LEAF PATHOGENS OF *EUCALYPTUS* IN SOUTH AFRICA WITH PARTICULAR REFERENCE TO *TERATOSPHAERIA DESTRUCTANS*

By

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DECLARATION

I, Emeldah Ingrid Rikhotso, declare that the dissertation, which I hereby submit for the degree *Magister Scientiae* at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE:

DATE:

TABLE OF CONTENTS

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PREFACE

Eucalyptus is the largest genus within the *Myrtaceae* with more than 700 species; most of which are endemic to Australia while a few are native to Papua New Guinea and the South East Asian islands. Species of *Eucalyptus* occur over a wide variety of environmental conditions and are thus grown throughout tropical, subtropical and temperate regions as exotics. They are mostly grown for construction and furniture timber and pulp for the production of paper and viscose, while extractives are used as medicinal oils, and in the cosmetics and food industries. In South Africa, *Eucalyptus* plantations cover approximately 520 000 ha, with most being planted in the KwaZulu-Natal and Mpumalanga provinces. The species that are commonly planted in South Africa include *Eucalyptus dunnii*, *E. macarthurrii*, *E. smithii*, as well as hybrids of *E. grandis* x *E. nitens* and *E. grandis* x *E. urophylla*.

The sustainability of plantations of *Eucalyptus* species is greatly threatened by the global increase of pests and pathogens. This includes pests and pathogens native to the exotic environment where eucalypts are grown, as well as introduced pests and pathogens from a range of other regions. Some of these diseases have necessitated costly species changes for commercial growers.

Leaf pathogens considered to be important include *Austropuccinia psidii*, species of *Calonectria*, *Coniella*, *Quambalaria*, *Teratosphaeria* and the bacterial species, *Pantoea*. These leaf pathogens cause diseases such as leaf and shoot blight, leaf spot and leaf blotch, which may cause shoot death, tip die-back and defoliation which ultimately result in growth and volume loss and potentially plant death.

Management of plantation diseases requires an in-depth knowledge of the pathogens, including but not limited to, their identities, their geographic distribution, population diversity, life cycles and epidemiology. The last comprehensive survey of foliar diseases of *Eucalyptus* species in commercial plantations in the KwaZulu-Natal, Mpumalanga and Limpopo Provinces of South Africa was conducted more than 20 years ago. Since then *Eucalyptus* genotypes grown in these areas have changed and significant changes in rainfall and temperature have been recorded in these regions. This dissertation, therefore, aimed to identify the most prevalent foliar pathogens of *Eucalyptus* species in the KwaZulu-Natal, Mpumalanga and Limpopo Provinces of South Africa. Secondly, it aimed to investigate some

aspects of the epidemiology of one of the currently most important foliar pathogen of *Eucalyptus* in South Africa, *Teratosphaeria destructans*.

The research conducted for this dissertation was divided into three chapters. Chapter One summarizes the most important *Eucalyptus* leaf pathogens in all *Eucalyptus*-growing regions in the world. The review provides a foundation for further studies conducted in South Africa regarding *Eucalyptus* leaf pathogens. A list of leaf diseases caused by various pathogens on *Eucalyptus* around the world is included.

Chapter two aimed to identify the most prevalent leaf diseases of *Eucalyptus* species and their hybrids in the KwaZulu-Natal, Limpopo and Mpumalanga provinces of South Africa. Symptoms and DNA sequence data were used to identify the leaf pathogens. Information generated in this chapter will be useful for biosecurity as well as the identification of focus points for the development of management strategies to reduce disease impacts in South African plantations.

Chapter three focuses on the epidemiology of the important leaf pathogen, *Teratosophaeria destructans*. The discovery of the leaf and shoot blight pathogen *T. destructans* in South Africa in 2015, and its subsequent impact, plus the results obtained from chapter two pertaining to its distribution and abundance in the country, identified the need for rapid screening techniques to select disease *Eucalyptus* genotypes tolerant to this pathogen. This requires knowledge of its epidemiology and infection biology. The optimum conditions that favour conidial germination, culture growth and infection of *T. destructans* were, therefore, investigated to provide a foundation for future studies, including the possible development of screening techniques against this pathogen.

In this manuscript, scientific names at all ranks are set in italics in accordance to the suggestion by Thines *et al*. (2020).

CHAPTER 1

LEAF PATHOGENS OF *EUCALYPTUS*: A GLOBAL PERSPECTIVE OF THE MAJOR DISEASES

INTRODUCTION

Eucalypts are woody plants in the *Myrtaceae* family. The group includes seven closely related genera, *Arillastrum* Pancher & Baill., *Allosyncarpia* S.T. Blake, *Angophora* Cav., *Corymbia* Hill & Johnson, *Eucalyptopsis* C.T. White, *Eucalyptus* L'Hér. and *Stockwellia* D.J. Carr, S.G.M. Carr & B. Hyland (Ladiges *et al.* 2003), which together include more than 800 species (Richardson and Rejmánek 2011). *Eucalyptus* is the largest genus within the *Myrtaceae* with more than 700 species (Thornhill *et al.* 2019). Most species of *Eucalyptus* are native to Australia (Luzar 2007), while a few, such as *E. papuana*, *E. deglupta*, *E. pellita* (Carr 1972), *E. tereticornis*, *E. rudis*, *E. gomphocephala* and *E. urophylla* (Laurenson *et al.* 1997), are native to Papua New Guinea (PNG) and other South East Asian islands (Grattapaglia *et al.* 2012, Thornhill *et al.* 2019). Species of *Eucalyptus* grow naturally in a wide variety of environments, and are, therefore, adapted to a large diversity of climatic conditions (Luzar 2007). Several species are grown widely in the tropical, subtropical and temperate regions of the world, as exotics in commercial plantations and woodlots (Huang *et al.* 2014). These plantations cover more than 20 million hectares globally (FAO 2016, Laclau 2018). Species of *Eucalyptus* have been selected for plantation forestry because of their rapid growth and ability to survive and recover from periods of harsh environmental conditions (Eldridge *et al.* 1994). They are grown for several products, including pulp for paper and viscose (Turnbull 2000), medicinal oils (Jun *et al.* 2013), cosmetics, the food industry, construction timber (Bennett 2010) and medium-density fiberboard (de Campos and Lahr 2004).

In South Africa, *E. globulus* was the first species of *Eucalyptus* to be introduced to the Cape Colony in 1828 (Poynton 1960). Other species were planted in botanic gardens in the 1850s and along railway lines for the provision of fuel for steam locomotives (Turnbull 1999). Later, in 1875, larger plantations were established for pulp, land reclamation purposes, oil products and the production of timber for furniture, mining and general construction purposes (Albaugh *et al.* 2013). By the 1930s, there was a great interest in *Eucalyptus* oil which was

used for medicinal purposes (Poynton 1979). Plantations of *Eucalyptus* currently cover approximately 520 000 ha in South Africa, which equates to 43.7% of the total plantation area in the country (Godsmark and Oberholzer 2018). Most of these plantations are in the KwaZulu-Natal (56.9%) and Mpumalanga (34.4%) Provinces (Godsmark and Oberholzer 2018). Initially, commercial plantations were dominated by *E. grandis* and *E. nitens* species as well as *E. grandis* x *E. camaldulensis* hybrids (Verryn 2000). However, due to high susceptibility to introduced pests and pathogens and poor environmental adaptability, the planting of pure *E. grandis* was largely discontinued (du Toit *et al.* 2017) and is currently used mostly as a hybrid with other species of *Eucalyptus* (Van den Berg 2017). Other species of *Eucalyptus* that are commonly planted in South Africa include *E. badjensis, E. dunnii, E. macarthurrii, E. smithii* and the hybrid *E. grandis* x *E. urophylla* (Dovey 2014). Currently, *E. dunni* is the most commonly planted species because it has a range of pulping properties that are suitable for dissolving wood pulp and craft pulping processes. It is also popular for having a naturally good form, high wood density, adaptability to a range of site conditions and current lack of major disease problems (Dovey 2014).

An increase in the diversity and abundance of pests and pathogens threatens the health status, sustainability and productivity of *Eucalyptus* forestry globally (Wingfield *et al.* 2015). Initially, when *Eucalyptus* species were first planted as non-native trees in commercial plantations they experienced very little disease and pest problems (Wingfield 2003, Wingfield *et al.* 2008, Hurley *et al.* 2016). This is attributed to the fact that they were removed from their natural enemies in their region of origin (Wingfield *et al.* 2008). However, globally, as time has passed, these trees have been affected by increasing numbers of pest and disease problems (Burgess and Wingfield 2017). This increase in the number of pathogens and insect pests has been ascribed to globalization and human activities, such as the increase in international trade, and the movement of people, plant products and live plants between countries and continents (Palm 1999, Wingfield 1999, Wingfield *et al.* 2001, Burgess and Wingfield 2002, Brasier 2008, Andjic *et al.* 2011). In addition to co-evolved pests and pathogens spreading globally, native pests and pathogens in areas where *Eucalyptus* has been introduced have started adapting to, and affecting, these trees, adding to increased disease and pest pressures (Wingfield *et al.* 2001, Wingfield 2003, Wingfield *et al.* 2008, Paine *et al.* 2011).

Several leaf pathogens have been reported to affect species of *Eucalyptus* globally. These include *Austropuccinia psidii*, species of *Calonectria*, *Coniella*, *Teratosphaeria*, and bacterial

species of *Pantoea* to name a few (Crous *et al.* 1989, Coutinho *et al.* 1998, Coutinho *et al.* 2000, Crous *et al.* 2019). Leaf pathogens cause leaf blight and leaf spot diseases, which may cause shoot die-back and defoliation, thereby reducing tree growth and in some circumstances causing tree death (Carnegie and Ades 2002, Crous *et al.* 2004, Balmelli *et al.* 2013, Balmelli *et al.* 2016, Smith *et al.* 2016, Pérez *et al.* 2017). Species of *Teratosphaeria*, previously known as *Mycosphaerella*, for example, are notorious for the leaf spot, blight and blotch diseases they cause on species of *Eucalyptus* globally (Crous 1998, Park *et al.* 2000, Old *et al.* 2003, Burgess *et al.* 2007, Hunter *et al.* 2011, Andjic *et al.* 2019). *Teratosphaeria nubilosa*, is commonly credited as being a major reason for the termination of the planting of *E. globulus* in South Africa (Lundquist *et al.* 1987). Similarly, in South America, thousands of dollars are spent annually on the management of the rust fungus *A. psidii* through the use of chemical sprays (Masson *et al.* 2013).

The aim of this review is to summarize information regarding the most important leaf pathogens of *Eucalyptus* species globally in order to establish a foundation for studies to investigate leaf diseases of these trees in South Africa. While more than 80 species of fungi, rust and bacteria have been reported from leaves of *Eucalyptus* globally (Table 1), not all are considered important pathogens and many have been recorded only once. This review will, therefore, focus on discussing those of economic importance to *Eucalyptus* globally as well as in South Africa.

IMPORTANT LEAF PATHOGENS OF PLANTATION GROWN *EUCALYPTUS*

Teratosphaeria species

Previously, one of the best known and most important foliar diseases of *Eucalyptus* species globally was known as Mycosphaerella Leaf Disease (MLD) or Mycosphaerella Leaf Blotch (MLB). The disease was ascribed to several species of *Mycosphaerella* (*Capnodiales*) (Hunter *et al.* 2011). The taxonomy of this genus has, however, undergone dramatic revisions and the pathogens previously classified in the genus *Mycosphaerella* now reside in several families within the *Capnodiales* (Crous *et al.* 2009b, Li *et al.* 2012, Quaedvlieg *et al.* 2014, Videira *et al.* 2017). These include genera in the *Mycosphaerellaceae* and *Teratosphaeriaceae*. Some of the most economically damaging leaf pathogens of *Eucalyptus*

species currently reside in the genus *Teratosphaeria* and the disease previously known as MLD is now called Teratosphaeria Leaf Disease (TLD) (Crous *et al.* 2009a).

Over 150 species of *Teratosphaeria* are associated with TLD (Hunter *et al.* 2011). Some of the species do not cause significant damage to *Eucalyptus* plantations (Maxwell *et al.* 2003), while others are serious pathogens which cause leaf, bud and shoot blight. These include *T. destructans*, *T. eucalypti, T. pseudoeucalypti and T. viscida* which cause problems in tropical and subtropical areas, and *T. cryptica* and *T. nubilosa* that cause problems in temperate regions (Andjic et al 2019). Together these pathogens are considered to be some of the most important species causing leaf and shoot blight diseases on *Eucalyptus* in temperate (Burgess and Wingfield 2017), tropical and sub-tropical areas (Andjic *et al.* 2011). Over the years, TLD pathogens have spread globally, most likely through trade in live plants (Crous 1998, Hunter *et al.* 2011). The disease is now known from all continents and multiple countries where *Eucalyptus* is grown (Table 1).

Symptoms of TLD include leaf spots (Fig. 1a) and leaf, bud and shoot blight, defoliation and death of susceptible plants (Andjic *et al.* 2007, Andjic *et al.* 2010b). The first symptoms of infection begin with circular-irregular brown-purple leaf spots that are surrounded by red-brown margins (Wingfield *et al.* 1996, Burgess *et al.* 2006, Andjic *et al.* 2010b). Spore masses and conidia form on the abaxial and, on some hosts, also the adaxial leaf surfaces (Andjic *et al.* 2019). Leaf blight damages large areas on both sides of the lamina, producing necrotic lesions (Old *et al.* 2003, Andjic *et al.* 2019). Leaves may become malformed and, in severe cases, infection may interfere with normal growth and alter the tree structure (Lundquist *et al.* 1987). Premature defoliation may result in tree death (Park and Keane 1982b, Carnegie 2000, Hunter *et al.* 2011).

The infection process of all TLD species begins when a conidium lands on either the abaxial or adaxial leaf surfaces from which it enters the leaves through the stomata (Park and Keane 1982b, Andjic *et al.* 2019). Park (1988) conducted an inoculation experiment of *E. globulus* seedlings to determine the infection requirements of *T. cryptica* and *T. nubilosa*. The results showed that germination and infection takes place with 5-7 days leaf wetness at temperatures ranging from 15-20°C (Park 1988a). However, the length of the leaf wetness treatment required for infection to take place may vary with the temperature and inoculum concentration. Infection and disease development takes place during the vegetative period when susceptible young leaves are being produced.

Eucalyptus are especially susceptible to TLD during their juvenile leaf stage with infection leading to reduced photosynthetic capacity, defoliation and stunting (Park and Keane 1982b, Lundquist *et al.* 1987, Carnegie and Ades 2002, Milgate *et al.* 2005a, Pinkard and Mohammed 2006, Pérez *et al.* 2017). The disease can lead to a decrease in green leaf tissue and lower photosynthesis rates (Farrar 1992, Shtienberg 1992). In Australia, a study was conducted to investigate the effects of TLD on the photosynthetic processes of *E. globulus* and it was shown to reduce the photosynthetic capacity irrespective of the level of infection (Pinkard and Mohammed 2006). This usually results in decreased wood volume production and the production of smaller leaves, indicating that the trees are experiencing a shortage of assimilate for growth which further reduces stem growth (Carnegie and Ades 2002, Tejedor 2004, Milgate *et al.* 2005a). In Spain, a study was conducted to examine the loss of biomass and energy in *E. globulus* plantations. After the crown damage index (CDI) and the tree height were evaluated, biomass losses were determined for each part of the tree separately and 83 000 to 184 000 MJ ha^{-1} energy loss was confirmed as a result of TLD (Pérez *et al.* 2017). In Ecuador, infection in *E. globulus* plantations resulted in diminished tree growth (Bernreiter *et al.* 2016). Tree death caused by TLD may occur as a result of repeated foliar infections, extensive defoliation and reduction in tree size combined with frost damage in cold areas (Park and Keane 1982b, Carnegie and Keane 1994, Carnegie 2002).

Teratosphaeria cryptica has been reported causing the most damaging outbreaks on *E. delegatensis*, *E. globulus*, *E. nitens* and *E. regnans* in forest plantations of New Zealand (Hunter *et al.* 2011) and on at least 30 *Eucalyptus* species in forest plantations of Australia (Carnegie *et al.* 2011). *Teratosphaeria cryptica* is able to infect both the young and mature leaves, leaving the lamina crinkled resulting in an appearance commonly referred to as "crinkle leaf disease" (Park and Keane 1982b). This pathogen has caused severe outbreaks in Australia which have led to the reduced photosynthetic leaf area, premature defoliation and reduced growth rates (Park 1988a). In the 1970s, *T. cryptica* was considered to be the most damaging pathogen of *Eucalyptus* in New Zealand (Ganapathi 1979, Dick 1982, Hood *et al.* 2002). Due to their susceptibility to *T. cryptica*, *E. tereticornis* and *E. camaldulensis* are no longer planted extensively in Queensland (Carnegie *et al.* 2011).

