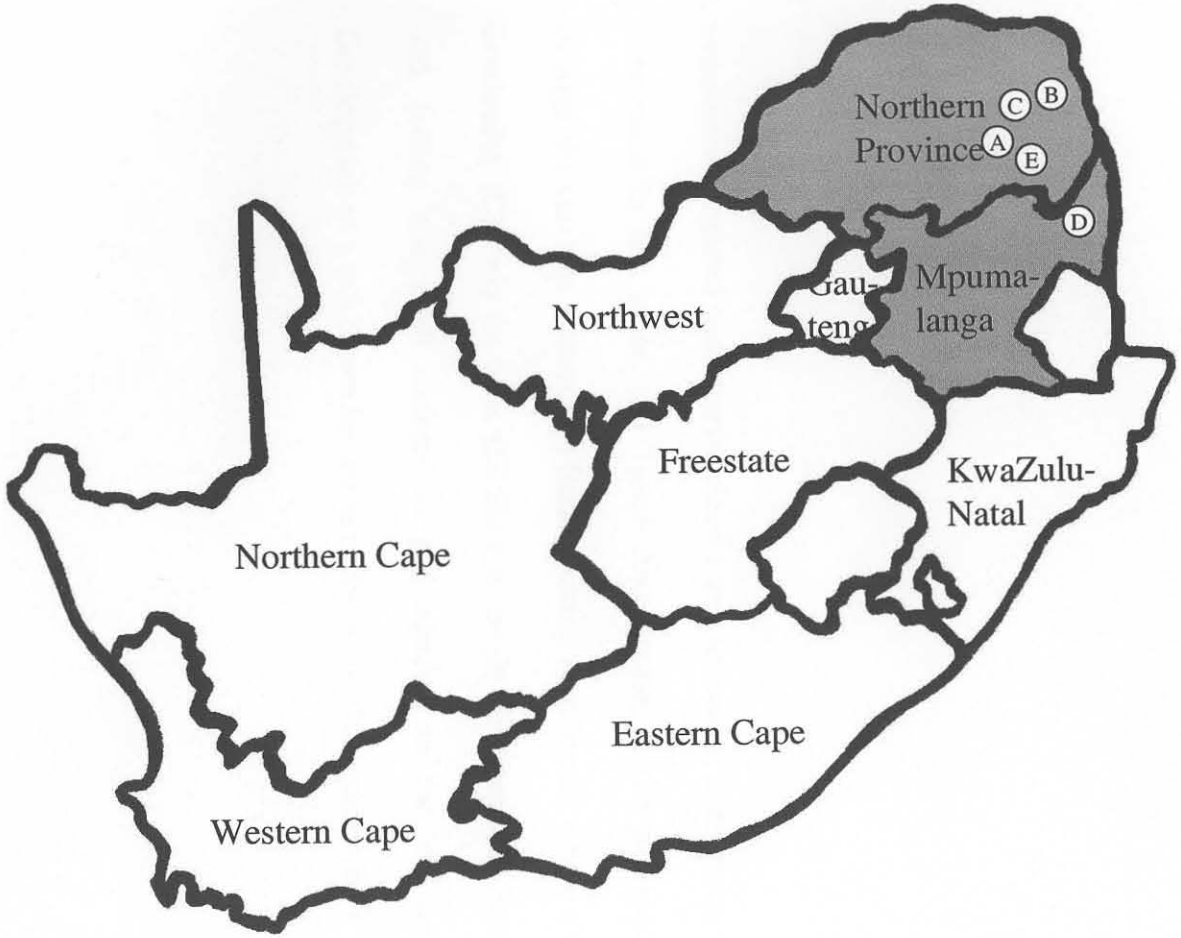


Figure 1. Map of South Africa showing the nine provinces including the Northern province and Mpumalanga, the main mango producing regions. The areas from which isolated were obtained are indicated as (A) Mariepskop, (B) Constantia, (C) Letsetele Valley, (D) Malelane and (E) Hoedspruit.