Since its first report from Australia (Cooke 1893), *T. nubilosa* has been reported from a number of countries in Africa, Europe, New Zealand and South America where it causes major disease epidemics on cold tolerant *Eucalyptus* species such as *E. nitens* (Hunter *et al.*

2009). Currently, the known host range of *T. nubilosa* has increased drastically throughout various parts of the world, and includes *E. bicostata*, *E. botryoides*, *E. bridgesiana*, *E. cypellocarpa*, *E. delegatensis*, *E. dunnii*, *E. grandis*, *E. gunnii*, *E. nitens*, *E. quadrangulata*, *E. viminalis*, *E. urophylla* and *E. grandis* × *E. resinifera*, with *E. globulus* and *E. nitens* considered to be the most susceptible species (Carnegie and Keane 1994, Crous 1998, Crous *et al.* 2004, Hunter *et al.* 2004b, Hunter *et al.* 2011). *Teratosphaeria nubilosa* was also reported on *E. delegatensis* where it reached epidemic levels in over 1000 ha of commercial plantations in New Zealand (Cheah and Hartill 1987). In Australia, *T. nubilosa* caused outbreaks which resulted in more than 95% defoliation of young, intermediate and adult leaves of *E. globulus* plantations (Crous *et al.* 2007b). In the 1930s, *T. nubilosa* caused significant infections on *E. globulus* in South Africa, which led to the discontinuation of its use in the country (Lundquist *et al.* 1987).

Since its first discovery in Indonesia where it was found to be an aggressive pathogen on *E. grandis* (Wingfield *et al.* 1996), *T. destructans* has been reported from a number of countries in Asia (Old *et al.* 2003, Burgess *et al.* 2006), as well as South Africa (Greyling *et al.* 2016). In Asia, it has been reported on *E. camaldulensis*, *E. grandis*, *E. urophylla* and their hybrids (Barber 2004). In South Africa, it was reported on a *E. grandis x E. urophylla* hybrid (Greyling *et al.* 2016). Its ability to cause extensive damage in *Eucalyptus* plantations has resulted in this pathogen being recognized as one of the most destructive pathogens on *E. grandis* and its hybrids in the tropics and sub tropics (Andjic *et al.* 2011).

Teratosphaeria eucalypti has caused significant damage on juvenile *E. nitens* leaves in New Zealand and on *E. nitens* and *E. nitens* x *E. nobilis* hybrids in Australia (Carnegie 2007). In Australia, it causes major damage resulting in more than 95% leaf area loss and complete defoliation leading to tree death (Carnegie 2007). In New Zealand, it also causes severe leaf damage which resulted in total defoliation of *E. nitens* plantations (Dick 1982). As a result of this stress, *E. nitens* became susceptible to stem fungi which led to top death and tree mortality (Carnegie 2007).

Since its first report in Australia on *E. grandis* x *E. camaldulensis* (Andjic *et al.* 2010a), *T. pseudoeucalypti* has been reported from South America (Cândido *et al.* 2014, Soria *et al.* 2014, Ramos and Perez 2015). *Teratosphaeria pseudoeucalypti* has been found on *E. camaldulensis*, *E. dalrympleana*, *E. grandis*, *E. globulus*, *E. nitens*, *E. viminalis*, *E. grandis* x *E. camaldulensis* and *E. grandis* x *E. teretricornis* causing severe damage (Ramos and Perez

2015). In Australia, *T. pseudoeucalypti* was found causing severe outbreaks and damage on *E. grandis* x *E. camaldulensis* resulting in defoliation of more than 75% which has since increased annually (Andjic *et al.* 2010b). Due to re-occurring infections, some areas are no longer available for commercial plantations of susceptible species such as *E. grandis* and its hybrids.

Management of TLD includes the selection as well as planting of tolerant genotypes, the use of fungicides, adjusting fertilizer regimes in plantations and quarantine. Differences in susceptibility between species, provenances, families and hybrids have been identified and are used commonly in plantation forestry (Dungey *et al.* 1997, Hood *et al.* 2002, Milgate *et al.* 2005a). For example, in Uruguay, hybrids between *E. globulus* (susceptible to *T. nubilosa*) and *E. grandis* (resistant to *T. nubilosa*) displayed high levels of resistance to TLD (Hunter *et al.* 2009). Hybrids of *E. globulus* and *E. nitens* were however found to be more susceptible than either parent which then led to the decision to stop planting these hybrids in areas where TLD is severe (Carnegie and Ades 2002). In Australia, a disease assessment scale was developed to establish the susceptibility of *E. globulus* and *E. nitens* subspecies and provenances to damage caused *by T. cryptica* (Carnegie and Ades 2005). As a result, *E. globulus* subsp. *bicostata* was found to be significantly more susceptible than *E. globulus* subsp. *globulus*, *E. globulus* subsp. *pseudoglobulus* and *E. globulus* subsp. *maidenii* (Carnegie and Ades 2005). However, no significant differences in susceptibility were found between the *E. nitens* provenances. In South Africa, the leaf spot severity and defoliation of 11 *E. nitens* provenances were evaluated using disease assessment diagrams and significant variation in disease severity and susceptibility were found among these provenances (Lundquist *et al.* 1987). The development and selection of resistant material is thus a priority and a long term management tool that should be applied for successful management (Wingfield *et al.* 2013).

The application of fungicides has shown efficacy in controlling severe TLD infections without negatively impacting the tree growth. In Brazil, two *E. dunnii* clones infected by TLD, were controlled by spraying two fungicide applications of azoxystrobin + cyproconazole and trifloxystrobin + tebuconazole (Garrett *et al.* 2018). This resulted in a successful reduction in TLD severity in the apical and middle branches without affecting the tree growth (Garrett *et al.* 2018). In some circumstances however, repeated applications of fungicides can have a significantly positive effect on tree growth. In Australia, a two-year

trial was conducted on a *E. globulus* plantation that had suffered severe defoliation from TLD (Smith *et al.* 2016). The results revealed that the disease reduced growth when the severity level exceeded 20% and after repeated applications of the fungicide, the tree volume had increased by 17% and the height had increased in growth (Smith *et al.* 2016). In Australia, consecutive applications of benomyl and chlorothalonil were applied on young and mature *E. globulus* over 17 months. The results showed that the development of TLD was significantly reduced and defoliation was also reduced by up to 50% while the height increment and diameter were increased by 13% and 4% respectively (Carnegie and Ades 2002).

To reduce fungicide costs, Park (1988b) suggested the application of fungicides should only be undertaken during the vegetative period of the *Eucalyptus* species. When symptoms of TLD start to develop in the lower crown, it is ideal to implement an early bottom-up orientated fungicide application (Carnegie and Ades 2002). The application of fungicides is however commercially and economically unviable especially in plantations where TLD causes severe outbreaks (Smith *et al.* 2016). This can thus be alternated with other control measures.

The rapid spread of species that cause TLD has been attributed to the movement of plant material between countries and continents. It is therefore essential to inspect plant material for the presence of pathogen propagules before moving them (Andjic *et al.* 2011). In cases where infection is latent, seeds and plants should also be tested for the presence of species of *Teratosphaeria* by using species-specific primers that have been previously developed for *Eucalyptus*-infecting species (Kularatne *et al.* 2004).

Austropuccinia psidii

Austropuccinia psidii, previously known as *Puccinia psidii*, is a rust fungus (*Sphaerophragmiaceae*, *Pucciniales*) that causes serious damage to various species of *Myrtaceae* globally (Coutinho *et al.* 1998, Glen *et al.* 2007, Carnegie *et al.* 2010, Morin *et al.* 2012, Carnegie *et al.* 2016). It was first described from guava, *Psidium guajava* L., in Brazil (Winter 1884), which is where one of the common names for the disease it causes, guava rust, originated. Thereafter it was reported from various species of *Myrtaceae*, including *Eucalyptus* in Brazil (Joffily 1944). This disease is now more commonly referred to as myrtle rust, reflecting its broad host range in the myrtle family.

Currently, *A. psidii* has been reported on approximately 460 species that reside in 73 genera of the *Myrtaceae* (Carnegie and Lidbetter 2012, Roux *et al.* 2013, Giblin and Carnegie 2014, Carnegie *et al.* 2016, McTaggart *et al.* 2016, Beenken 2017, Beresford *et al.* 2018). *Austropuccinia psidii* is known from: Africa - South Africa (Roux *et al.* 2013); Australia (Simpson *et al.* 2006); Asia - China (Zhuang and Wei 2011), India (Walker 1983), Indonesia (McTaggart *et al.* 2016), Japan (Kawanishi *et al.* 2009), Singapore (du Plessis *et al.* 2017) and Taiwan (Wang 1992); Central America and Caribbean - Barbados (Baker and Dale 1948), British Virgin Islands (EPPO 2020), Costa Rica (Di Stéfano *et al.* 1998), Cuba (Seaver and Chardón 1926), Dominica (Baker and Dale 1948), Guatemala (Schieber and Sanchez 1968), Jamaica (Smith 1935), Panama (CABI and EPPO 2014), Puerto Rico (MacLachlan 1938), Trinidad and Tobago (Baker and Dale 1951) and U.S. Virgin Islands (CABI and EPPO 2014); New Caledonia (Giblin 2013); New Zealand (Beresford *et al.* 2018); North America - Dominican Republic (CABI and EPPO 2014), Mexico (CABI and EPPO 2014) and USA (Marlatt and Kimbrough 1979); South America - Argentina (Di Fonzo 1946), Brazil (Winter 1884), Colombia (Rodas *et al.* 2015), Ecuador (Stevenson 1926), Paraguay (Silva *et al.* 2020), Uruguay (Pérez *et al.* 2011) and Venezuela (CABI and EPPO 2014).

Austropuccinia psidii is an autoecious, macrocyclic rust, which means that it has the ability to complete its life cycle on *Myrtaceae*, without the need for alternate hosts (Glen *et al.* 2007, Morin *et al.* 2014, McTaggart *et al.* 2017). The life cycle begins with infections by wind dispersed basidiospores, which are produced by teliospores *in situ* in telia (McTaggart *et al.* 2017). After infection, hyphae emerging from two basidiospores are hypothesized to merge to produce a dikaryotic hymenium which then produces either uredinia or telia (Maier *et al.* 2016, McTaggart *et al.* 2016). Telia produce sexual teliospores, in which recombination occurs and genotypic diversity is produced (McTaggart *et al.* 2017). Telia may be produced directly, may form from uredinia, or not at all (McTaggart *et al.* 2017). Uredinia produce asexual urediniospores which disperse in wind to start new infections and it is this spore stage that is responsible for rapid epidemic development.

Austropuccinia psidii requires high relative humidity and low light conditions for spore germination and plant infection. The environmental conditions for spore production vary between each of the spore stages: uredinial stage (15-20°C), telial stage (21-25°C) and basidiospore stage (21°C) (Piza and Ribeiro 1988, McTaggart *et al.* 2017). For disease development, high relative humidity (~80%), leaf wetness and high inoculum concentration

is essential during stages of active host growth (Blum and Dianese 2001). In the event of drought or insufficient rain, fog and dew can provide enough moisture for infection and the survival of the spores (Pegg *et al.* 2014).

Several strains or "biotypes" of *A. psidii* have been identified based on cross-inoculation studies, and, more recently, analysis of microsatellite markers (Graça 2011). For example, it has been shown that isolates of *A. psidii* from *Pimenta dioica* and *Syzygium jambos* did not infect *Psidium guajava* (Graça *et al.* 2013). Microsatellite markers were used to identify a single strain of *A. psidii* that has spread widely to several locations globally. This strain is commonly referred to as the pandemic biotype (Ross-Davis *et al.* 2014, Granados *et al.* 2017). So far, only two strains have been detected outside of South America - the pandemic strain which occurs in multiple regions, and the South African strain, which has only been found here (Roux *et al.* 2016). Microsatellite markers revealed several unique multilocus genotypes (MLGs) from the Americas and one from Hawaii. These grouped isolates into nine distinct genetic clusters: C1 - diverse hosts (Costa Rica, Jamaica, Mexico, Puerto Rico and USA), C2 - *Eucalyptus* spp. (Brazil and Uruguay) and *Syzygium jambos* (Brazil), C3 - *Eucalyptus* spp. (Brazil), C4 - diverse hosts (USA-Florida), C5 - *Syzygium cumini* (Brazil), C6 - *Psidium guineense* (Brazil), C7 - *Eugenia uniflora* (Brazil), C8 - *Pimenta dioica* (Jamaica) and *Myrrhinium atropurpureum* (Uruguay) and C9 - *Myrciaria cauliflora* (Brazil) (Stewart *et al.* 2018). The MLGs on diverse hosts in Hawaii (C1) and Florida (C4) belong to the pandemic biotype that is also found in Australia (Sandhu *et al.* 2016), China (Machado *et al.* 2015), Colombia (Granados *et al.* 2017), Indonesia (McTaggart *et al.* 2016), New Caledonia (Giblin 2013), New Zealand (du Plessis *et al.* 2019) and Singapore (du Plessis *et al.* 2019). Based on the high genetic diversity of *A. psidii* in South America, as well as its long history in that region, it is hypothesized that the fungus is native there (Tommerup *et al.* 2003). In Brazil, for example, *A. psidii* populations are strongly genetically differentiated by host, and multiple host associated MLGs have been found (Graça *et al.* 2013). A hypothesis suggesting that the fungus spread from guava to eucalypts (*Allosyncarpia*, *Angophora*, *Arillastrum*, *Corymbia*, *Eucalyptus*, *Eucalyptopsis and Stockwellia*) was strongly rejected after the population structure of *A. psidii* was determined using microsatellite markers, and the results revealed large genetic distances between the guava and eucalypt populations, further suggesting that *A. psidii* populations have strong host associations (Graça *et al.* 2013).

Infection by *A. psidii* results in leaf spots (Fig. 1a) and, in severe cases, shoot blight and plant death. It mostly attacks young, tender plant material, including leaves, shoots, flower buds,

flowers and fruits (Coutinho *et al.* 1998, Pegg *et al.* 2014). The disease is easily recognized by the yellow masses of urediniospores produced by the fungus (Figure 1a). In severe cases, and where multiple infections occur over more than one season, *A. psidii* has resulted in plant death (Telechea *et al.* 2003, Tommerup *et al.* 2003, Carnegie *et al.* 2016). *Austropuccinia psidii* led to the demise of the allspice (*Pimenta dioica*) industry in Jamaica (MacLachlan 1938). Similarly, it has necessitated expensive fungicide spray programs in guava and *Eucalyptus* industries in South America (Ferrari *et al.* 1997, de Goes *et al.* 2004). In environments where the pathogen is a non-native invasive, such as Australia, it has a significant impact on native *Myrtaceae*, driving already threatened species into extinction (Pegg *et al.* 2014, Carnegie *et al.* 2016). It is also threatening emerging businesses in Australia, such as the lemon myrtle oil industry (Carnegie and Cooper 2011).

Austropuccinia psidii is considered an important pathogen of *Eucalyptus* and poses a threat to commercial plantations globally (Grgurinovic *et al.* 2006, Glen *et al.* 2007). It causes significant losses in nurseries and plantations of *Eucalyptus* in a number of South American countries (Ferreira 1983, Telechea *et al.* 2003, Tommerup *et al.* 2003, Rodas *et al.* 2015). In Brazil, *A. psidii* is considered the most damaging disease in *Eucalyptus* nurseries and plantations with several outbreaks having resulted in severe damage on trees younger than two years old (Ferreira 1983, Junghans *et al.* 2003, Alfenas *et al.* 2005). In Uruguay, this pathogen has caused significant losses of one year old *Eucalyptus* (Telechea *et al.* 2003), and also threatens the *Eucalyptus* forestry industry in neighbouring countries (Pérez *et al.* 2011). To date, *A. psidii* has not been reported to affect species of *Eucalyptus* or their hybrids in South Africa (Roux *et al.* 2016). Similarly, in Australia no reports of damage to *Eucalyptus* plantations have been received, suggesting that the South African and pandemic strains may not be aggressive on these hosts.

Management of *A. psidii* is complex and requires a combination of international and national interventions and management tools. On an international level, regulations to manage plant trade and plant movement between countries is necessary and strict border control should be implemented to avoid the entrance of *A. psidii* into new geographic regions or to prevent the introduction of additional *A. psidii* strains into countries where the pathogen already occurs (Pegg *et al.* 2014). In the event of detection in a limited area, immediate eradication should be attempted, because *A. psidii* produces air-borne spores which spread rapidly (Soewarto *et al.* 2018). However, successful eradication of the pathogen has not been achieved to date.

Once the pathogen has entered the country, chemical (Old *et al.* 2003) and biological control applications (Glen *et al.* 2007) and the use of resistant plant material (Graça 2011, Graça *et al.* 2013) become the main tools to manage the disease. Regular application of non-systemic fungicides such as copper oxychloride, mancozeb and propiconazole can be used as a preventative or curative measure for the control of *A. psidii* on infected plant material (Carnegie *et al.* 2016). Systemic fungicides such as azoxystrobin, triadimenol and tebuconazole have a curative effect by reducing the number of sori and preventing pustule formation on the leaf area (Glen *et al.* 2007). A number of potential biological control agents have been identified. *Fusarium decemcellulare* was demonstrated to serve as a hyper parasite of *A. psidii* in Brazil (Amorim *et al.* 1993), while *Pseudomonas aeruginosa* has been used to induce systemic resistance in *E. grandis* x *E. urophylla* (Teixeira *et al.* 2005, Glen *et al.* 2007). Further work is essential, however, before recommending them as biological control agents.