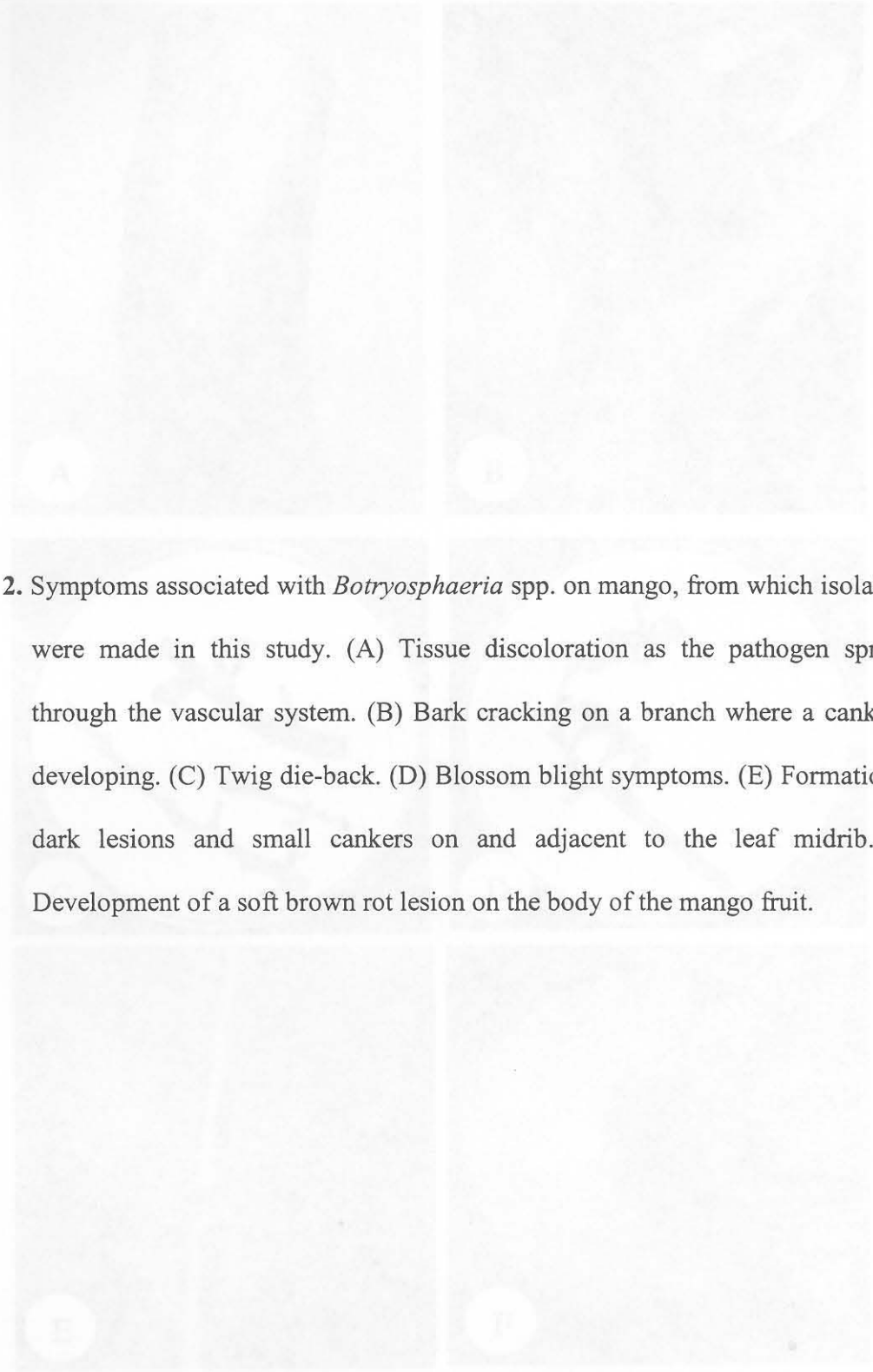


Figure 2. Symptoms associated with *Botryosphaeria* spp. on mango, from which isolations were made in this study. (A) Tissue discoloration as the pathogen spreads through the vascular system. (B) Bark cracking on a branch where a canker is developing. (C) Twig die-back. (D) Blossom blight symptoms. (E) Formation of dark lesions and small cankers on and adjacent to the leaf midrib. (F) Development of a soft brown rot lesion on the body of the mango fruit.

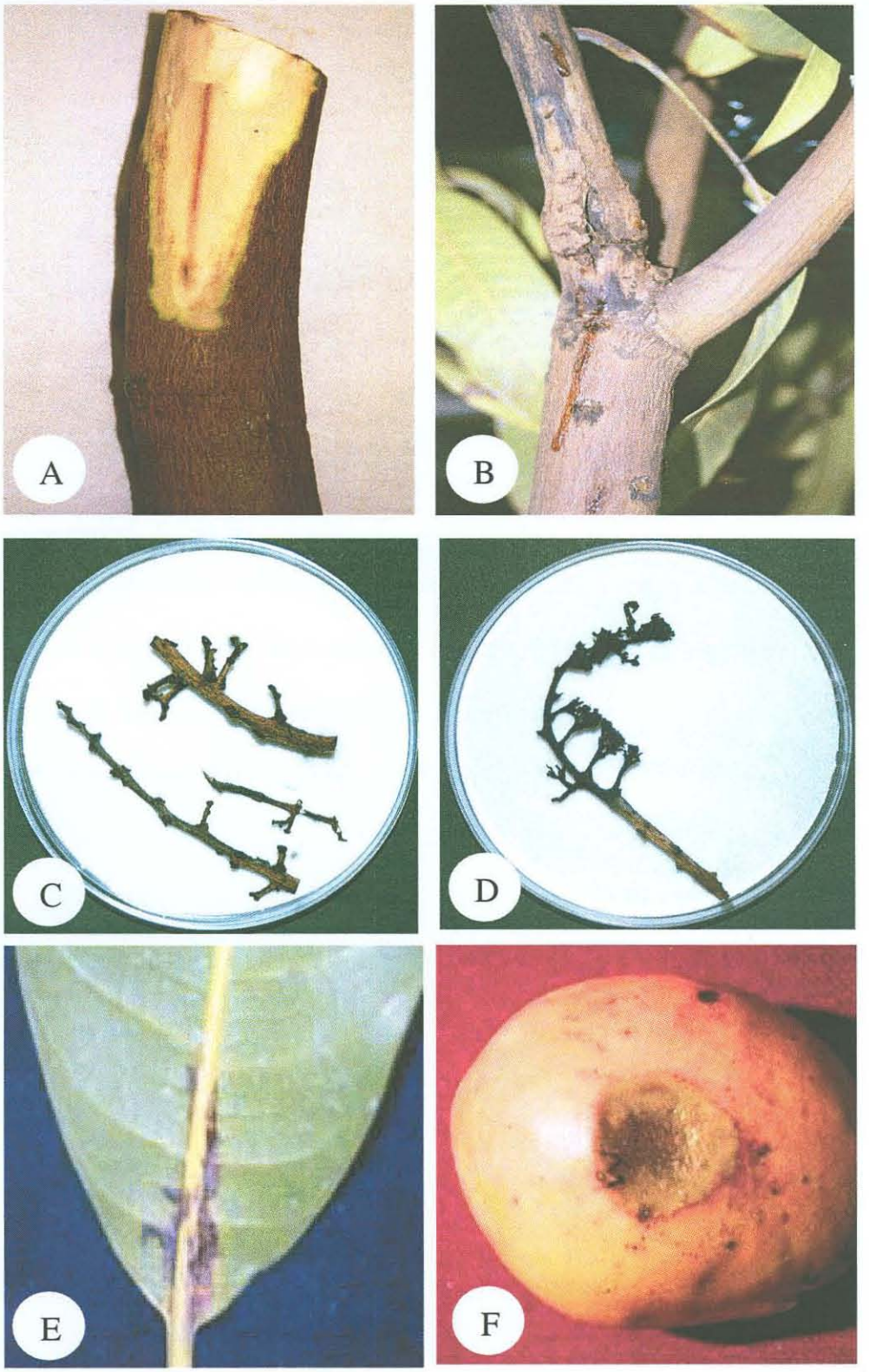


Figure 3. A 3% agarose gel indicating polymorphic banding patterns after digestion of the PCR amplicon with the restriction enzyme *Cfo*I. Lane 1 represents the 100bp marker. Lanes 2-12 represents (A) *Fusicoccum* parvum, (B) *F. indigoticum*, (C) *F. mangiferum,* (D) *F. aesculi*, (E) *F. bacilliforme* and (F) *B. rhodina*.

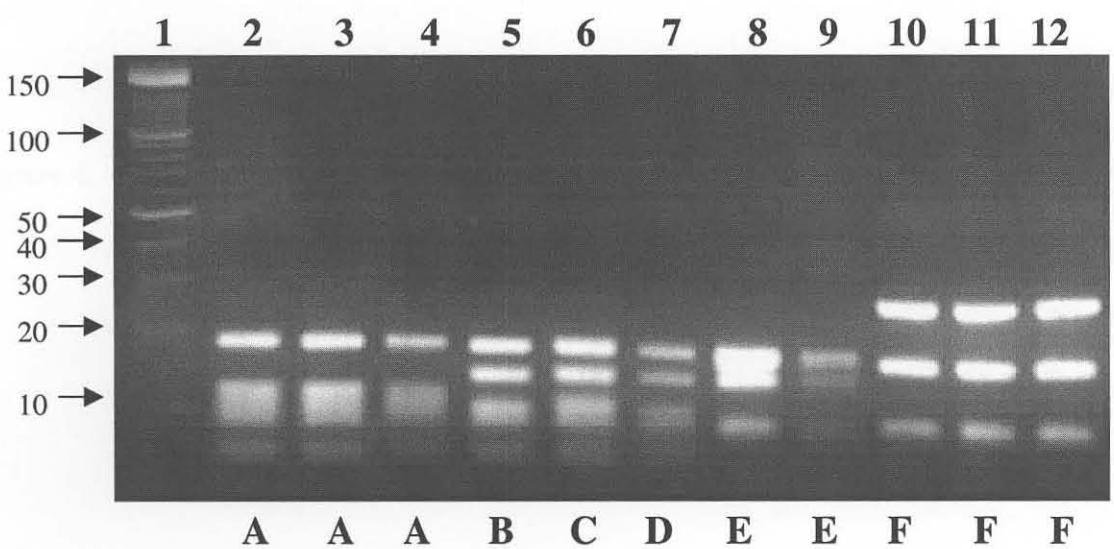

1 2 3 4 5 6 7 8 9 10 11 12
150
100
50
40
30
20
10
A A A B C D E E F F F

Figure 4. A 3% agarose gel indicating polymorphic banding patterns after digestion of the PCR amplicon with the restriction enzyme *Alu*I. Lane 1 represents the 100bp marker and lanes 2-4 represent (A) *F. indigoticum*, (B) *F. aesculi* and (C) *F. mangiferum*.