The use of resistant material is a long-term and an economically viable approach in controlling *A. psidii* (Glen *et al.* 2007). In Uruguay, artificial inoculations revealed variation in susceptibility of *Eucalyptus* species and this was used to select resistant species to be considered for planting (Pérez *et al.* 2011). The development of molecular markers to identify resistance and susceptibility in hosts have aided in the early selection of resistant material and can be used to speed up resistance breeding efforts (Junghans *et al.* 2003, Laia *et al.* 2015).

Calonectria species

Calonectria (De Notaris 1867) is a genus that resides in the *Nectriaceae* (Rogerson 1970). This genus includes pathogens which cause root, shoot and foliar diseases of forestry trees (Crous 2002). Species of *Calonectria* are pathogenic on a number of hosts and have been recorded to cause diseases on approximately 335 species which reside in 100 plant families (Crous 2002, Lombard *et al.* 2010a). Hosts of this genus include important agricultural crops (Dianese *et al.* 1986, Gai *et al.* 1992, Silva *et al.* 2001), ornamental plants (Reis *et al.* 2004, Poltronieri *et al.* 2011) and forest trees (Alfenas *et al.* 2013b, Alfenas *et al.* 2013c).

Species of *Calonectria* cause a disease of *Eucalyptus* commonly referred to as Calonectria Leaf Blight (CLB) disease (Sharma and Mohanan 1991, Booth *et al.* 2000, Crous 2002, Rodas *et al.* 2005) which has been reported from commercial plantations and nurseries from multiple regions of the world. These include: Africa - Republic of Congo (Roux *et al.* 2000),

Madagascar (Crous and Swart 1995), South Africa (Lundquist and Baxter 1985) and Zambia (Chungu *et al.* 2010); Australia (Pitkethley 1976); Asia - China (Wang 1992), India (Peerally 1974d) and Indonesia (Crous *et al.* 1998); North America - USA (Uchida and Aragaki 1997); South America - Argentina (Crous and Kang 2001), Brazil (Batista 1951) and Colombia (Crous and Kang 2001); and Vanuatu (Ivory *et al.* 1993).

Species of *Calonectria* are characterized by a sexual morphological state which has pigmented, scaly to warty perithecia containing fusiform ascospore walls (Crous 2002) and a *Cylindrocladium* asexual state which is recognized by the production of cylindrical septate conidia (Lombard *et al.* 2016). They reside in two main phylogenetic groups (Lombard *et al.* 2010). These groups are referred to as the Prolate group, which includes species of *Calonectria* that have clavate to pyriform to ellipsoidal vesicles, and the Sphaero -Naviculate group which includes species of *Calonectria* characterized by sphaeropedunculate and naviculate vesicles (Lombard *et al.* 2010). The Prolate group is represented by nine species complexes namely: *Ca. brassicae*, *Ca. candelabrum*, *Ca. colhounii*, *Ca. cylindrospora*, *Ca. gracilipes*, *Ca. mexicana*, *Ca. pteridis*, *Ca. retaudii* and *Ca. spathiphylli*. The Sphaero-Naviculate group is represented by the *Ca. kyotensis* and *Ca. naviculata* species complexes (Lombard *et al.* 2010, Liu *et al.* 2020). The majority of the species associated with CLB disease of *Eucalyptus* in Asia (Booth *et al.* 2000, Lombard *et al.* 2010d, Li *et al.* 2017), Australia (Crous 2002) and Colombia (Rodas *et al.* 2005) reside in the *Ca. candelabrum* and *Ca. reteaudii* species complexes, while those associated with CLB in Brazil reside in the *Ca. pteridis* species complex (Graça *et al.* 2009, Alfenas *et al.* 2015, Freitas *et al.* 2019, Pham *et al.* 2019). In Africa, however, species associated with CLB reside in several species complexes, namely the *Ca. candelabrum* (Chungu *et al.* 2010, Crous *et al.* 2013), *Ca. colhounii* (Crous and Wingfield 1994), *Ca. mexicana* (Lombard *et al.* 2011), *Ca. reteaudii* (Peerally 1974d, Crous and Swart 1995), and *Ca. spathiphylli* species complexes (Schoch *et al.* 1999). All of the above mentioned species belong in the Prolate group. *Calonectria reteaudii* (residing in the *Ca. reteaudii* species complex) is considered the most dominant plantation pathogen responsible for CLB, especially in South America (Rodas *et al.* 2005, Alfenas *et al.* 2015) and South East Asia (Lombard *et al.* 2010a, Chen *et al.* 2011) where it threatens plantation productivity and wood production. In nurseries, *Ca. pauciramosa* (residing in the *Ca. candelabrum* species complex) is the most dominant pathogen responsible for CLB in Australia, Italy, South Africa, Spain and USA (Polizzi and Crous

1999, Schoch *et al.* 1999, Koike and Crous 2001, Lombard *et al.* 2010a, Liu *et al.* 2020) where it threatens the production of cuttings.

Disease symptoms of CLB include leaf, shoot and seedling blight (Fig. 1c), defoliation, leaf blotch and leaf spot (Sharma *et al.* 1984, Crous *et al.* 1991, Crous 2002, Lombard *et al.* 2010a). Symptoms of CLB begin with leaf spots on young and mature leaves which develop when conditions are favourable (Crous 2002), appearing first as small circular irregular greyish necrotic spots on water-soaked lesions (Old *et al.* 2003). Favourable conditions include warm temperatures (above 16°C), wet and humid conditions, heavy shade and high seedling density (Crous *et al.* 1991, Crous 2002, Vitale *et al.* 2013). Thereafter, white spore masses and mycelia can be observed on the lesion margins followed by the development of large irregular lesions that can cover the entire leaf surface (Old *et al.* 2003). The development of shoot lesions can result in leaf blight or defoliation (Crous 2002, Old *et al.* 2003). Trees that are highly infested with CLB may have a deformed structure and growth reduction (Old *et al.* 2003).

Some species of *Calonectria* are important nursery pathogens of *Eucalyptus* (Polizzi and Catara 2001). Symptoms found to be associated with diseases in seedling production in nurseries include root, collar and crown rots, and seedling blight (Lombard et al. 2010). Symptoms start developing at an early growth stage, and include chlorotic leaves, necrotic crowns, and stem and root rot (Crous *et al.* 1991, Crous 2002). Infection in nurseries may result in seedling death (Vitale *et al.* 2013). In Australia and Southern Africa, infection of *Eucalyptus* by species of *Calonectria* is mostly limited to forestry nurseries and is seldom found in plantations (Crous 2002, Lombard *et al.* 2010a, Lombard *et al.* 2010b). Some nursery pathogens have also been detected in plantations, for example *Ca. pauciramosa*, which was reported as a plantation pathogen in China (Chen *et al.* 2011).

Species of *Calonectria* have a polycyclic life cycle (Crous 2002), producing more than one infection cycle per crop cycle. Ascospores are produced in perithecia that form on plant material. Once they are matured they are discharged into the air where they are carried by wind to susceptible host plants on which they will germinate (Yu and Elliott 2013). Conidia of *Calonectria* are produced on leaf surfaces or stems and disperse shorter distances in water splash from rain or irrigation (Crous *et al.* 1991, Yu and Elliott 2013). Clusters of chlamydospores, called microsclerotia, survive on plant debris until the diseased plant

material falls to the ground which is when they are released into the soil. Once they are in the soil, they can survive for long periods before initiating the next infection cycle (Crous 2002).

To effectively manage *Calonectria* foliar diseases, a combination of chemical control (Crous 2002) and selection of resistant material is required. Various methods of chemical application have been proposed and preventative methods were found to be more effective as opposed to curative methods (Henricot *et al.* 2008). In Italy, 11 fungicides were tested to control leaf spots on *Eucalyptus* seedlings caused by three species of *Calonectria* in nurseries (Aiello *et al.* 2013). Most of these fungicides were found to be effective in reducing the populations of some species of *Calonectria*; however, repeated applications should be avoided as it can result in phytotoxicity (Vitale 2003) and copper accumulation in the soil (Vitale 2013). The use of chemical control is expensive and not entirely effective to control some aggressive species of *Calonectria* (Sharma and Mohanan 1991). It should thus be applied in an integrated approach together with resistant material for effective management. The selection of resistant plant material is the more effective long term approach for management of CLB (Sharma and Mohanan 1991).

Quambalaria species

Species of *Quambalaria* (*Basidiomycota*, *Microstromatales*, *Quambalariaceae*) cause leaf, shoot and stem diseases of eucalypts (*Corymbia* and *Eucalyptus*) (Walker and Bertus 1971, De Beer *et al.* 2006, Roux *et al.* 2006, Paap *et al.* 2008, Chen *et al.* 2017). The first species of *Quambalaria* was described in Australia in the 1950s, causing shoot blight on seedlings of what was then known as *Eucalyptus* (now *Corymbia*) (Walker and Bertus 1971). They have since been reported from eucalypts in Africa - South Africa (Wingfield *et al.* 1993) and Uganda (Roux and Slippers 2007); Asia - China (Zhou *et al.* 2007); Europe - Portugal (Bragança *et al.* 2016); South America - Brazil (Alfenas *et al.* 2001b) and Uruguay (Bettucci *et al.* 1999).

The taxonomy of the genus has undergone several changes over time. Initially the fungus was thought be an ascomycete and the first *Quambalaria* species was described as *Ramularia pitereka* (Walker and Bertus 1971). Other synonyms for the species in the genus include *Sporothrix* (Braun 1995) and *Fugomyces* (Sigler *et al.* 1990). The genus *Quambalaria* was first introduced in 2000 (Simpson 2000). It is only since the early 2000s that the pathogen was known to be a basidiomycete and classified into the *Microstomatales* (De Beer *et al.*

2006). Currently, eight species of *Quambalaria* have been identified from eucalypts (Chen *et al.* 2017). These are *Q. coyrecup, Q. cyanescens, Q. eucalypti, Q. pitereka, Q. pusilla, Q. rugosae, Q. simpsonii* and *Q. tasmaniae*

The pathogenicity and host specificity of the different species varies. *Quambalaria coyrecup* causes canker diseases and shoot blight of both *Corymbia* and *Eucalyptus* (Paap *et al.* 2008). *Quambalaria cyanescens* was originally found on human skin and was later considered to be a saprophyte feeding off dead plant material (de Hoog and de Vries 1973). However, it has also been found on live *Corymbia* where it co-occurs with *Q. coyrecup* and *Q. pitereka* (Paap *et al.* 2008). *Quambalaria eucalypti* was first reported from a *Eucalyptus* clonal nursery in South Africa causing leaf spots and serious shoot infections (Wingfield *et al.* 1993). *Quambalaria pitereka* has been found on species of *Angophora*, *Blakella* and *Corymbia* causing shoot blight and was associated with stem cankers in Australia (Simpson 2000, Pegg *et al.* 2008). The taxonomic status of *Q. pusilla* is currently unresolved (Braun 1995, De Beer *et al.* 2006) and cannot be confirmed because the type culture was contaminated, thus DNA could not be extracted (De Beer *et al.* 2006, Pegg *et al.* 2008). *Quambalaria simpsonii* was previously found on stem cankers of *Eucalyptus* species where it co-occurred with *Teratosphaeria zuluensis* (Chen *et al.* 2017). Due to an overlap in potential symptoms of the two pathogens its pathogenicity is not yet clearly established (Cheewangkoon *et al.* 2010). *Quambalaria rugosae* and *Q. tasmaniae* have recently been identified from Australia causing leaf spot on *Eucalyptus* spp. (Crous *et al.* 2019). Information regarding the severity of these two new species is not known however, phylogenetically, *Q. rugosae* is closely related to *Q. pitereka* while *Q. tasmaniae* is closely related to *Q. eucalypti* (Crous *et al.* 2019).

Quambalaria eucalypti and *Q. pitereka* cause one of the most important diseases of plantation grown eucalypts, called Quambalaria leaf and shoot blight (Simpson 2000, Pegg *et al.* 2008). Quambalaria leaf and shoot blight disease occurs on commercial seedlings (Wingfield *et al.* 1993) as well as in plantations (Roux *et al.* 2006). Infection of leaves result in distortion and necrotic lesions (Pegg *et al.* 2008), often characterized by the presence of powdery white fungal spore masses (Fig. 1d) that are found on both surfaces of the leaves (Alfenas *et al.* 2004). Spore masses can also extend to the edges of the leaves and the midribs (Pegg *et al.* 2009a). Infection on juvenile leaves could result in lesions that are associated with wounds caused by various insects (Pegg *et al.* 2008). Infection of stems begins with bark lesions, followed by formation of white spore masses on the lesions. Cankers are found in

plantations where they are associated with bark splitting and in nurseries where they cause die-back of seedlings and shoot death (Roux *et al.* 2006, Bragança *et al.* 2016).

Infection takes place when the basidiospores adhere to and penetrate the leaf surface through the stomata or fresh wounds (Mafia *et al.* 2009, Pegg *et al.* 2009b, Pegg 2011). Germination and infection takes place on both the abaxial and adaxial leaf surfaces which lead to the development of tiny lesions on young leaves and stems within five days. These eventually grow into large sporulating lesions characterized by white pustules (Pegg *et al.* 2009b). Favourable conditions for infection of Quambalaria leaf and shoot blight in Australia were found to be between 10 and 35°C for *Q. eucalypti* and 20-25°C for *Q. pitereka* with a relative humidity of more than 90% (Pegg *et al.* 2009b, Pegg 2011). In Brazil, the optimum conditions were found to be 25°C with a high relative humidity when incubated in the dark for 3-10 days for both *Q. eucalypti* and *Q. pitereka* (Alfenas *et al.* 2001b).

Quambalaria leaf and shoot disease has had a significant impact on the growth and production of eucalypts in various countries (Pegg *et al.* 2009a). In Australia, *Q. pitereka* and Q. *cyanescens* were found causing severe shoot blight and stem cankers on *Corymbia* species while *Q. eucalypti* was found causing shoot and leaf blight on *Eucalyptus* plantations and on a single *Corymbia* hybrid. This resulted in premature leaf senescence, loss of apical dominance and extensive canker diseases (Pegg *et al.* 2008). In Brazil, *Q. eucalypti* is one of the most important *Eucalyptus* nursery diseases, causing stem girdling on seedlings. It has also spread throughout the major *Eucalyptus* plantation areas where it causes leaf and shoot blight on stumps and on clonal propagation material of *Eucalyptus* (Alfenas *et al.* 2001b, Alfenas *et al.* 2004, Mafia *et al.* 2009). In South Africa, *Q. eucalypti* is mostly a problem in nurseries causing extensive shoot and leaf die-back, but it has also been reported causing stem canker and leaf death of *E. nitens* under field conditions (Roux *et al.* 2006). In Uruguay, *Q. eucalypti* has been found infecting twigs of *Eucalyptus* with no detrimental effect (Bettucci *et al.* 1999), but has undergone a host shift to a native tree, *Myrceugenia glaucescens*, to give rise to a new host-pathogen interaction (Pérez *et al.* 2008).
In China, three *Quambalaria* species were reported from *Corymbia* and *Eucalyptus* hosts, these are *Q. eucalypti*, *Q. pitereka* and *Q. simpsonii* (Chen *et al.* 2017). After *Q. pitereka* was isolated from multiple *C. citriodora* provenances, it was concluded that it may have the potential to actively spread between different regions (Chen *et al.* 2017). *Quambalaria eucalypti* is considered to be an emerging pathogen because of its ability to infect the leaves

and stems of a number of host species (Chen *et al.* 2017). The impacts caused by *Quambalaria* species require rigorous management to maintain the growth and production of eucalypts.

To best manage Quambalaria leaf and shoot blight, quarantine measures, selection of resistant plant material and chemical control should be considered (Roux *et al.* 2006). In this regard, quarantine measures are applicable by restricting potential pests and pathogens from entering specific areas to avoid infection by *Quambalaria* (Roux 2006, Pegg 2008). The selection of resistant plant material should form the basis of disease management. For example, Pegg *et al*. (2011) conducted pathogenicity trials in order to test the resistance of *Corymbia* species to *Q. pitereka* and the results revealed that *C. citriodora* was highly resistant while *C. vagrieta* and *C. henryi* were the most susceptible. Foliar pathogenicity trials that were conducted in South Africa revealed that *E. grandis* hybrids were less susceptible to *Q. eucalypti* than *E. grandis* and *E. smithii* (Roux *et al.* 2006). Various fungicides have also been tested for the management of Quambalaria leaf and shoot blight disease, with some being able to significantly reduce infection and to some extent, eradicate infection by the pathogen (Ferreira *et al.* 2008). Another management option would be the removal of susceptible clones from sites; this however, is only a temporary solution which becomes ineffective over time. For example, in South Africa, after all the susceptible clones were removed, Quambalaria leaf and shoot blight reappeared in the same area causing more damage to other *Eucalyptus* species (Wingfield *et al.* 1993, Roux *et al.* 2006). Adequate information regarding the biological cycle and the epidemiology of *Quambalaria* species is of significant importance as it would help with the successful management of Quambalaria leaf and shoot blight disease (Pegg *et al.* 2009a).