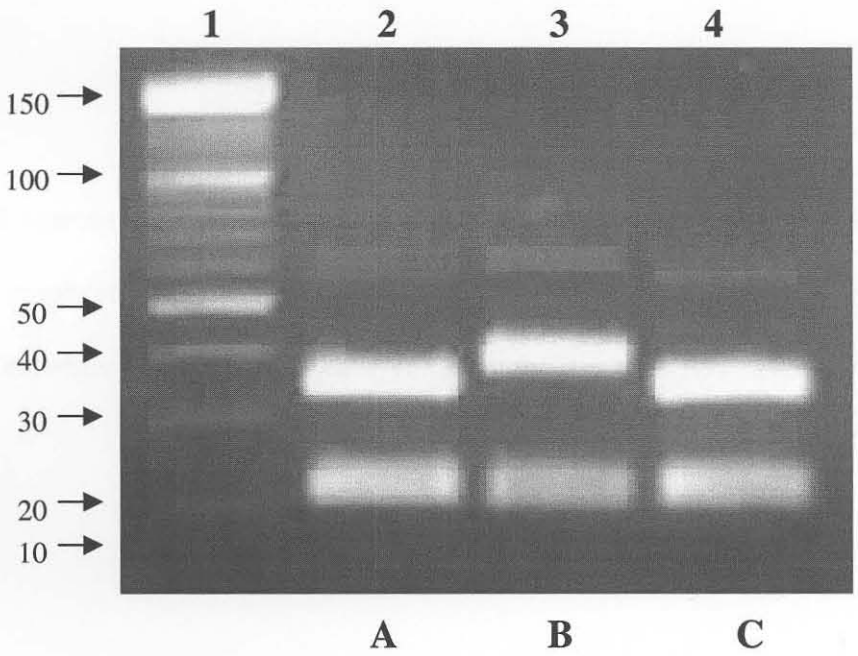

1
2
3
4
150
100
50
40
30
20
10
A
B
C

Figure 5. A 3% agarose gel indicating polymorphic banding patterns after digestion of the PCR amplicon with the restriction enzyme *Bst*71I. Lane 1 represents the 100bp marker, while lanes 2-3 represent (A) *F. indigoticum* and (B) *F. mangiferum.*

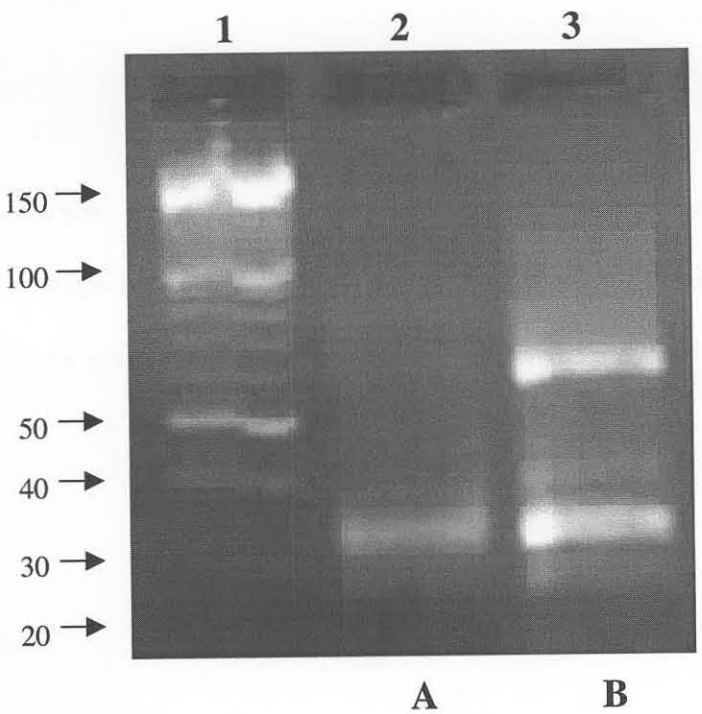

1
2
3
150
100
50
40
30
20
A
B

Figure 6. Restriction enzyme maps generated to identify the *Botryosphaeria* spp. from mango. The maps indicate product sizes generated after digestion of the ITS PCR amplicons (indicating ITS1, 5.8S and ITS4) with (A) *Cfo*I, (B) *Alu*I and (C) *Bst*71I.

A. CfoI

Botryosphaeria parva

121 ↓ 22 ↓ 182 ↓ 90 ↓ 64 ↓ 72 (550)

F. indigoticum

143 ↓ 182 ↓ 90 ↓ 64 ↓ 71 (550)

F. bacilliforme

147 ↓ 181 ↓ 89 ↓ 138 (555)

F. mangiferum

145 ↓ 182 ↓ 90 ↓ 64 ↓ 74 (555)

F. aesculi

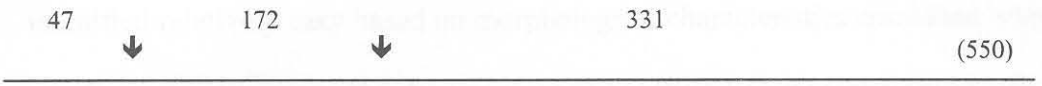
146 ↓ 180 ↓ 88 ↓ 70 ↓ 72 (556)