Bacterial leaf blight

Bacterial blight of eucalypts is a major leaf and shoot disease that may result in leaf drop and shoot death (Coutinho *et al.* 2002). The causal agents of bacterial blight are gram negative bacteria that reside in three genera, *Pantoea* (*Enterobacteriaceae*), *Pseudomonas* (*Pseudomonadaceae*) and *Xanthomonas* (*Xanthomonadaceae*) (Gavini *et al.* 1989, Vauterin *et al.* 1995, Palleroni 2008). Different bacterial species have been identified as the causal agents or found to be associated with the disease. These include *Pantoea vagans* in Argentina (Brady *et al.* 2009), *Xanthomonas dyei* pv. *eucalypti* in Australia (Truman 1974),

Pseudomonas cichorii, *X. axonopodis* and *X. axonopodis* pv. *eucalyptorum* in Brazil (Pomella *et al.* 1995, Gonçalves *et al.* 2008, Ferraz *et al.* 2018), *P. rodasii* in Colombia (Brady *et al.* 2012), *P. rwandensis* in Rwanda (Brady *et al.* 2012), *P. ananatis*, *P. wallisii* and *X. vasicola* in South Africa (Coutinho *et al.* 2002, Brady *et al.* 2012, Coutinho *et al.* 2015), *X. axonopodis* pv. *eucalypti* in Thailand (Pothiluk *et al.* 2013), *P. deleyi* and *P. vagans* in Uganda (Brady *et al.* 2009), and *P. eucalypti* and *P. vagans* in Uruguay (Brady *et al.* 2009).

These different species cause disease on a wide range of hosts including agricultural crops such as maize, onion, rice, tomato and sugarcane, fruit such as cantaloupe, citrus, melons and pineapple, and forest trees such as eucalypts (*Corymbia* and *Eucalyptus*) (Pomella *et al.* 1995, Coutinho and Venter 2009, Young *et al.* 2010). Some of these bacterial pathogens are able to move between host species, for example, *X. vasicola* was found to cause a devastating outbreak on a newly established *E. grandis* plantation in South Africa, representing a significant host jump from a sugarcane plot nearby (Coutinho *et al.* 2015).

Bacterial leaf blight disease was first reported from eucalypts in the 1970s causing die-back on *Corymbia* in Australia (Truman 1974). It has since been reported from South America - Paraguay (Ferreira *et al.* 2001) and Uruguay (Alfenas *et al.* 2001a). The eucalypt clones, hybrids and species that are infected by these bacterial blight species include *C. citriodora, C. maculata*, *E. camaldulensis*, *E. camaldulensis* x *E. deglupta*, *E. cloeziana*, *E. dunni*, *E. globulus*, *E. globulus* subsp. *maidenii*, *E. grandis*, *E. grandis* x *E. camaldulensis*, *E. grandis* x *E. urophylla*, *E. nitens*, *E. robusta*, *E. saligna*, *E. smithii*, and *E. urophylla* x *maidenii* (Truman 1974, Pomella *et al.* 1995, Gonçalves *et al.* 2008, Pothiluk *et al.* 2013, Coutinho *et al.* 2015). Symptoms that are caused by the bacteria, e. g. *Pantoea* species, on the same host may differ between countries; this could potentially be as a result of the different environmental conditions that enhance infection (Coutinho and Venter 2009).

Symptoms of bacterial leaf blight disease of eucalypts include leaf spots, leaf blight (Fig. 1e) and die-back (Coutinho *et al.* 2002, Coutinho *et al.* 2011). Symptoms are characterized by water-soaked, angular and interveinal spots which develop into necrotic lesions that are associated with chlorotic, reddish lesion margins, or a halo of bacterial residue (Gonçalves *et al.* 2008, Ferraz *et al.* 2018). The lesions are distributed along the main vein, on the leaf surface and on the leaf edges (Gonçalves *et al.* 2008, Coutinho *et al.* 2011). The lesions then spread from the main veins into the petioles, resulting in die-back (Coutinho *et al.* 2015). In severe cases, when conditions are favourable, infection results in defoliation, early

senescence of the infected leaves, stunting, and malformation – trees develop a bushy appearance (Coutinho *et al.* 2002, Coutinho *et al.* 2011, Ferraz *et al.* 2018).

Species that cause bacterial blight enter the host through flowers (by insects that collect pollen or nectar) or wounds caused either by mechanical injury, feeding insects or contact between plants (Serrano 1928, Hasegawa *et al.* 2003). Once the bacterium has entered the host, moderate temperatures (20 - 25°C) and high relative humidity is required for symptom development to take place (Coutinho *et al.* 2002, Paccola-Meirelles *et al.* 2002) which, when severe, could result in extensive damage.

Bacterial blight species have caused significant economic and financial losses in nurseries and plantations of *Eucalyptus* (Gonçalves *et al.* 2008). In severe cases, seedlings, ramets and cuttings in nurseries may die, resulting in lost propagative material, whereas in the field, trees may become stunted and develop a malformed, multi-stemmed appearance (Coutinho *et al.* 2015). In Brazil, millions of dollars were lost as a result of bacterial blight infection on *Eucalyptus* stumps and rooted cuttings in nurseries which eventually could not be transplanted and thus had to be discarded (Alfenas *et al.* 2009). Unless these bacterial blight species are controlled, they will continue hindering the ability to produce vegetative material for rooting, thus negatively impacting the forestry industry.

In Brazil, variability of susceptibility within hosts was observed when strains previously isolated from *Eucalyptus* showed varying pathogenicity and aggressiveness when inoculated onto *E. urophylla* x *globulus* hybrids (Ferraz *et al.* 2018). It is also known that differences in susceptibility exist among *E. grandis* clones specifically, thus rapid screening techniques could be used to select material that is tolerant to the disease (Coutinho *et al.* 2002). These screening techniques could assist in identifying the most resistant material that can be used to reduce impact caused by this disease. This has been achieved by selecting resistant material from a cutting production nursery and by artificially inoculating different species and hybrids with the most aggressive strain and choosing the most resistant cultivar (Coutinho and Venter 2009).

Another possible measure that could be considered is the eradication or removal of diseased plant material in order to maintain hygiene (Coutinho and Venter 2009). For example, after *Eucalyptus* stumps and cuttings were severely infected by bacterial blight disease, they had to

be removed and be discarded (Alfenas *et al.* 2009). This however, is a time-consuming approach which could be applicable in dire cases.

LEAF DISEASES OF *EUCALYPTUS* IN SOUTH AFRICA

The first detailed surveys of *Eucalyptus* leaf pathogens conducted in South African plantations were in the late 1980s (Crous *et al.* 1989c). A number of previously unreported leaf pathogens from different genera were observed. Pathogens that were identified included species of the following genera: *Aulographina*, *Cercospora*, *Coniothyrium*, *Cylindrocladium*, *Cyllindrocladiella*, *Fairmaniella*, *Guignardia*, *Harknessia*, *Mycosphaerella*, *Pestalotiopsis*, *Phaeoseptoria*, *Pseudocercospora*, *Readeriella*, *Seimatosporium*, *Sphaerotheca*, and *Thyriopsis* (Crous *et al.* 1989, Crous *et al.* 1989a). Some of these leaf pathogens were found to cause severe leaf spotting, extensive defoliation and loss of juvenile leaves, while others were not of great economic importance (Crous *et al.* 1989, Crous *et al.* 1989c). More recently, species of *Coniella*, *Cryptosporiopsis* and *Microsphaeropsis*, as well as *Pantoea ananatis*, *Quambalaria eucalypti*, *Teratosphaeria destructans*, and *Xanthomonas vasicola* were also reported from South Africa (Wingfield *et al.* 1993, Coutinho *et al.* 2002, Coutinho *et al.* 2015, Greyling *et al.* 2016).

The most important leaf diseases of *Eucalyptus* in South Africa are those caused by species of *Teratosphaeria*. As noted above, the presence of *T. nubilosa* contributed to the discontinuation of planting of *E. globulus* in South Africa during the 1930s (Lundquist *et al.* 1987) and has necessitated changes in the provenances of *E. nitens* planted in the country (Purnell and Lundquist 1986). In the early 2000s, surveys of species of *Teratosphaeria* affecting several *Eucalyptus* species including *E. nitens* were conducted in the Eastern Cape, KwaZulu-Natal and Limpopo, where *Eucalyptus* was mostly grown and where TLD is most severe (Hunter *et al.* 2004a, Hunter *et al.* 2004b). As a result, six species were recognized namely *T. ellipsoidea*, *T. fori*, *T. irregulariramosa*, *T. juvenis*, *T. lateralis*, *T. marksii* and *T. nubilosa* which was revealed to be the main causal agent of TLD, highlighting its importance in commercial plantations.

The most recent detailed surveys of *Eucalyptus* leaf pathogens in South Africa, with the exception of the work of Hunter et al. (2004b) on *E. nitens*, were conducted in the 1980s.

Information pertaining to leaf pathogens of commercial *E. grandis* hybrids in the eastern part of South Africa, in the Provinces of KwaZulu-Natal, Limpopo and Mpumalanga, is limited. It is possible that there may be unreported *Eucalyptus* leaf pathogens in these areas, as was highlighted by the recent discovery of *T. destructans* in the Zululand region (Greyling *et al.* 2016). New diseases may also have emerged on *Eucalyptus* species that have been planted in the country as native fungi could adapt to these trees. It is, therefore, important to conduct surveys to address this lack of information on the current status of *Eucalyptus* diseases in the major commercial plantation regions of the country.

CONCLUSIONS

Species of *Eucalyptus* are among the most widely planted trees globally. They are planted and grown in commercial plantations for various purposes, such as for the timber, fibre, paper and pulp industries. The first plantations of *Eucalyptus* were mostly free from diseases due to separation of the trees from their natural enemies in their countries of origin. As the production of *Eucalyptus* species increased, so did the number of pests and diseases affecting these trees in areas where they were introduced. Over the decades a number of important leaf pathogens have made their mark on plantations of *Eucalyptus* trees, resulting in defoliation and in severe cases tree death.

Several leaf pathogens have been reported from South Africa, both from commercial *Eucalyptus* plantations, as well as from ornamental and unmanaged stands. Some of these pathogens have had serious impacts on the industry. Among these, species of *Teratosphaeria* have had the most significant impact on the industry, with more than 20 species reported from these trees in South Africa. One *Teratosphaeria* species, *T. nubilosa* was even responsible for the discontinuation of the planting of *E. globulus* in the country.

No structured or detailed surveys have been conducted of the leaf pathogens affecting commercially grown *Eucalyptus* species in South Africa for more than two decades. The recent discovery of the non-native leaf pathogen, *T. destructans*, in the country has highlighted the need to investigate the current situation regarding leaf pathogens of *Eucalyptus* in South Africa. It is highly possible that there are unreported leaf pathogens which may in future result in significant losses to the forestry industry. Timely detection of

such pathogens will allow the reduction of losses as early management actions may be implemented.

The aims of the research reported on in the following chapters of this dissertation were: (1) to survey commercial *Eucalyptus* plantations in the major plantation forestry areas of the eastern part of South Africa (KwaZulu-Natal, Mpumalanga and Limpopo Provinces) for the presence of leaf diseases. Particular focus was placed on sub-tropical species and hybrids of *Eucalyptus* as previous surveys focused mainly on cold tolerant species and (2) to conduct studies to better understand the biology of *Teratosphaeria destructans*, with particular focus on studies to investigate optimal conditions for its growth, germination and infection.

TABLE 1: *Eucalyptus* leaf pathogens on a global scale: with a complete distribution and host list

Order	Causal Pathogen	*Eucalyptus* host	Disease description	Distribution	References
		Fungal leaf pathogens			
Amphisphaeriales	*Bartalinia terricola*	*E. exerta, E. tereticornis, E. urophylla*	Leaf spots	India	Luke and Devi 1979
	Seimatosporium brevilatum	*E. globulus, E. nitens, E. regnans*	Leaf spots	Australia	Swart and Griffiths 1974, Marks *et al*. 1982
	Seimatosporium eucalypti	*E. globulus , E. maculata, E. nitens, E. regnans, E. smithii,*	Leaf spots	Australia, South Africa	Swart 1982b, Crous *et al*. 1990
	Seimatosporium falcatum	*E. delegatensis, E. dives, E. radiata, E. regnans*	Leaf spots	Australia	Shoemaker 1964, Marks *et al*. 1982
	Seimatosporium fusisporum	*E. nitens, E. polyanthemos, E. regnans*	Leaf spots	Australia	Swart and Griffiths 1974, Marks *et al*. 1982, Swart 1982b
	Seimatosporium lichenicola	*E. globulus*	Leaf spots	USA	Gibson 1975
Asterinales	*Blastacervulus eucalypti*	*E. obliqua,E. robertsonii*	Leaf spots	Australia	Swart 1988, Cheewangkoon *et al*. 2009, Giraldo *et al*. 2017
	Blastacervulus robbenensis	*Eucalyptus* sp.	Leaf spots	Cyprus, South Africa	Crous *et al*. 1994, Crous *et al*. 2007b, Cheewangkoon *et al*. 2012
Botryosphaeriales	*Botryosphaeria ribis*	*E. camaldulensis, E. cladocalyx, E. globulus, E. grandis, E. nitens*	Leaf blight	Argentina, Brazil, India, Iran, Madagascar, Mauritius, South Africa, Uruguay	Grossenbacher and Duggar 1911, Punithalingam and Holliday 1973, Morgan-Jones and White 1987, Sinclair *et al*. 1987, Crous *et al*. 1989b
	Dothiorella eucalypti	*E. globulus*	Leaf spots	Portugal	Gibson 1975
	Guignardia citricarpa	*E. grandis*	Leaf spots	Malaysia, South Africa	Gibson 1975

	Microdiplodia microsporella	*E. globulus*	Leaf blight	Mauritius	Allescher 1901
	Phyllosticta eucalypti	*E. globulus*	Leaf spots	Algeria, Australia, Denmark, Portugal, Spain, USA	Ellis and Everhart 1897, Thüm 1879, Gibson 1975
Cantharellales	*Rhizoctonia solani*	*E. grandis, E. tereticornis*	Leaf blight	India	Kühn 1858
Capnodiales	*Alysidiella eucalypti*	*E. dunnii*	Leaf spots	Uruguay, South Africa	Crous and Wingfield 2006, Cheewangkoon and Crous 2012
	Alysidiella kleinziense	*E. dunnii*	Leaf spots	South Africa	Crous and Pretorius 2007
	Alysidiella parasitica	*E. dunnii*	Leaf spots	South Africa	Summerell *et al*. 2006
	Alysidiella suttonii	*E. dunnii*	Leaf spots	Cyprus	Cheewangkoon and Crous 2012
	Amycosphaerella africana	*E. viminalis*	Leaf spots	South Africa	Quaedvlieg & Crous,
	Aulographina eucalypti	*E. andrewsii, E. botryoides, E. delegatensis, E. elata, E. fastigata, E. ficifolia, E. fraxinoides, E. globulus subsp. maidenii, E. globulus subsp. globulus, E. macathurrii, E. muelleriana, E. nitens, E.obligua, E. pilularis, E. regnans, E. resinfera, E. saligna, E. stellulata*	Leaf spots	Australia, Hawaii, New Zealand, South Africa	Doige 1950, Doige *et al*. 1953, Cooke and Massee 1960, Idczak 1975; Dick 1982, Marks *et al.* 1982, Wall and Keane 1984, Lundquist and Baxter 1985, Swart 1986b, Swart 1988
	Davisoniella eucalypti	*E. marginata*	Leaf spots	Australia	Swart 1988
	Mycosphaerella delegatensis	*E. delegatensis, E. obliqua*	Leaf spots	Australia	Park and Keane 1984
	Mycosphaerella fori	*E. grandis*	Leaf spots	South Africa	Hunter *et al*. 2004
	Mycosphaerella marskii	*E. camaldulensis, E. cloeziana, E. dunnii, E. globulus* x *E. camaldulensis E. grandis, E. grandis* x *E. camaldulensis, E. grandis* x *E. resinifera, E. globulus, E. nitens,*	Leaf spots	Australia, South Africa	Carnegie and Keane 1994, Hunter *et al*. 2004
	Mycosphaerella martinae	*E. globulus*	Leaf spots	Australia	Hansford 1954
	Mycosphaerella swartii	*E. delegatensis, E. dives, E. obliqua, E. radiata*	Leaf spots	Australia, New Zealand	Dick 1982, Park and Keane 1982a, Park and Keane 1984, Swart and