B. rhodina

284 92 ↓ 138 ↓ (514)

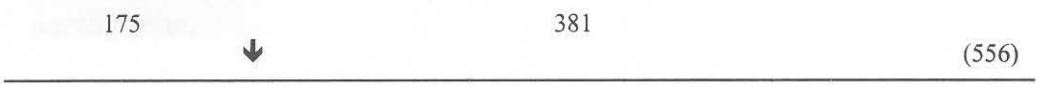
B. *Alu* I

F. indigoticum



F. mangiferum

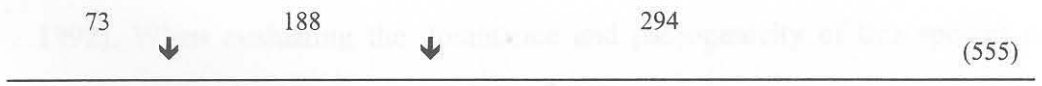
F. aesculi



C. *Bst* 71I

F. indigoticum

F. mangiferum



GENERAL DISCUSSION

In this study, it is clearly shown that four *Botryosphaeria* spp. occur on mango in South Africa. This is the first time that the taxonomy of these fungi on mango has been studied in South Africa. Results for this study will facilitate further research and more effective management of *Botryosphaeria* diseases associated with mango. The four species can be identified relatively easy based on morphological characteristics combined with ITS and ß-tubulin gene sequences. The development of the PCR-RFLP identification system will also facilitate future identification of these *Botryosphaeria* spp. from mango. These species represent *F. parvum*, *Lasiodiplodia theobromae* (*B. rhodina*) and two undescribed *Fusicoccum* spp., for which names are provided here, namely *F. indigoticum* and *F. bacilliforme*.

The majority of isolates collected from symptomatic and asymptomatic tissues in this study reside as *B. parva*. *Botryosphaeria parva (*previously known as *B. ribis)* is a well-known pathogen of many woody plants world-wide (Von Arx, 1987; Punithalingham, 1980). On mango, this fungus has commonly been described as *Dothiorella dominicana*, but has now been correctly identified as *F. parvum* in South Africa, which is most frequently encountered in nature (Johnson, 1992; Slippers *et al.*, 2001). *Fusicoccum parvum* has regularly been isolated from mango in various countries and is considered the primary causal agent of pre- and postharvest disease (Darvas, 1991; Ramos *et al.*, 1991; Johnson, 1992). When evaluating the dominance and pathogenicity of this species on mango, it became evident that this is the most important causal agent of mango diseases in any area and on any cultivar in South Africa. *Botryosphaeria parva* was more frequently isolated than any other *Botryosphaeria* spp. from all plant parts, but mostly from symptomatic fruit

and branches. We therefore, consider *B. parva* to be the main cause of *Botryosphaeria* diseases on mango trees in orchards and on fruit in South Africa. When developing control strategies and other management practices, the presence of this species should, therefore, be closely considered.

Botryosphaeria rhodina was the second most dominant species isolated from mango in South Africa. This species was mostly obtained from fruit rots and asymptomatic plant tissue. The fact that this species was infrequent or absent from any symptomatic plant parts other than the fruit, suggests that it is probably insignificant in causing tree diseases. These findings are similar to those from previously published literature, where *B. rhodina* is well-documented as an endophyte and is described as the most common fruit rot pathogen of many fruit crops, including mango (Punitalingham, 1980; Sanchote, 1991; Johnson, 1992). The *B. rhodina* isolates were easily identified based on morphological characters and identifications were easily confirmed using DNA sequence data and PCR-RFLP. Although this species is commonly isolated together with *Botryosphaeria* spp. having hyaline conidia, it tends to dominate only in warmer, tropical regions (Brown & Britton, 1986; Johnson, 1992). This suggests that different *Botryosphaeria* spp. may respond to the environment and host differently. Pathogen reaction to seasonal variation should thus be considered before final conclusions are made regarding the role of this and other *Botryosphaeria* spp. in disease (Britton & Hendrix, 1986; Brown-Rytlewski & McManus, 2000). The dominant occurrence, together with the pathogenicity of *B. rhodina,* especially on fruit is thus important to consider when developing disease control strategies.

Both molecular and morphological data confirmed that a unique *Botryosphaeria* spp. was isolated in this study, which represent a previously undescribed taxon, which was assigned

the name *F. indigoticum*. The conidial morphology of this new species resembles that of *B. parva* to some degree, but it remains distinct in cultural and conidial morphology. Using morphology on its own to distinguish *Botryosphaeria* spp. may be confusing and it is recommended that the PCR-RFLP system additionally be used for reliable identification. *Fusicoccum indigoticum, B. parva* and *B. rhodina* were also found to be equally pathogenic to mango trees in this study. Variation in virulence of this species between the two pathogenicity trials can be attributed to the fact that symptom expression can be influenced by environmental conditions, as is the case with *B. rhodina* (Johnson, 1992). *Fusicoccum indigoticum* is, however, rarely found in mango orchards or on fruit and is therefor not considered an important *Botryosphaeria* spp. affecting mango in South Africa.