				Walker 1988
Mycosphaerella walkeri	*E. cladocalyx, E. globoidea, E. globulus, E. gomphocephala, E. nitens, E. polyanthemo*	Leaf spots	Australia, Chile, Colombia, Ecuador, New Zealand, Portugal	Gibson 1975, Fripp and Forrester 1981, Dick 1982, Park and Keane 1982a, Park and Keane 1984, Sinclair *et al*. 1987, Swart and Walker 1988
Pallidocercospora heimii	*E. dunnii, E. oblique, E. urophylla*	Leaf spots	Madagascar, Uruguay	Bouriquet and Crous 1995, Perez et al. 2009b
Parapenidiella tasmaniensis	*E. globulus* x *E. nitens*	Leaf blight	Australia	Crous *et al*. 1989
Pseudocercospora acerosa	*E. baxteri, E. nitens, E. verrucata*	Leaf spots	New Zealand	Braun and Dick 2002
Pseudocercospora crousii	*E. dendromorpha, E. fastigata, E. muelleriana, E. pilularis, E. regnans, E. stenostoma*	Leaf spots	New Zealand	Braun and Dick 2002
Pseudocercospora eucalyptuorum	*E. botryoides, E. delegatensis, E. dendromorpha, E. fastigata, E. fraxinoides, E. globoidea, E. globulus, E. nicholii, E. nitens, E. obliqua, E. ovata, E. radiata, E. regnans*	Leaf spots	New Zealand	Crous *et al*. 1989
Pseudocercospora pseudobasitruncata	*E. nitens*	Leaf spots	New Zealand	Braun and Dick 2000
Pseudocercospora subulata	*E. nitens*	Leaf spots	New Zealand	Yuan *et al*. 2000
Sonderhenia eucalypticola	*E. fraxinoides, E. globulus subsp. globulus, E. nicholii, E. nitens, E. sideroxylon*	Leaf spots	New Zealand	Swart and Walker 1988
Sonderhenia eucalyptorum	*E. delegatensis, E. elata, E. fastigata, E. fraxinoides, E. globoidea, E. johntonii, E. leucoxylon, E. maculate, E. muelleriana, E. oblique, E. regnans, E. viminalis*	Leaf spots	New Zealand	Swart and Walker 1988
Teratosphaeria ambiphylla	*E. globulus*	Leaf spots	Australia	Maxwell *et al*. 2003

Teratosphaeria australiensis	*E. ficifolia*	Leaf blight	Australia	Sutton 1974
Teratosphaeria cryptica	*E. alba, E. bridgesiana, E. camaldulensis, E. cinerea, E. cloeziana, E. cordata, E. crenulata, E. dalrympleana delegatensis, E.dendromorpha, E. diversicolor, E. dunnii, E. fastigata, E. fraxinoides, E. glonulus, E. globulus subsp. maidenii, E. globulus* x *E. nitens, E. grandis* x *E. camaldulensis, E. gunnii, E. macarthuri, E. nitens, E. obliqua, E. ovate, E. regnans, E. saligna*	Crinkle leaf, leaf spots	Australia, New Zealand	Hansford 1956, Ganapti 1979
Teratosphaeria destructans	*E. camaldulensis, E. grandis, E. grandis* x *E. urophylla, E. urophylla*	Leaf spots, leaf blight, shoot and tip death, defoliation	China, Indonesia, Laos, Thailand, Timor, South Africa, Vietnam	Wingfield *et al*. 1996, Old *et al*. 2003a, Burgess *et al*. 2006, Barber et al. 2012, Greyling *et al*. 2016
Teratosphaeria eucalypti	*E. aggregata, E. alba, E. albens, E. botryoides, E. bridgesiana, E. camaldulensis, E. camphora, E. cinerea, E. crebra, E. cypellocarpa, E. fastigata, E. ficifolia, E. globulus, E. grandis, E. gunnii, E. largiflorens, E. longiflora, E. moluccana, E. nitens, E. nitens* x *nobilis, E. obliqua, E. ovate, E. paniculata, E. pauciflora, E. platypus, E. punctata, E. regnans, E. robusta, E. rudis, E. saligna, E. stellulata, E. tereticornis, E. viminalis*	Leaf blight, leaf spots, defoliation, shoot blight	Argentina, Australia, Brazil, India, Italy, New Zealand, Peru, Paraguay, Taiwan, Zaire	Cooke 1889, Dick 1982, Gadgil and Dick 1983, Park and Keane 1984, Miller *et al*. 1992, Park *et al*. 2000, Carnegie 2007b
Teratosphaeria molleriana	*E. globulus, E. grandis, E.*	Leaf spots	Africa, Brazil, Europe,	Lindau 1897, Doige 1950, Doige *et*

	maidenii, *E. resinifera*		Portugal, South Africa, USA	*al*. 1953, Gibson 1975, Lundquist and Baxter 1985, Park and Kean 1982b, Park and Kean 1984
Teratosphaeria nubilosa	*E. bicostata* , *E. botryoides*, *E. bridgesiana*, *E. camaldulensis*, *E. cypellocarpa*, *E. dunni*, *E. globulus*, *E. grandis*, *E. grandis x E. resinifera*, *E. gunnii*, *E. johnstonii*, *E. nitens*, *E. pilularis*, *E. quadrangulata*, *E. urophylla* x E. *globulus*, *E. viminalis*,	Leaf spots, defoliation, leaf blotch	Australia, Brazil, Ethiopia, Kenya, New Zealand, Portugal, South Africa, Spain, Tanzania, Uruguay, Zambia	Cooke 1893, Hansford 1956, Dick 1982, Cheah and Hartill 1987, Carnegie and Keane 1994, Crous 1998, Crous *et al*. 2004a, Maxwell 2004, Milgate *et al*. 2005a, Gezahgne *et al*. 2006; Carnegie 2007b; Hunter *et al*. 2008
Teratosphaeria ovata	*E. cladocalyx*, *E. dives*, *E. lehmannii*, *E. leucoxylon*, *E. macrorhyncha*, *E. obliqua*	Leaf spots	Australia, New Zealand, South Africa	Swart 1986a, Crous *et al*. 1988, Wingfield 1987
Teratosphaericola pseudoafricana	*E. globulus*	Leaf spots	Zambia	Crous *et al*. 2006d
Teratosphaeria pseudoeucalypti	*E. botryoides*, *E. camaldulensis*, *E. grandis*, *E. grandis* x *E. camaldulensis*, *E. grandis x E. tereticornis*, *E. globulus*, *E. macarthurii*, *E. maidenii*, *E. tereticornis*	Leaf blight, defoliation	Australia	Andjic *et al*. 2010
Teratosphaeria pseudonubilosa	*E. globulus*	Leaf spots	Australia	Perez *et al*. 2014
Teratosphaeria stellenboschiana	*Eucalyptus sp.*	Leaf spots	South Africa	Crous *et al*. 2007a
Teratosphaeria suberosa	*E. agglomerata*, *E. cloeziana*, *E. dunnii*, *E. globulus*, *E. grandis*, *E. grandis* x *E. camaldulensis*, *E. laevopinea*, *E. moluccana*, *E. muelleriana*, *E. nitens*, *E. nitens* x *E. nobilis*, *E. punctata*, *E. saligna*, *E. tereticornis*, *E. viminalis*	Leaf spots	Australia, Brazil, Colombia, Indonesia, New Zealand	Crous *et al*. 1993a, Balmelli *et a*l. 2004, Alfenas *et al*. 2009

	Teratosphaeria suttonii	*E. aggregata, E. ampifolia, E. aromaphloia, E. benthamii, E. camaldulensis, E. cephalocarpa, E. cinerea, E. cordata, E. cypellocarpa, E. dalrympleana subsp. dalrympleana, E. glaucescens, E. globoidea, E. globulus, E. globulus subsp. globulus, E. globulus subsp. maidenii, E. grandis, E. gunnii, E. kitsoniana, E. macarthurii, E. nicholii, E. nitens, E. obliqua, E. ovata, E. perriniana, E. sideroxylon, E. urnigera, E. viminalis subsp. viminalis.*	Leaf spots, defoliation	Argentina, Australia, Bhutan, Brazil, China, Ethiopia, Hong Kong, India, Indonesia, Italy, Madagascar, Malawi, Myanmar, New Zealand, Philippines, Taiwan, Tanzania, USA, Zambia South Africa	Hansford 1957, Crous and Wingfield 1997, Knipscheer *et al*. 1990, Nichol *et al*. 1992, Brown 2000, Park *et al*. 2000, Old *et al*. 2003, Carnegie 2007b
	Teratosphaeria toledana	*Eucalyptus sp.*	Leaf spots	Spain	Crous *et al*. 2004b
	Teratosphaeria velox	*E. miniata*	Leaf spots	Australia	Crous *et al*. 2009a
	Teratosphaeria verrucosa	*E. cladocalyx*	Leaf spots	South Africa	Crous *et al*. 2009a
Chaetosphaeriales	*Codinaea septata*	*E. grandis*	Leaf spots	Brazil, South Africa	Sutton and Hodges 1975; Crous *et al*. 1990
Diaporthales	*Apoharknessia insueta*	*E. grandis, E. pellita, E. robusta*	Leaf spots	Brazil, Mauritius	Orian 1933, Crous 1993, Lee *et al*. 2004
	Harknessia eucalypti	*E. globulus, E. grandis, E. maidenii, E. nitens, E. trabuti, E. viminalis*	Leaf and shoot blight	Europe , Italy, Portugal, South Africa, USA , Zambia	Cooke 1881, Gibson 1975, Sutton 1971b, Crous *et al*. 1989a, Crous *et al*. 1989b
	Harknessia fumaginea	*E. pilulans*	Shoot tip blight	Australia, Brazil	Sutton 1975, Sutton and Alcorn 1975
	Harknessia globosa	*E. globulus, E. grandis*	Leaf spots	New Zealand, South Africa	Sutton 1971, Crous *et al*. 1989a, Crous *et al*. 1989b
	Harknessia hawaiiensis	*E. grandis, E. robusta*	Leaf spots	Brazil, Hawaii, Zimbabwe	Sutton 1971b, Gibson 1975

	Harknessia uromycoides	*E. globulus, E. odoratus, E. scarbo, E. viminalis*	Leaf spots	Argentina, Australia, Portugal, South Africa, Spain, USA	Doidge 1950, Doige *et al*. 1953, Sutton 1971b; Gibson 1975
	Coniella africana	*E. nitens*	Leaf spots	South Africa	Crous 1990
	Coniella eucalyptigena	*E. brassiana*	Leaf spots	Malaysia	Wingfield 2014
	Coniella eucalyptorum	*E. phylla, E. grandis, E. grandis x E. tereticornis, E. grandis x E. urophylla, E. camaldulensis ssp. stimulata*	Spots, principally on older leaves	Australia, Brazil, Chile, Indonesia, Mexico, Vietnam	Thu and Gibbs 1999, Van Niekerk *et al*. 2004, Wingfield 2011
	Coniella fusiformis	*E. pellita*	Spots, principally on older leaves.	Australia	Alvarez *et al*. 2016
	Coniella paracastaneicola	*Eucalyptus sp.*	Spots, principally on older leaves	Australia	Alvarez *et al*. 2016
	Coniella quercicola	*Eucalyptus sp.*	Spots, principally on older leaves	Indonesia	Wingfield 2011
	Coniella wangiensis	*Eucalyptus sp.*	Spots, principally on older leaves	Australia	Crous and Summerrell 2012
	Phomopsis eucalypti	*E. exerta, E. grandis, E. tereticornis, E. urophylla*	Leaf spots	India	Zerova 1940
Dothidiales	*Pachysacca eucalypti*	*E. diversifolia, E. rostrata, E. viminalis*	Leaf spots	Australia	Sydow 1930
	Pachysacca pusilla	*E. regnans, E. rossii, E. botryoides, E. fastigata, E. delegatensis, E. viminalis*	Leaf spots	Australia, New Zealand	Swart 1982
	Pachysacca samuelii	*E. goniocalyx, E. obliqua, E. odorata, E. radiata, E. rostrata, E. sieberi*	Leaf spots	Australia	Swart 1982
Erysiphales	*Erysiphe cichoracearum*	*E. creba, E. globulus, E. porosa, E. viridis*	Leaf spots, malformation of leaves and shoots	Britain , California, New Zealand, USA	De Candolle 1805, Boesewinkel 1981
	Podosphaera aphanis	*E. albens, E. diversifolia*	Powdery leaf patches	Australia, New Zealand, Japan	Boesewinkel 1981, Braun and Takamatsu 2000, Cunnington *et al*. 2003, Tanda and Hirose 2003
	Podosphaera macularis	*E. gunnii, E. nitens, E. perriniana*	Powdery leaf patches	Germany	Braun and Takamatsu 2000

	Podosphaera pannosa	*E. camaldulensis, E. moluccana, E. albens, E. globulus, E. gunii, E. maidenii*	Powdery leaf patches, leaf malformation	Argentina, Australia, Brazil, Denmark, Italy, Korea, Poland, Portugal, South Africa	De bary 1870, Grasso 1948, Glasscock and Rosser 1958, Spaulding 1961, Gibson 1975, Boesewinkel 1981, Crous *et al*. 1989, Cunnington *et al*. 2003, Delhey *et al*. 2003, Cho *et al*. 2016,
Helotiales	*Botrytis cinerea*	*E. alba, E. botrioides, E. citriodora, E. globulus, E. rostrata, E, tereticornis*	Leaf spots, damping off. Leaf blight, die-back, grey mould	Argentina, Brazil, South Africa	Abrahao, 1948, Hepting 1971, Raggi, 1947, Wingfield 1987
	Calonectria aciculata	*E. urophylla x E. grandis*	Leaf spots, leaf and shoot blight	China	Li *et al*. 2017
	Calonectria braviensis	*E. urophylla*	Leaf spots, leaf and shoot blight	Vietnam	Pham *et al*. 2019
	Calonectria brasiliensis	*E. grandis*	Leaf spots, leaf and shoot blight	Brazil	Lombard *et al*. 2010a
	Calonectria crotalariae	*E. camaldulensis, E. grandis, E. saligna, E. rudis, E. robusta, E. tereticornis*	Leaf spots, leaf and shoot blight	USA	Bell and Sobers 1966
	Calonectria crousiana	*E. grandis*	Leaf spots, leaf and shoot blight	China	Chen *et al*. 2011
	Calonectria cylindrospora	*E. alba, E. citriodora, E. grandis, E. ma culata, E. saligna*	Leaf spots, leaf and shoot blight	Brazil	Morgan 1892
	Calonectria eucalypti	*E. grandis*	Leaf spots, leaf and shoot blight	Indonesia	Lombard *et al*. 2010b
	Calonectria floridana	*E. grandis, E.robusta, E. rudis, E. saligna*	Leaf spots, leaf and shoot blight	USA	Gibson 1975
	Calonectria foliicola	*E. urophylla* x *grandis*	Leaf spots, leaf and shoot blight	China	Lombard *et al*. 2015
	Calonectria fujianensis	*E. grandis*	Leaf spots, leaf and shoot blight	China	Chen *et al*. 2011
	Calonectria lauri	*E. globulus*	Leaf spots, leaf and shoot blight	Brazil, India, Kenya, Malaysia	Boedijn and Reitsma 1950, Figueiredo and Cruz 1963, Reddy 1974, Mohanan 1982
	Calonectria microconidialis	*E. urophylla x grandis*	Leaf spots, leaf and	China	Lombard *et al*. 2015

			shoot blight		
	Calonectria macroconidialis	*E. grandis*	Leaf spots, leaf and shoot blight	South Africa	Crous *et al*. 1993, Crous *et al*. 1999
	Calonectria multiseptata	*E. grandis*		Indonesia	Crous *et al*. 1998
	Calonectria pauciramosa	*E. grandis, E. dunnii, E. urophylla x grandis*	Leaf spot, leaf and shoot blight	Australia, Brazil, China, Italy, South Africa, South Africa, Spain, Vietnam, USA	Polizzi and Crous 1999, Schoch et al. 1999, Schoch *et al*. 1999, Koike and Crous 2001, Crous *et al*. 2002, Lombard *et al*. 2010a, Lombard *et al*. 2010d, Chen *et al*. 2011, Lombard *et al*. 2015
	Calonectria pteridis	*E. grandis, E. robusta*	Leaf spots, leaf blight	Brazil, USA	Wolf 1926, Ferreira *et al*. 1995, Fernandes *et al*. 2016
	Calonectria quinqueseptata	*E. robusta*	Leaf spots	Brazil, India, Indonesia, Malaysia, Mauritius	Peerally 1973, Pitkethley 1976, Lanier 1986, Lombard *et al*. 2010a
	Calonectroa reteaudii	*E. camaldulensis*	Leaf spots, leaf blight, seedling blight, shoot blightlarge nectrotic lesions-leaf blotch	Australia, India, Laos, Madagascar, Mauritius, Vietnam	Booth 1966, Peerally 1974d, Pitkethley 1976, Sharma and Mohana 1982, Crous and Swart 1995, Booth *et al*. 2000, Barber *et al*. 2012
	Calonectria seminaria	*E. urophylla x grandis*	Leaf spots, leaf blight	China	Lombard *et al*, 2015
	Cylindrocladiella camelliae	*E. grandis*	Leaf spots	South Africa	Boesewinkel 1982
	Cylindrocladiella lageniformis	*Eucalyptus sp.*	Leaf spots	Brazil, South Africa	Crous and Wingfield 1993
	Cylindrocladiella peruviana	*Eucalyptus sp.*	Leaf spots	South Africa	Boesewinkel 1982
	Trimmatostroma bifarium	*E. delegatensis, E. fastigata, E. fraxinoides, E. nitens, E. obliqua, E. regnans, E. sieberi*	Leafspots	New Zealand	Dick 1983, Gadgil and Dick 1983
	Trimmatostroma excentricum	*E. delegatensis, E. fastigata, E. obliqua, E. regnans, E. pauciflora subsp. Niphophila, E. sieberi*	Leaf spots	Fiji, New Zealand	Sutton and Ganapathi 1978, Dick 1982, Park and Keane 1982a
Microstromatales	*Quambalaria eucalypti*	*E. dunnii, E. globulus, E. grandis, E. grandis* x *E.*	Leaf spots, leaf and shoot blight, branch	Australia, Brazil, China, South Africa, Portugal	Wingfield *et al*. 1993, Simpson 2000, Alfenas *et al*. 2001b, Pegg *et*

		camaldulensis, E. longirostrata, E. microcorys, E. nitens, E. saligna x *maidenii*	and stem cankers		*al*. 2008, Chen *et al*. 2017
	Quambalaria rugosae	*E. rugosa*	Leaf spots	Australia	Crous *et al*. 2019
	Quambalaria simpsonii	*E. urophylla* x *E. grandis*	Leaf spots	China	Cheewangkoon 2009
	Quambalaria tasmaniae	*Eucalyptus sp.*	Leaf spots	Australia	Crous *et al*. 2019
Mycrothyriales	*Microthyrium eucalypti*	*E. delegatensis, E. fastigata, E. globulus, E. polyanthemos, E. regnans*	Leaf blight	USA	Hennings 1901
Myriangiales	*Elsinoë eucalypti*	*E. delegatensis*	Dark maroon leaf spots	Brazil, New Zealand	Hansford 1954
Phacidiales	*Ceuthospora innumera*	*E. regnans*	Leaf spots	Australia	Massee 1899
Phyllachorales	*Corynespora cassiicola*	*E. grandis, E. grandis x E. globulus, E. grandis x E. urophylla,*	Spots and leaf blight	Brazil, India, Portugal	Wei 1950, Wilson and Rema Devi 1966, Reis *et al*. 2014
	Hendersonia eucalyptina	*E. globulus*	Leaf spots	Portugal	Gibson 1975
	Ophiodothella longispora	*E. goniocalyx*	Leaf spots	Australia	Swart 1982
	Rehmiodothis eucalypti	*E. cloeziana, E. delegatensis*	Leaf spots	Australia	Swart 1987
	Rehmiodothis inaequali	*E. cloeziana, E. delegatensis*	Leaf spots	Australia	Swart 1987
Rust pathogens					
Pucciniales	*Austropuccinia psidii*	*E. botryoides, E. camaldulensis, E. citriodora, E. cladocalyx, E. cloeziana, E. deglupta, E. dunnii, E. globulus, E. grandis, E. nitens,E. pellita, E. pilularis, E. punctata, E. robusta, E. saligna, E. tereticornis*	Leaf spots, leaf and shoot blight, shoot death	Brazil, India, Uruguay	Winter 1884, De Castro *et al*. 1983, Dianese *et al*. 1984, Ferreira 1983, Gibson 1975, Telechea *et al*. 2003
	Phakopsora myrtacearum	*E. cloeziana, E. grandis, E. nitens*	Small leaf spots on older leaves	Kenya, Mozambique, South Africa	Maier *et al*. 2015
Rhytismatales	*Rhytisma eucalypti*	*E. diversifolia*	Leaf spots	Australia	Hennings 1901
Xylariales	*Bgadiella eucalypti*	*E. globulus*	Leaf spots	Australia	Crous et al. 2017a

	Bagadiella koalae	*E. globulus*	Leaf spots	Australia	Crous et al. 2011a
	Bagadiella lunata	*E. globulus*	Leaf spots	Australia	Cheewangkoon and Crous 2009
	Bagadiella victoriae	*E. globulus*	Leaf spots	Australia	Crous *et al*. 2011a
	Clypeophysalospora latitans	*E. deanei, E. tereticornis, E. bicostata*	Leaf spots	France, Portugal, South Africa	Swart 1981
	Neophysalospora eucalypti	*E. camaldulensis, E. cloeziana, E. globulus, E. grandis x E. camaldulensis, E. grandis x E. urophylla, E. urophylla, E. urophylla x maidenii, E. robusta, E. saligna*	Leaf spots	Indonesia, South Africa	Crous and Wingfield 2014
	Pestalotiopsis disseminata	*E. citriodora*	Leaf spots, leaf blight	India, South Africa	Steyaert 1948, Doige 1950, Doige *et al*. 1953, Lundquist and Baxter 1985
	Pestalotiopsis funerea	*E. globulus*	Leaf spots , leaf	Africa, Asia, Australia, Europe, South Africa, USA	Steyaert 1949, Mordue 1976, Upadhyay and Dwivedi 1977, Upadhyay and Arora 1980, Upadhyay 1981, Upadhyay 1984, Lanier 1986
	Phaeothyriolum microthyroides	*E. botryoides, E. delegatensis, E. fastigata, E. ficifolia, E. fraxinoides, E. johnstonii, E. maculata, E. nitens, E. quadrangulata*	Leaf spots, leaf blotch	New Zealand	Park and Keane 1982
	Phomopsis eucalypti	*E. exerta, E. grandis, E. tereticornis, E. urophylla*	Leaf spots	India	Zerova 1940
Bacterial leaf pathogens					
Enterobacteriales	*Pantoea agglomerans*	*E. grandis, E. urophylla, E. grandis x E. globulus*	Bacterial blight and die-back	Brazil	Ewing and Fife 1972
	Pantoea ananatis	*E. camaldulensis x E. deglupta, E. cloeziana, E. dunni, E. globulus, E. globulus subsp. maidenii, E. grandis, E. grandis, E. dunni, E. nitens, E. smithii, E.*	Bacterial blight and die-back	South Africa, Uruguay	Coutinho *et al*. 2002, Telechea *et al*. 2003

		grandis x *E. camaldulensis, E. grandis, E. urophylla, E. robusta, E. saligna, E. smithii, and E. urophylla x maidenii*			
	Pantoea deleyi	*E. brassica, E. globulus* x *E. grandis*	Bacterial blight and die-back	Uganda	Brady *et al*. 2009
	Pantoea eucalypti	*E. camaldulensis, E. dunnii*	Bacterial blight and die-back	Uruguay, South Africa	Brady *et al*. 2009
	Pantoea rodasii	*E. robusta, E. saligna*	Bacterial blight and die-back	Colombia	Brady *et al*. 2012
	Pantoea rwandensis	*E. saligna, E. brassica,*	Bacterial blight and die-back	Rwanda	Brady *et al*. 2012
	Pantoea vagans	*E. robusta, E. saligna, E. dunnii*	Bacterial blight and die-back	Argentina, Colombia, Thailand, Uganda, Uruguay	Brady *et al*. 2009
	Pantoea wallisii	*E. globulus x E. grandis*	Bacterial blight and die-back	South Africa	Brady *et al*. 2012
Pseudomonadales	*Pseudomonas cichorii*	*E. grandis*	Bacterial blight	Brazil, Argentina, Paraguay, Uruguay	Pomella *et al*. 1995
Xanthomonadales	*Xanthomonas axonopodis*	*E. camaldulensis, E. cloeziana, E. globulus, E. grandis x E. urophylla, E. robusta, E. saligna, E. urophylla x E. maidenii,*	Leaf spots and bacterial blight	Brazil	Reis *et al*. 1996
	Xanthomonas axonopodis pv. *eucalypti*	*E. camaldulensis x E. deglupta, E. grandis*	Leaf spots and bacterial blight	Thailand	Pothiluk *et al*. 2013
	Xanthomonas vasicola	*E. grandis*	Leaf spots and bacterial blight	South Africa	Coutinho *et al*. 2015

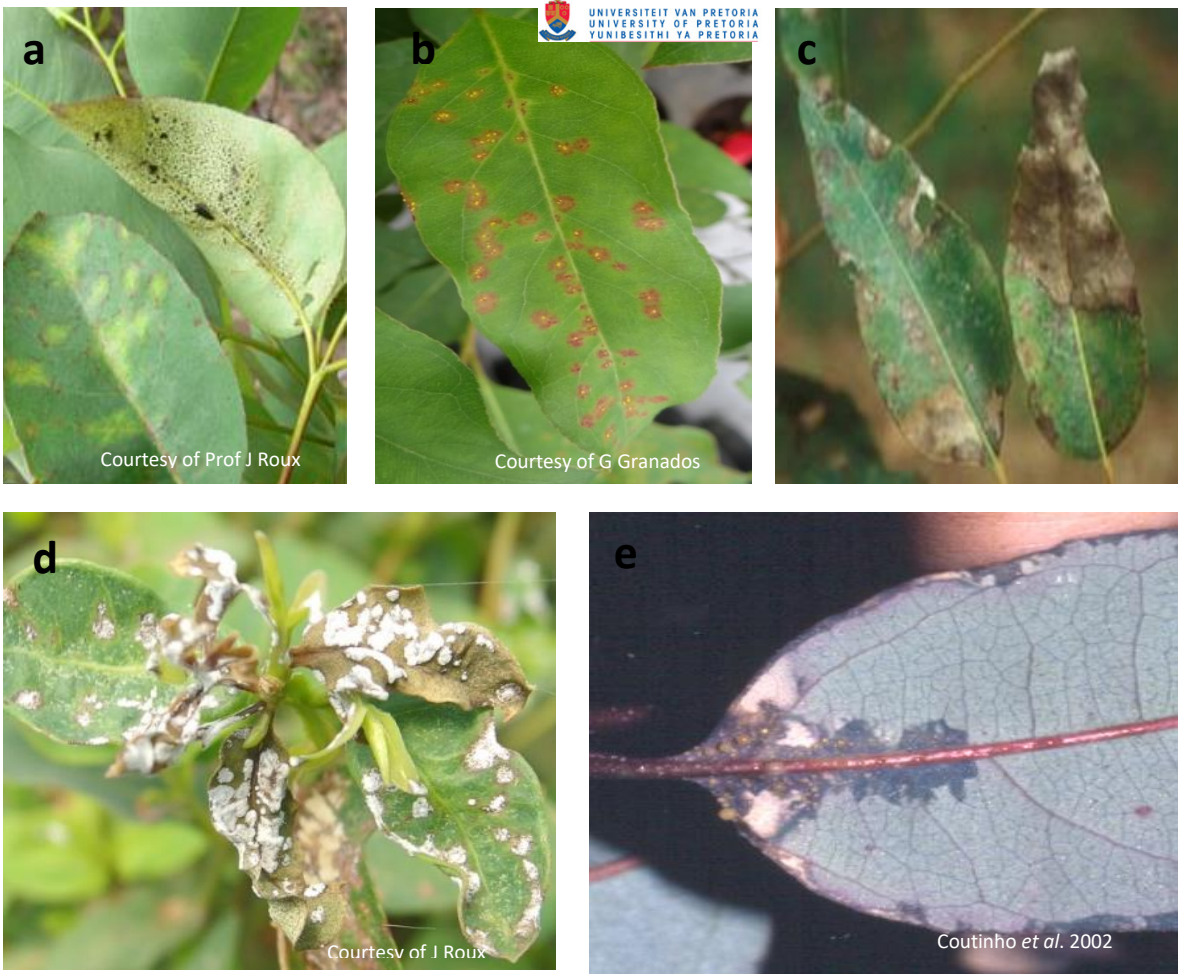

Fig. 1. Disease symptoms found on *Eucalyptus*. (a) Teratosphaeria Leaf Disease caused by *Teratosphaeria destructans*, (b) Rust disease caused by *Austropuccinia psidii*, (c) Calonectria Leaf Blight caused by *Calonectria* species, (d) Quambalaria leaf and shoot blight caused by *Quambalaria eucalypti*, (e) Bacterial blight caused by *Pantoea ananatis.*

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CHAPTER 2

IDENTIFICATION OF *EUCALYPTUS* LEAF PATHOGENS IN SOUTH AFRICA

Abstract: Species of *Eucalyptus* have been successfully grown in South Africa for more than a century. However, the number of pests and pathogens affecting these trees continue to increase. This results from accidental introductions into the country as well as from movements from native environments to non-native environments. The last detailed surveys of leaf diseases affecting *Eucalyptus* species in commercial plantations in eastern South Africa were last conducted in the 1980s and there may be new leaf diseases affecting trees in this region. To monitor the current situation regarding leaf diseases of these trees in commercial plantations in South Africa, surveys were conducted in the major *Eucalyptus* growing regions of the KwaZulu-Natal, Mpumalanga and Limpopo Provinces. Diseases and their causal agents were identified based on symptoms, morphology and DNA sequence data. Based on leaf symptoms observed in the field and nursery leaf diseases were identified as representing *Aulographina* sp., bacterial leaf blight, *Calonectria* sp., *Coniella* sp., Destructans leaf blight, Phakopsora rust, powdery mildew, Teratosphaeria epicoccoides leaf spot and Quambalaria leaf blight. The support of DNA sequence data further confirmed the identification of *Ca. pauciramosa*, *Q. eucalypti* and *T. destructans* as causal agents. The causal agents of Phakopsora rust, Coniella leaf spot, bacterial blight and powdery mildew could not be identified and thus these diseases are therefore reported based only on field leaf symptoms. The findings of this study give a preliminary update regarding the current status of leaf pathogens in the major plantation regions of South Africa. It highlights the importance of Destructans leaf blight and Quambalaria leaf blight diseases and presents the first report of a Calonectria leaf disease in a commercial plantation in the country.

INTRODUCTION

Eucalyptus is the largest tree genus within the *Myrtaceae*, with over 700 species grown worldwide (Grattapaglia *et al.* 2012). Most *Eucalyptus* species are native to Australia, while a few are native to Indonesia, the Philippines and Papua New Guinea (Paine *et al.* 2011, Grattapaglia *et al.* 2012, Thornhill *et al.* 2019). However, due to the fact that *Eucalyptus* species are hardy and adaptable to a wide range of climates (Eldridge *et al.* 1994, Luzar 2007), they are widely planted as a hardwood timber species and as a source of biomass (fuels), wood (dyes, gold prospecting, musical instruments and sugar production), oil (antiseptics, cosmetics and food supplements), and pulp (paper and viscose) (Turnbull 2000, Wingfield *et al.* 2008, Bennett 2010, Jun *et al.* 2013).

Species of *Eucalyptus* have been established in many countries where they are planted as exotic plantation trees (Huang *et al.* 2014). In South Africa, *Eucalyptus* plantations cover approximately 520 000 ha (43.7 % of total forestry plantations), with KwaZulu-Natal being the major *Eucalyptus* growing region (Godsmark and Oberholzer 2018). Currently, in South Africa, the most commonly planted species of *Eucalyptus* include *E. dunnii*, *E. macarthurii*, *E. nitens*, *E. smithii* and the hybrids *E. grandis* x *E. nitens* and *E. grandis* x *E. urophylla* (Dovey 2014, Vecchio *et al.* 2016, Musengi and Archibald 2017, Van den Berg 2017, Hirsch *et al.* 2019). The rapid growth and success of *Eucalyptus* trees in South Africa, which is a non-native environment, has been ascribed to the absence of their natural pests and pathogens from their native environment (Keane and Crawley 2002, Wingfield 2003, Wingfield *et al.* 2008).

The number of pests and pathogens affecting *Eucalyptus* trees in South Africa, and globally, has increased significantly over the past two decades (Wingfield 2003, Wingfield *et al.* 2008, Hurley *et al.* 2016, Burgess and Wingfield 2017). This is as a result of the accidental movement of pests and pathogens of *Eucalyptus* species from their native habitat into non-native habitats. It is in these non-native habitats where the trees are being grown in genetically uniform stands, causing outbreaks and significant economic losses (Burgess and Wingfield 2017). The spread of pathogens into areas where they have not been recorded before has been attributed to globalization and the movement of plants and plant products between countries (Palm 1999, Wingfield 1999, Wingfield *et al.* 2001, Burgess and Wingfield 2002). Adding to the pest and pathogen pressure on *Eucalyptus* in their new countries are insects and fungi that undergo a host-

range expansion from the native vegetation (Slippers *et al.* 2005, Wingfield *et al.* 2008). A summary of the major plantation forestry diseases in South Africa, published in 2012 lists five insect pests and 21 microbial pathogens of *Eucalyptus* and its hybrids in the country (Roux *et al.* 2012). This number has since increased with pathogen reports of *Teratosphaeria destructans* (Greyling *et al.* 2016), *Phakopsora myrtacearum* (Maier *et al.* 2016) and most recently, *Ceratocystis* wilt (Roux *et al.* 2020). Additional insect pest reports of *Glycaspis brimblecombei* (Tribe 2015), *Ophelimus maskelli* and *Spondyliaspis* cf. *plicatuloides* (Bush *et al.* 2016) have also increased the number of insect pests on these trees in South Africa.

Globally, several *Eucalyptus* leaf pathogens have been reported to cause diseases that can result in shoot die-back, defoliation and even tree death. These leaf pathogens include species of *Calonectria*, *Coniella*, *Pantoea*, *Quambalaria*, *Teratosphaeria* as well as *Austropuccinia psidii* (Walker and Bertus 1971, Crous *et al.* 1989, Coutinho *et al.* 1998, Old *et al.* 2003, Crous *et al.* 2004b, Crous *et al.* 2019). In South Africa, several *Eucalyptus* leaf pathogens have been reported (Crous *et al.* 1988, Crous *et al.* 1989a, Crous *et al.* 1989b, Crous *et al.* 1989c, Crous *et al.* 2019). However, the majority have to date not resulted in significant economic losses in plantations. The exception is *Teratosphaeria nubilosa*, the causal agent of the disease previously known as Mycosphaerella Leaf Blotch (MLB) (Lundquist *et al.* 1987). In forestry nurseries, *Calonectria pauciramosa* (Crous 2002), *Pantoea ananatis* (Coutinho *et al.* 2002), *Sphaerotheca pannosa* (powdery mildew) (Viljoen *et al.* 1992) and *Quambalaria eucalypti* (Wingfield *et al.* 1993) cause economic losses by causing leaf drop and plant death.