Two South African isolates obtained in this study closely resembled two isolates that were described by Johnson *et al.* (1991) as unknown species from a mango stem end rot pathogen survey in Australia. The fungus was not formally described, but was referred to as *Dothiorella* 'long' (Johnson *et al.*, 1991; Johnson, 1992). *Dothiorella* 'long' has, however, been shown to belong to the genus *Fusicoccum* (Slippers *et al.*, 2001). Sequence data, however, confirmed that the isolates from Australia and South Africa were not identical and could reside as different species, if more isolates are obtained. In this study, however, isolates in this group has cylindrical to bacilliform conidia and produce a yellow pigment in the growth medium and is, therefore, considered as the same species. Mycelial clumps are also produced in concentric rings, which is very different to any of the other *Botryosphaeria* sp. We have, therefore, provided the name *Fusicoccum bacilliforme* for all isolates falling within this group. This species was, however, isolated only from diseased mango branches. The smaller lesions, lower virulence and low isolation frequency of *F. bacilliforme*, as well as the variation in pathogenicity of isolates, suggests that this species

is a weak pathogen that does not contribute significantly to mango diseases in South Africa.

Botryosphaeria spp. were isolated in varying frequencies from different commercial mango cultivars cultivated in South Africa and sampled during this study. The cultivars Sensation and Tommy Atkins yielded the highest frequency of *Botryoshaeria* spp. The cultivars Keitt and Kent indicated a very low isolation frequency in this study, which correlates well with previous findings that these species are more disease tolerant in the orchards. With the inoculation trials, Tommy Atkins and Keitt were respectively chosen for their disease susceptibility and tolerance ability. Under controlled glasshouse conditions, however, these cultivars showed no difference in their susceptibility to *Botryosphaeria* diseases. The resistance of cultivars should, therefore, be tested under normal environmental conditions for a true reflection of disease resistance to various pathogens.

Botryosphaeria spp. were isolated from all mango producing regions of South Africa surveyed in this study. The highest incidence of *Botryosphaeria* spp. were found in the Letsetele Valley, Hoedspruit and Mariepskop areas. The use of weather data can attribute to the estimation of environmental conditions, which may influence the incidence of disease such as higher rainfall in the Letsetele Valley region (South African Weather Buro). Close correlation of production with environmental conditions in mango production regions may give a broader view of the optimal environmental conditions which may favour *Botryosphaeria* disease development in orchards.

A small number of asymptomatic isolations yielded the species *B. parva* and *B. rhodina*, which are known endophytes of mango and other woody hosts world-wide. The endophytic status of these fungi have been investigated previously (McPartland & Schoeneweiss, 1986; Johnson et al., 1991), where it was shown that the endophytic phase can be found in all mango plant parts. In this study, we have, however, confirmed that these fungi can also be pathogenic on all plant parts screened in this survey. Control of this fungi can, therefore, not be restricted to a specific area on the tree, due to the movement thereof, and systemic control should be the focus point.

In this study, we conclude that four *Botryosphaeria* spp. occur on mango in South Africa, of which two species are new to science. *Botryosphaeria parva*, *B. rhodina*, *F. indigoticum* and *F. bacilliforme* is easily identified with the use of a PCR-RFLP identification system developed in this study. The pathogenic ability of the *Botryosphaeria* spp. on mango in South Africa suggests that most of these species has the ability to cause diseases and should be consider when management strategies are implemented in the mango industry. Other *Botryosphaeria* spp. have, however, also been implicated as causal agents of diseases on mango in other countries, such as *F. mangiferum* (known as *D. mangiferae*) and *F. aesculi* (known as *D. aromatica*) (Johnson, 1992; Slippers *et al.*, 2001), but these species are easily distinguished from the four South African species with the PCR-RFLP identification system. The PCR-RFLP technique overcomes the difficulties experienced using morphological characteristics to identify *Botryosphaeria* spp. from mango. It is simple and rapid and negates problems experienced when needing to sequence DNA from large numbers of isolates.

No true disease tolerant cultivars could be identified in this study, but further field trials are needed to evaluate resistance under normal field conditions. Currently, however, the most effective means of control of *Botryosphaeria* diseases can be achieved through increasing plant vigour by reducing stress. This is expected to minimise disease incidence due to *Botryosphaeria* spp. and impact on mango quality and production. Care should, however, be taken to prevent the introduction of foreign species such as *F. aesculi* and *F. mangiferum* that currently do not occur in South Africa. The implementation of effective quarantine strategies and the screening of foreign material with the PCR-RFLP system may provide a useful method implemented in sanitation and management practices for these species world-wide.

REFERENCES

Britton, K.O. & Hendrix, F.F. 1986. Population dynamics of *Botryosphaeria* spp. in Peach gummosis cankers. *Plant Disease* 70: 134-136.

Brown-Rytlewski, D.E. & McManus, P.S. 2000. Virulence of *Botryosphaeria dothidea* and *Botryosphaeria obtusa* on apple and management of stem cankers with fungicides. *Plant Disease* 84: 1031-1037.