Since increased global movement of pathogens increase the risk of new introductions of *Eucalyptus* leaf pathogens into countries, it is necessary to continuously survey plantations and nurseries for new introductions and/or host jumps from the native vegetation. It has been more than twenty years since the last focused survey of leaf diseases affecting *Eucalyptus* species and their hybrids have been conducted in the major plantation growing regions of South Africa. In this period the genotype composition of South African plantations has also changed significantly. Some of the new genotypes may be more susceptible to previously unknown pests and diseases, such as the recently detected Destructans leaf blight disease (Greyling *et al*. 2016). The aim of this research chapter was therefor to conduct a survey of *Eucalyptus* species and their hybrids in the major plantation growing areas of the KwaZulu-Natal, Limpopo and Mpumalanga Provinces.

This was done in order to investigate the current status of leaf diseases of commercially planted *Eucalyptus* species in these plantation growing regions. Causal agents of leaf diseases were identified based on symptoms, spore morphology and where cultures could be obtained, the use of DNA sequence data.

MATERIALS AND METHODS

Field and nursery surveys

Surveys were conducted in *Eucalyptus* nurseries and plantations in the KwaZulu-Natal (March 2016, April 2016 and February 2017), Limpopo (March 2016) and Mpumalanga (April 2016 and February 2017) provinces (Table 1). Surveys were mostly limited to compartments with trees younger than two years of age in order to facilitate visualization of symptoms as well as sample collection. Trees older than two years were typically in excess of 4 meters tall, making sampling of leaves difficult. To obtain samples, a haphazard sampling strategy was applied. Multiple rows of trees/seedlings were randomly selected per visited compartment/nursery and plants were inspected for the presence of leaf diseases. At least 30 minutes was spent inspecting trees per compartment, with at least two groups of observers at each time. Approximately ten symptomatic leaves, representing each symptom type, in reach of the collector's height, were sampled from at least three trees per disease type, per compartment. Leaves bearing disease symptoms were collected and placed in brown paper bags.

Pathogen isolation and identification

Leaf disease symptoms and spore morphology

All isolates obtained in this study were tentatively designated into different taxa based on their morphological characteristics as observed from leaf disease symptoms and spores on the leaf samples. This was done through microscopic examination of the fungal structures from leaf samples. Using a sterile needle, fungal structures were picked up and mounted onto a glass slide in distilled water. The morphological characteristics were studied using a Nikon Eclipse compound microscope. Representative isolates of each morphological group (based on spores), symptom type and location were selected for species level identification using DNA sequencing.

Culture morphology

Sampled leaves were examined under a dissecting microscope (ZEISS Stereo Discovery.v8) and primary isolations were made from fruiting structures visible in disease symptoms on the leaves. Where fruiting structures were dry and not sporulating leaf material was placed in moist chambers to induce sporulation. Spores of the observed fungi were aseptically picked up from the leaf surfaces using a sterilized needle and transferred to 2% Malt Extract Agar (20g/L Biolab malt extract, 15g/L, Biolab agar, Midrand, South Africa) containing 100mg/L streptomycin (Biotech laboratories, Midrand) (MEAS). Streptomycin was used to reduce bacterial contamination of fungal cultures. MEAS plates were incubated at room temperature (25°C) until fungal growth was observed. To obtain pure cultures, single hyphal tips and/or spore drops were transferred to fresh MEAS using a dissecting microscope and incubated under the same conditions.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from purified fungal cultures using Prepman ULTRA™ Sample Preparation Reagent (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions. The ITS and 5.8S gene region was sequence for all isolates as this is the generally accepted fungal barcording region. Additional gene regions were thereafter sequenced, where required, and based on the appropriate gene regions as indicated in published scientific literature for each fungal genus. Multiple gene regions for each morphological group were amplified and sequenced using previously published primers (Table 2) and slightly adjusted protocols (Aghayeva *et al*. 2004, van Niekerk *et al*. 2004, Andjic *et al*. 2007a and Liu *et al*. 2015). The protocols were adjusted according to the specific annealing temperatures used for each gene region per morphological group as listed in Table 2. The PCR reaction mixtures used to amplify the different gene regions consisted of 0.3 units MyTaq™ DNA Polymerase (Bioline, Inc, USA), 5 x MyTaq™ PCR Buffer (Bioline, Inc, USA), 10 mM of each primer and approximately 30 ng fungal genomic DNA in a final reaction volume of 25 µL.

Amplified PCR fragments were purified using ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA, USA) as per manufacturer's specifications and sequenced in both directions using the same primers used for amplification. Fragments were sequenced using the Big Dye Terminator sequencing kit v.3.1 (Applied Biosystems, USA) according to the manufacturer's specifications. The sequencing PCR condition in all cases was

1 cycle of 5 s at 96 °C, followed by 28 cycles of 10 s at 96 °C, 5 s at the specific annealing temperature (Table 2) and 4 min at 60 °C and holding at 4 °C. Sequences were obtained by running samples on an ABI PRISM™ 3100 DNA sequencer (Applied Biosystems, USA).

DNA sequence assembly, editing and BLASTN

DNA sequences were edited and consensus sequences obtained using BioEdit sequence alignment editor v. 7.2 (Alzohairy 2011). The sequences were then used in BLASTN searches on the National Center for Biotechnology Information (NCBI) GenBank database (http://www.ncbi.nlm.nih.gov). Sequences for all gene regions were used. Firstly, ITS gene sequences were used to obtain an indication of the genus and closest species identify of each isolate. Secondly, blast searches were used to assist in selection of sequences of highly similar isolates for inclusion in phylogenetic analyses. Results of the Blast searches were used to further trace sequences of type specimens and published sequence data sets with which to compare sequences obtained in the current study.

Phylogenetic analysis

Sequence data sets were compiled based on published literature for each genus (Van Niekerk 2004, Andjic *et al*. 2016, Lombard *et al*. 2016, Chen *et al*. 2017, Liu *et al*. 2020) by obtaining sequences for all appropriate type species from GenBank. These sequences were aligned using the default settings on MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server) (Katoh & Standley 2013) and edited manually in MEGA v. 7 (Kumar et al. 2016). Both the individual and combined trees were considered in the analysis. For the individual trees, the BLASTN test was done as a first level of indication and to build a dataset. For each genus, the individual gene regions were first analyzed separately and then a concatenated (combined) data set was constructed and analyzed for confirmation. The most appropriate nucleotide substitution model for each data set was obtained by implementing the Bayesian information criterion selection strategy in jModeltest v. 2.1.5 (Posada 2008). To determine whether the individual data sets can be combined, the partition homogeneity test (PHT) was applied using PAUP 4.0b10 (Swofford 2003). Maximum likelihood analyses using custom models obtained with JModelTest were conducted using raxmlGUI v. 1.5 (Michalak 2012). Confidence levels for the tree branch nodes were determined with 1000 bootstrap replicates. Depending on the genus, trees were rooted to

various species (*Curvicladiella cignea*, *Readeriella angustia* and *Sympodiomycopsis kandeliae*) based on the close relationships with the ingroup. The resulting trees were illustrated using MEGA v. 7 (Kumar *et al*. 2016) and edited on Microsoft PowerPoint 2010.

RESULTS

Field and nursery surveys

A total of 350 trees/seedlings were sampled for identification of the causal agents of observed diseases. Disease symptoms observed in nurseries were characteristic of bacterial blight, powdery mildew and Quambalaria leaf and shoot blight. Leaf disease symptoms encountered in the plantations were typical of *Coniella* and *Phakopsora* species, while symptoms of *Aulographina*, *Calonectria* and *Teratosphaeria* species were observed in both nurseries and plantations. Symptoms caused by *Teratosphaeria* species included those typical of Destructans leaf blight and T. epicoccoides leaf spot. No large-scale or serious leaf diseases, resulting in leaf blight and death, were encountered during the field surveys in commercial plantations in KwaZulu-Natal, Limpopo or Mpumalanga.

The exception was Destructans leaf blight which was found to cause leaf blight and shoot tip death on single trees in compartments in Mkhondo and Whiteriver, Mpumalanga and in Cramond and Hilton and Pietermaritzburg, KwaZulu-Natal. This disease was characterized by pale green to yellow lesions (Fig. 1A) with black pycnidia exuding spores in black cirri on the abaxial leaf surfaces (Fig. 1B). In severe cases, the lesions covered almost the entire leaf and the pycnidia were moist.

Symptoms resembling leaf spots caused by *T. epicoccoides* were characterized by brown to black spore masses covering the abaxial leaf surface (Fig. 1C) with purple leaf spots which expanded and coalesced on the adaxial surface of the leaves (Fig. 1D). Infection was found mostly on older leaves. These were found in the nurseries in Pietermaritzburg and in plantations at Cramond and Mtubatuba, KwaZulu-Natal and Magoebaskloof, Limpopo.

Aulographina leaf spot symptoms were found on seedlings in the nurseries in Pietermaritzburg, KwaZulu-Natal and on plantation trees in Louis Trichardt, Limpopo and in Barberton,

Mpumalanga. These symptoms were characterized by spots that had raised, crusty centers with black fruiting bodies scattered in them (Fig. 1E). In severe cases, the leaves had a slightly disfigured appearance.

Calonectria leaf spot was characterized by large brown lesions with microscopic white spore masses and aerial mycelia on the margin of lesions (Fig. 1F). Leaves that were severely infected by *Calonectria* had blight covering almost the entire leaf surface (Fig. 1G). This disease was observed in the KwaZulu-Natal province from the nurseries in Hilton and Pietermaritzburg and the plantations at Cramond.

Coniella leaf spot was characterized by pale brown to black fruiting bodies which were formed in concentric rings on both sides of the leaves (Fig. 1H). Infections were found mostly on older leaves which in severe conditions were partially curled up at the tip. In the KwaZulu-Natal province, it was found in a nursery at Wartburg and plantations near Cramond. In Louis Trichardt, Limpopo and in Amsterdam, Mpumalanga provinces, it was found in the plantations.

Quambalaria leaf and shoot blight disease was characterized by the occurrence of white masses of conidia (Fig. 1I). Infection spread from the young leaves to the petioles in severe cases. In KwaZulu-Natal, it was found on nursery seedlings at Cramond and in plantations Wartburg Wartburg and Pietermaritzburg respectively.

Bacterial blight disease was characterized by water soaked lesions scattered on the leaf surface as well as slight leaf distortion (Fig. 1J). In severe cases, infection spread from the petiole resulting in necrotic areas. This disease was observed on seedlings in a nursery at Wartburg, KwaZulu-Natal province.

Powdery mildew was characterized by powdery white patches that covered the leaves (Fig. 1K). In severe conditions, the white mycelia covered the entire leaf surface. It was observed on seedlings in the nurseries in Wartburg and Pietermaritzburg, KwaZulu-Natal and in Ngodwana, Mpumalanga province.

Symptoms of *Phakopsora* rust were observed on plantation trees at Cramond, KwaZulu-Natal. These symptoms were characterized by small leaf spots with cream to white coloured spore

masses in them (Fig. 1L). Infection was mostly found on older leaves which in severe cases resulted in necrotic areas.

Pathogen isolation and identification

Leaf disease symptoms and spore morphology

Based on leaf disease symptoms eight diseases and their causal agents were observed on *Eucalyptus* plants. These were diseases caused by species of *Aulographina*, *Calonectria* (Fig. 2A), *Coniella* (Fig. 2B), *Phakopsora*, *Quambalaria* (Fig. 2D) and *Teratosphaeria* (Fig. 2E). Additionally, powdery mildew and bacterial blight disease were also observed. During the microscopic examinations of fungal structures associated with leaf spot and leaf blight symptoms *Pestalotiopsis* species (Fig 2C) were also observed associated with the above mentioned fungi. Based on leaf disease symptoms and spore morphology, only those considered economically important pathogens were identified further. At the time of the identification process in 2016, *Pestalotiopsis* was not considered of economic importance on *Eucalyptus* therefore no further identification work was done for this pathogen.

Culture morphology

Attempts to obtain fungal cultures from all observed symptoms were not successful. Furthermore, due to the obligate nature of some pathogens, e.g. the causal agents of powdery mildew and Phakopsora rust, cultures were not obtained for these pathogens. Fungal cultures representing five different leaf disease symptoms were successfully obtained. These were for Coniella, T. epicoccoides leaf spot, Calonectria, Teratosphaeria and Quambalaria leaf and shoot blight. Only fungi considered of higher importance, based on observed symptoms in the field and literature were selected. As a result, isolates from symptoms typical of *T. epicoccoides* leaf spot were not included in further studies. Based on the above, a total of 158 isolates were obtained from which 41 (17 for *Calonectria*, 7 for *Coniella*, 12 for *Teratosphaeria* and 5 for *Quambalaria*) representative isolates based on location and host were selected. These were divided into four different morphology groups. Stock cultures, from which sub-cultures were made for further study, were maintained on 2% MEA in a refrigerator for the duration of the study.

Cultures obtained from *Calonectria* symptoms could be grouped into two morphological groups. Isolates in Group 1 were characterized by white to brown mycelium with feathery irregular brown margins and white spore masses on the upper surface (Fig. 3A) becoming brown in the center of the reverse side of the plate (Fig. 3B). Group 2 had feathery irregular mycelium at the edges and yellow spore masses on the upper surface (Fig. 3C) and were white on the reverse side of the plate (Fig. 3D). Some of these group 2 cultures had irregular mycelia with white spore masses on top (Fig. 3E) and white-yellow on the reverse side of the plate (Fig. 3F).

Cultures of *Coniella* species grouped in a single morphological group and were characterized by fluffy white aerial mycelium that was spreading from the center and alternating with black spore masses arranged in concentric zones (Fig. 3G). On the reverse side of the plate they were white with black concentric zones (Fig. 3H). The culture morphology seemed to be as expected based on the literature.

Quambalaria cultures grouped in a single morphological group and were characterized by white mycelium on the upper surface (Fig. 3I). On the reverse side of the plate they had a dark appearance (Fig. 3J). The culture morphology of these fungi was as expected, based on the literature.

Cultures that were obtained from Destructans leaf blight were grouped in a single morphological group and characterized by pink cultures with black spore masses on the upper surface (Fig. 3K). On the reverse side of the plate they had an olive-black appearance (Fig. 3L). Based on literature, the morphology of these cultures was as expected.

DNA sequence assembly, editing and BLASTN

Out of a total of 41 isolates, 34 sequences were obtained for the ITS and 5.8S gene region. This gene region was sequenced for the *Coniella*, *Quambalaria*, *Teratosphaeria* isolates as well as one of the two morphological groups obtained from Calonectria leaf blight symptoms. Average band sizes for all genera ranged from 485 to 910 bp and were all as expected based on published literature.

DNA sequences obtained for the *tub2* gene region were a total of 29, of which all were successful. This gene region was sequenced for the two *Calonectria* morphological groups,

Coniella and *Teratosphaeria* isolates. The average band sizes for all genera ranged from 470 - 670 bp in size, as expected from literature.

For the *cmdA* and *his3* gene regions, a total of 17 sequences each were successfully obtained. These gene regions were sequenced only for the two *Calonectria* morphological groups. The average band sizes for the *cmdA* gene region ranged from 430 -750 bp, while those from the *his3* gene region ranged from 420 - 550 bp, as expected from published literature.

A total of seven sequences were successfully obtained for the LSU gene region. This gene region was sequenced only for the *Coniella* isolates. The average band sizes ranged from 848 - 870 bp, which was not as expected because according to published literature, LSU sequences of *Coniella* have approximately 1 200 bp.

For the *tef1* gene region, a total of 36 sequences were successfully obtained. This gene region was sequenced for the two *Calonectria* morphology groups, *Coniella* and *Teratosphaeria* isolates. The average band sizes for all genera ranged from 250 - 500 bp, as expected from published literature.

The *tub2*, *cmdA*, *his3* and *tef1* BLASTN search results for the two *Calonectria* morphological groups (17 isolates) revealed that seven of these isolates were closely related to *Calonectria* species (Table 3) with 100% similarity. BLASTN results for *his3* showed that the closest match for the remaining ten isolates was *Cylindrocladiella* species although similarity percentages were only approximately 90% (Table 3). However, BLASTN search results of the ITS and 5.8S, *tub2*, *cmdA* and *tef1* gene regions revealed that those ten isolates were instead close to those of *Chlonostachys* species (Table 3) with 100% sequence similarities. The discrepancy in BLASTN results and subsequent checking of cultures of the 10 isolates showed that they were contaminated and these 10 isolates could not be studied further.

BLASTN search results of ITS and 5.8S, LSU and *tef1* for the seven *Coniella* isolates revealed that they were all closely related to species of *Pestalotiopsis* (Table 3). The similarity percentages for all three gene regions were approximately 100%. These results were not as expected because these isolates were thought to be those of *Coniella* species.