Darvas, J.M. 1991. *Dothiorella dominicana*, a new mango pathogen in South Africa. *Phytophylactica* 23: 295-298.

Johnson, G.I. 1992. Biology and control of stem end rot pathogens of mango. Ph.D. thesis. University of Queensland, Queensland, Australia.

Johnson, G.I., Cooke, A.W., Mead, A.J. & Wells, I.A. 1991. Stem end rot of mango in Australia: Causes and control. *Acta Horticulturae* 291: 288-295.

McPartland, J.M. & Schoeneweiss, D.F. 1984. Hyphal morphology of *Botryosphaeria dothidea* in vessels of unstressed and drought-stressed stems of *Betula alba*. *Phytopathology* 74: 358-362.

Punithalingam, E. 1980. Plant diseases attributed to *Botryodiplodia theobromae* Pat., J. Cramer. Germany. 2, 42-43 pp.

Ramos, L. J., Lara, S.P., McMillan Jr, R.T. & Narayanan, K.R. 1991. Tip dieback of mango (*Mangiferae indica*) caused by *Botryosphaeria ribis*. *Plant Disease* 75: 315-318.

Sangchote, S. 1991. *Botryodiplodia* stem end rot of mango and its control. *Acta Horticulturae* 291: 296–304.

Slippers, B., Johnson, G.I., Cooke, A.W., Crous, P.W., Coutinho, T.A., Wingfield, B.D. & Wingfield, M.J. 2001. Taxonomy of *Botryosphaeria* spp. causing stem end rot of

mango in Australia. In: Proceedings of the 13th American Plant Pathological Society. 25-29 August. Cairns, Australia.

Von Arx, J.A. 1987. Plant-pathogenic fungi. 203-204 pp. J. Cramer, Berlin, Germany.

SUMMARY

In Chapter one of this thesis, the literature on *Botryosphaeria* spp. associated with mango is reviewed. From this review, it is clear that *Botryosphaeria* spp. are responsible for diseases on mango plants and fruit and cause major economic losses to this industry. Various fungal species have been associated with these diseases on mango. Due to the difficulty in distinguishing morphological characteristics, the taxonomy of *Botryosphaeria* spp. associated with these symptoms is confused. Identification has mainly been based on anamorph morphology. *Botryosphaeria* on mango are recognised to be endophytes and can become pathogenic under stress conditions or infect any plant parts directly through natural openings and wounds. Furthermore, published data have shown that limited control of *Botryosphaeria* diseases have been achieved thus far. There is thus a need to clarify the taxonomy of the *Botryosphaeria* spp. affecting mango and to utilise this knowledge in developing effective management strategies to control disease outbreaks.

In the second chapter of this thesis, *Botryosphaeria* spp. are identified from mango in South Africa. These species include *F. parvum*, *L. theobromae* (*B. rhodina*) and two undescribed species. The names *Fusicoccum indigoticum* and *F. bacilliforme*, are thus provided for them. The four species are further distinguished based on combined morphological and molecular data. In other parts of the world, other species such as *F. aesculi* and *F. mangiferum* are also common pathogens on mango, but they were not found in this study. Morphological characteristics that have traditionally been used to identify these *Botryosphaeria* spp. overlap in some instances. It is, therefore, shown that these morphological data must be combined with molecular characteristics to confirm species identity.

The pathogenicity of four *Botryosphaeria* spp. from mango in South Africa is evaluated in chapter three of this thesis. *Botryosphaeria parva*, *B. rhodina* and *F. indigoticum* were thus found to be equally pathogenic on two mango cultivars that are commonly planted in South Africa. *Fusicoccum bacilliforme* was least pathogenic and is most likely not contributing to disease. Results suggest that greenhouse trials do not necessarily reflect cultivar resistance in the field. Field trials are, therefore, needed to evaluate the pathogenic potential and cultivar resistance to these species, under normal environmental conditions.

In the fourth and final chapter, a PCR-RFLP based identification system for *Botryosphaeria* spp. from mango in South Africa and Australia is developed. The restriction enzyme *Cfo*I is able to distinguish all *Botryosphaeria* spp. from mango in South Africa. *Alu*I and *Bst*71I were, however, needed to differentiate the Australian species, *F. aesculi* and *F. mangiferum* respectively, from the South African isolates. This identification system was successfully applied in a survey of *Botryosphaeria* spp. conducted in South Africa. From these data it is evident that *B. parva* is the dominating species in South Africa, followed by *B. rhodina*, which is more important as a fruit than a mango tree pathogen. *Fusicoccum indigoticum* and *F. bacilliforme* occurred seldom and are apparently less important species on mango. Due to the difficulty in identifying *Botryosphaeria* spp. based on morphology and the cost of sequencing large numbers of isolates, the PCR-RFLP can be used to provide rapid and reliable identifications.

In this thesis, I hope to have set the foundation for further studies of *Botryosphaeria* spp. on mango in South Africa. Species occurring on this crop have been identified for the first time and hopefully these identities will clarify problems experiencedwith the epidemiology and control of the *Botryosphaeria* spp. on mango in South Africa. This information should

help to prevent the further spread of new and exotic pathogens to foreign countries and support programmes in quarantine and control.

OPSOMMING

In hoofstuk een van hierdie tesis word die literatuur hersien van *Botryosphaeria* spp. wat geassosieër word met mango. Vanuit hierdie literatuuroorsig is dit duidelik dat *Botryosphaeria* spp. verantwoordelik is vir wesenlike ekonomiese verliese in die mango industrie, as gevolg van siektes wat dit veroorsaak op mango plante en vrugte. Verskeie swam spesies is al voorheen geassosieër met hierdie siekte simptome op mango. As gevolg daarvan dat morfologiese karakters moeilik onderskeibaar is, is die taksonomie van hierdie *Botryosphaeria* spp., tans nie georden nie. Spesie identifikasie word hoofsaaklik gebaseer op anamorf morfologie. *Botryosphaeria* spp. is bekend as endofiete van mango, maar kan patogenies raak tydens ongunstige omstandighede vir die gasheer. Die patogeen kan ook natuurlike openinge en wonde van enige plant deel direk infekteer. Gepubliseerde data dui ook aan dat slegs beperkte beheer van *Botryosphaeria* siektes tans bahaal word. Daar is dus 'n aanvraag om die taksonomie van *Botryosphaeria* wat mango affekteer uit te klaar en hierdie kennis te gebruik in die ontwikkeling van effektiewe beheer strategieë om uitbrake van siektes.

In die tweede hoofstuk van hiedie tesis word die *Botryosphaeria* spp. vanaf mango in Suid-Afrika geïdentifiseer. Hierdie spesies sluit in, *F.parvum*, *L. theobromae* (*B. rhodina*), en twee onbeskryfde spesies. Die name, *F. indigoticum* en *F. bacilliforme* word aan hierdie spesie toegeken. Die vier spesies word onderskei deur morfologiese en molekulêre data te kombineer. In ander wêrelddele word spesies soos *F. aesculi* en *F. mangiferum* algemeen as mango patogene geisoleer. Hierdie laasgenoemde spesies is egter nie tydens hierdie studie in Suid-Afrika geidentifiseer nie. Morfologiese eienskappe wat tradisioneel gebruik

word vir *Botryosphaeria* spp. identifikasie is geneig om te oorvleul in sekere omstandighede. Dit is dus duidelik dat morfologiese eienskappe saam met molekulêre data gebruik moet word om spesies te identifiseer.

Die patogenisiteit van die vier *Botryosphaeria* spp. vanaf mango in Suid-Afrika is geëvalueer in hoofstuk drie van hierdie tesis. *Botryosphaeria parva*, *B. rhodina* en *F. indigoticum* was ewe patogenies op twee mango kultiwars wat algemeen in Suid-Afrika geplant word. *Fusicoccum bacilliforme* was die minste patogenies and dra moontlik nie by tot simptoom ontwikkeling nie. Die resultate van die studie dui ook aan dat glashuis eksperimente nie noodwendig kultiwar weerstandbiedendheid in die veld weerspieël nie. Veld eksperimente is dus nodig om die patogenisiteit en kultiwar weerstandbiedendheid van hierdie spesies te evalueer onder verskeie omgewings toestande.

In die vierde en finale hoofstuk van hierdie tesis is 'n PKR-RFLP identifikasie sisteem vir *Botryosphaeria* spp. vanaf mango in Suid-Afrika en Australië ontwikkel. Die restriksie ensiem *Cfo*I onderskei alle *Botryosphaeria* spp. vanaf mango in Suid-Afrika. *Alu*I en *Bst*71I was beide nodig om die Australiese spesies, *F. aesculi* en *F. mangiferum*, onderskeidelik van Suid-Afrikaanse isolate te onderskei. Hierdie tegniek is suksesvol toegepas tydens 'n *Botryosphaeria* spp. opname in Suid-Afrika. Vanuit hierdie data blyk dit dat *B. parva* die mees dominante spesie in Suid-Afrika is, gevolg deur *B. rhodina* wat 'n belangriker mango vrug patogeen as 'n mango boom patogeen blyk te wees. *Fusicoccum indigoticum* en *F. bacilliforme* het selde voor gekom en is duidelik minder belangrike spesies op mango. As gevolg van die problematiese identifikasie van *Botryosphaeria* spp. wat gebasseer is op morfologie en die kostes verbonde aan DNS

volgorde bepaling van groot getalle isolate, is die PKR-RFLP bruikbaar vir die spoedige en betroubare identifikasie van spesies.

Met hierdie tesis is daar 'n fondament gelê vir die verdere studie van *Botryosphaeria* spp. op mango in Suid-Afrika en elders. Spesies wat voorkom op hierdie gewas in Suid-Afrika is vir die eerste maal geïdentifiseer. Hierdie identifikasies sal help om die epidemiology van *Botryosphaeria* spp. uit te klaar en sodoende siekte beheer probleme met die swamme op mango in Suid-Afrika op te los. Hierdie inligting sal ook die verdere verspreiding van patogene na vreemde lande help bekamp, veral omdat dit programme in beheer en kwarentyn ondersteun.