Both BLASTN search results of the five *Quambalaria* isolates and those of the 12 *Teratopshaeria* isolates had expected results. ITS and 5.8S BLASTN search results revealed that all five *Quambalaria* isolates were closely related to species of *Quambalaria* (Table 4) with 99.74 - 100% sequence similarities. The ITS and 5.8S, *tub2* and *tef1* BLASTN search results revealed that all 12 *Teratosphaeria* isolates were related to *Teratosphaeria* species (Table 3) with 99.20 - 100% sequence similarities.

Phylogenetic analyses

Calonectria morphological group 1

The data sets of the first *Calonectria* morphological group had a total of 81 taxa for *tub2*, 72 for *his3*, 78 for *cmdA* and 83 taxa for *tef1* (Table 4). The PHT generated a P-value of 0.36 (P > 0.05), indicating that the data sets were combinable. All four gene regions amplified for this group (*tub2*, *cmdA*, *his3* and *tef1*) were combined into a concatenated data set to improve phylogenetic accuracy. The combined sequence dataset contained 67 taxa, including the outgroup taxa represented by *Curvicladiella cignea*. The GTR+I+G substitution model was best suited for all gene regions and the combined data set from which five phylogenetic trees were constructed.

The maximum likelihood phylogenies of the four gene regions as well as the combined data set grouped the isolates collected in this study with sequences belonging to *Ca. pauciramosa*, residing in the *Ca. candelabrum* species complex (ML bootstrap support = 70-94) (Fig. 4 - 8). These bootstrap values, which are above 70, provide good confidence that these isolates belong to the *Ca. pauciramosa* species. These results are as expected with the support of previously obtained results.

Calonectria morphological group 2

The data sets for the second *Calonectria* morphology group had a total of 53 taxa for the *his3* gene (Table 5). Datasets of the other gene regions (ITS and 5.8S, *tub2*, *cmdA* and *tef1*) were not included in the phylogenetic analyses due to their BLASTN results being different from those of *his3*. Subsequently, a combined data set could not be obtained due to conflicting results of unrelated species from all gene regions. The ingroup dataset was separated into 18 different

species and the isolates collected in this study. The outgroup taxa was represented by *Curvicladiella cignea*. The GTR+I+G model was best suited for the data set.

Phylogenetic tree of the *his3* gene region grouped the isolates collected in this study in a separate clade (ML bootstrap support = 99) (Fig 9). The bootstrap value gives very good confidence that the isolates collected in this study do not belong to the genus *Cylindrocladiella*. The BLASTN results obtained for the other gene regions confirmed that these isolates do not belong in either *Calonectria* or *Cylindrocladiella genera* nor were they related to them.

Coniella species

Phylogenetic analyses of the isolates that were thought to be those of the species of *Coniella* were not conducted. This was based on previously obtained BLASTN results which strongly suggest that there is no relation to the species of *Coniella*. Upon checking cultures, it was confirmed that these were contaminated with a *Pestalotiopsis* sp. based on spore morphology.

Quambalaria species

The dataset of the isolates that were related to *Quambalaria* species had a total of 86 taxa for the ITS and 5.8S gene region (Table 6) including the outgroup taxa. The ingroup taxa were

separated into seven major clades representing *Q. coyrecup*, *Q. cyanescens*, *Q. eucalypti*, *Q. pitereka*, *Q. purpurascens*, *Q. pusilla* and *Q. simpsonii*. The outgroup taxa were represented by *Sympodiomycopsis kandeliae*. The GTR+G model suited the ITS gene region best. There was no combined dataset because only one gene region (ITS and 5.8S) was sequenced; therefore, one phylogenetic tree was constructed. The phylogenetic tree grouped the isolates collected in this study with sequences belonging to *Q. eucalypti* (ML bootstrap support = 99) (Fig. 10). These bootstrap values, which are above 70, provide good confidence that these isolates belong to the *Q. eucalypti* species. These results support the previously obtained results.

Teratosphaeria species

The data sets of the isolates that were related to the species of *Teratosphaeria* had a total of 52 taxa for all gene regions (ITS and 5.8S, *tub2* and *tef1*) including the outgroup taxa (Table 7). The PHT generated a P-value of 0.31 (P > 0.05), indicating that the data sets are combinable. All three gene regions were combined into a concatenated data set that was constructed in order to improve the phylogenetic accuracy. The combined sequence also consisted of 52 taxa including the outgroup taxa. The ingroup taxa were separated into seven major clades representing *T. destructans*, *T. eucalypti*, *T. novaehollandiae*, *T. pseudoeucalpti*, *T. tiwiana* and *T. viscidus*. The outgroup taxa were represented by *Readeriella angustia*. The GTR+I+G model was best suited for all gene regions and the combined data set from which four phylogenetic trees were constructed.

The phylogenetic trees of the three gene regions as well as the combined datasets grouped the isolates collected in this study with sequences belonging to *T. destructans* (ML bootstrap support = 81 - 92) (Fig. 11 - 14). These bootstrap values, which are above 70, provide good confidence that these isolates are *T. destructans*. These results are consistent with previously obtained results.

DISCUSSION

This study provides results of a survey to investigate the current status of leaf diseases of commercially planted *Eucalyptus* species in the major plantation growing regions in South Africa. Use was made of symptoms observed on trees in the plantation and nursery, the morphology of fungal spores observed on the symptoms, culture morphology and DNA

sequence data to identify the putative fungal leaf pathogens. This is the first survey of *Eucalyptus* leaf pathogens to be conducted in the major *Eucalyptus* growing regions in South Africa (KwaZulu-Natal, Limpopo and Mpumalanga) since the 1980s. To our knowledge, this study is the first to report Calonectria leaf blight disease from *Eucalyptus* plantations in South Africa, where it was previously only found in nurseries. This study also revealed that *T. destructans* is no longer limited to the Zululand region of the KwaZulu-Natal Province, where it was initially discovered.

Symptoms observed in the field and nursery represented nine different leaf diseases namely Aulographina, Coniella, T. epicoccoides leaf spot; Calonectria, Destructans, Quambalaria leaf blight; bacterial blight, powdery mildew and Phakopsora rust. Spore and culture morphology indicated the following putative causal agents associated with leaf symptoms: *Calonectria* sp., *Coniella* sp., *Quambalaria* sp. and T. *destructans*. DNA sequence data further supported the identification of *Ca. pauciramosa*, *Q. eucalypti* and *T. destructans* as causal agents. *Pestalotiopsis* sp. and *Chlonostachys* sp. were also isolated but are contaminants of some of the leaf symptoms observed. Culture and/or DNA sequence data for symptoms associated with causal agents of Phakopsora rust, Coniella leaf spot, bacterial blight and powdery mildew could not be obtained and these diseases are therefore reported based only on field symptoms.

Culture morphology and initial BLASTN results suggested the presence of two fungal genera associated with Calonectria leaf blight symptoms. The majority of putative *Calonectria* isolates were identified as *Ca. pauciramosa*. This was supported by leaf symptoms, spore and culture morphology as well as the phylogenetic analyses of four gene regions and a combined dataset, with bootstrap support of >70. These isolates were obtained from *E. dunnii*, *E. grandis* x *E. nitens* and *E. grandis* x *E. urophylla* in plantations near Cramond and in a nursery in Hilton and in Pietermaritzburg, KwaZulu-Natal.

Calonectria pauciramosa resides in the *Ca. candelabrum* species complex which accommodates the largest number of *Calonectria* species, most of which are important pathogens that pose a serious threat to forest industries (Crous 2002, Lombard *et al.* 2010a, Lombard *et al.* 2011, Alfenas *et al.* 2015, Lombard *et al.* 2015a). Species residing in this species complex have a global distribution (Crous *et al.* 2013, Lombard *et al.* 2016, Liu *et al.* 2020). This study is the first, to our knowledge, to report Calonectria leaf blight disease from *Eucalyptus* plantations in South Africa.

BLASTN results of one gene region (*his3*) indicated the possible presence of a second genus associated with CLB on *Eucalyptus* species in this study, *Cylindrocladiella,* although with lower overall sequence similarity (90%)**.** BLASTN results of other gene regions sequenced (ITS and 5.8S, *tef1* and *tub2*) identified the same cultures as being a species of *Clonostachys* with higher sequence similarity (100%). Phylogenetic analyses of the *his3* gene region showed that these isolates group separately from *Cylindrocladiella* sequences, confirming the BLASTN results obtained with other gene regions. *Clonastachys* spp. are well-known mycoparasites (Rodríguez *et al.* 2011, Krauss *et al.* 2013, Nygren *et al.* 2018), suggesting that isolates of possible *Cylindrocladiella* obtained in this study were contaminated. The finding of the second genus could therefore not be confirmed.

Typical symptoms of Coniella leaf spot were observed on *E. cloeziana*, *E. dunnii*, *E. grandis* and *E. grandis* x *E. urophylla* in Wartburg and Cramond, KwaZulu-Natal, Louis Trichardt, Limpopo and Amsterdam, Mpumalanga. Based on examination of fungal spores from leaf samples during primary isolations, the cause of these symptoms were confirmed as being a species of *Coniella*. However, sequence results of ITS and 5.8S, LSU and *tef1* gene regions revealed that the cultures from which DNA was extracted represent a *Pestalotiopsis* species. This would indicate that during subsequent processes cultures became contaminated/mixed and DNA sequence data is, therefore, not available to confirm the species identity of the causal agent.

Quambalaria leaf spot symptoms were observed on *E. dunnii* and *E. grandis* x *E. nitens* in a nursery in Cramond and in Wartburg and in a plantation at Pietermaritzburg, KwaZulu-Natal Province. Isolates collected in this study were identified as *Q. eucalypti*. This was based on leaf symptoms, spore and culture morphology as well as on sequence data of the ITS and 5.8S gene regions with bootstrap support of > 70. These results confirm that *Q. eucalypti* is still the only *Quambalaria* species causing QLB in commercial nurseries and plantations South Africa.

Quambalaria eucalypti is an important species which causes Quambalaria leaf and shoot blight (QLB), a serious disease of *Eucalyptus* (Simpson 2000, Pegg *et al.* 2008). It was first reported from a clonal nursery causing leaf spots and shoot infections on *E. grandis* in KwaZulu-Natal (Wingfield *et al.* 1993). Since its first report from *E. grandis*, *Q. eucalypti* has spread to *E. nitens* in Mpumalanga (Roux *et al.* 2006). This, together with its movement from *Eucalyptus* nurseries to commercial plantations emphasizes its potential to cause QLB

across various *Eucalyptus* species regardless of tree age or climatic conditions. *Quambalaria eucalypti* is regarded as having the most extensive distribution (Crous *et al.* 2019), making it a very serious pathogen not only in South Africa but in other countries as well (Alfenas *et al.* 2001b, Zauza *et al.* 2003).

Leaf spots and blight symptoms typical of those caused by *Teratosphaeria* species represented the most common symptoms observed during the surveys. The symptoms broadly represented Destructans leaf blight disease and those typically caused by *T. epicoccoides*. As *T. destructans* is considered an economically important pathogen, isolates were identified to confirm causal agent. Andjic et al. (2016) highlighted the close relationship that exists among various *Teratosphaeria* species that are considered to be cryptic as they are morphologically identical. The analyses of the phylogenetic species recognition of the *Teratosphaeria* species collected in this study was thus a necessary exercise because the morphological species recognition approach on its own could have resulted in possible missing cryptic species in this group.

Leaf symptoms, spore and culture morphological characters, as well as DNA sequence data and phylogenetic analyses, provided clear evidence that the causal agent of the symptoms were *T. destructans*. These isolates were obtained from *E. grandis*, *E. grandis* x *E. nitens* and *E. grandis* x *E. urophylla* in a nursery in Hilton, Wartburg and Pietermaritzburg, KwaZulu-Natal, in the plantations at Cramond and Mtubatuba, KwaZulu-Natal and in Mkhondo and Whiteriver, Mpumalanga.

Teratosphaeria destructans was first reported in South Africa in 2016 (Greyling *et al.* 2016), representing a recent introduction into the country. Initially, *T. destructans* was known only from Zululand, South Africa on *E. grandis* x *E. urophylla* hybrids (Greyling *et al.* 2016). Data from this study indicate that the pathogen is now present in all major *Eucalyptus* growing areas of South Africa and infects a number of *E. grandis* and *E. urophylla* hybrids in these areas. Its widespread occurrence indicates that it is capable of infecting multiple eucalypt genotypes, as well as occur in various climatic regions of the country. Its impact may thus be higher than initially suggested. Population structure data of this pathogen showed low genotypic diversity which suggest limited introduction events from an unknown source (Havenga *et al.* 2020). This is a result of the movement of infected plants through international trade (Andjic *et al.* 2019). Therefore, not moving plants around between

countries should be a priority to prevent the dissemination of different pathogen genotypes into new areas.

The findings of this study give a preliminary update regarding the current status of leaf pathogens on different *Eucalyptus* species from some of the major *Eucalyptus*-growing regions in South Africa. Due to the superficial sampling (not sampling all current *Eucalyptus* species and varieties, nor all plantation regions) and problems experienced with contamination of cultures it is highly likely that that there may still be unreported *Eucalyptus* leaf pathogens in South Africa. The presence or association of putative *Cylindrocladiella* species with Calonectria leaf blight symptoms also needs further investigation. More intensive surveys would be needed to identify other possible leaf pathogens. Regular monitoring is also necessary to reduce the spread of these pathogens to other *Eucalyptus* species or even to other provinces.

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Table 1. Collection of all isolates obtained from surveys conducted in KwaZulu-Natal, Limpopo and Mpumalanga provinces that are included in the phylogenetic analyses.

Sample no	Species/ Hybrid	Field/Nursery	Area
Isolates resembling species of *Calonectria*			
2479	*E. grandis* x *E.nitens*	Nursery	Hilton, KwaZulu-Natal
2481	*E. grandis* x *E.nitens*	Nursery	Pietermaritzburg, KwaZulu-Natal
2453	*E. grandis* x *E.nitens*	Nursery	Wartburg, KwaZulu-Natal
24372	*E. grandis*	Nursery	Howick, KwaZulu-Natal
2425	*E. grandis* x *E. urophylla*	Plantation	Cramond, KwaZulu-Natal
2433	*E. grandis* x *E. urophylla*	Plantation	Cramond, KwaZulu-Natal
2435	*E. grandis* x *E. urophylla*	Plantation	Cramond, KwaZulu-Natal
2440	*E dunnii*	Plantation	Cramond, KwaZulu-Natal
2445	*E dunnii*	Plantation	Cramond, KwaZulu-Natal
2436	*E dunnii*	Plantation	Cramond, KwaZulu-Natal
24106	*E. grandis x E. camaldulensis*	Plantation	Louis Trichardt, Limpopo
24141	*E. cloeziana*	Plantation	Louis Trichardt, Limpopo
24174	*E. grandis* x *E. urophylla*	Plantation	Mtubatuba, KwaZulu-Natal
24277	*E. grandis* x *E. urophylla*	Plantation	Mtubatuba, KwaZulu-Natal
24369	*E. grandis*	Plantation	Magoebaskloof, Limpopo
24451	*E. grandis*	Plantation	Mkhondo, Mpumalanga
24488	*E. grandis*	Plantation	Barberton, Mpumalanga
Isolates resembling species of *Coniella*			
2421	*E. grandis* x *E. nitens*	Nursery	Wartburg, KwaZulu-Natal
2429	*E. grandis* x *E. nitens*	Nursery	Wartburg, KwaZulu-Natal
2441	*E. dunnii*	Plantation	Cramond, KwaZulu-Natal
24106	*E. grandis* x *camaldulensis*	Plantation	Louis Trichardt, Limpopo
24116	*E. grandis*	Plantation	Louis Trichardt, Limpopo
24117	*E. grandis*	Plantation	Louis Trichardt, Limpopo
24338	*E. grandis*	Plantation	Barberton, Mpumalanga
Isolates resembling species of *Quambalaria*			

241	*E. dunnii*	Nursery	Cramond, KwaZulu-Natal
2412	*E. grandis* x *E. nitens*	Nursery	Wartburg, KwaZulu-Natal
2415	*E. dunnii*	Nursery	Wartburg, KwaZulu-Natal
2484	*E. grandis* x *E. nitens*	Plantation	Pietermaritzburg, KwaZulu-Natal
2485	*E. grandis* x *E. nitens*	Plantation	Pietermaritzburg, KwaZulu-Natal
Isolates resembling species of *Teratopshaeria*			
2413	*E. grandis* x *E. urophylla*	Nursery	Wartburg, KwaZulu-Natal
2488	*E. grandis* x *E. nitens*	Nursery	Hilton, KwaZulu-Natal
2447	*E. grandis* x *E. nitens*	Plantation	Cramond, KwaZulu-Natal
2496	*E. grandis* x *E. urophylla*	Plantation	Pietermaritzburg, KwaZulu-Natal
24163	*E. grandis* x *E. urophylla*	Plantation	Mtubatuba, KwaZulu-Natal
24193	*E. grandis*	Plantation	Mtubatuba, KwaZulu-Natal
24194	*E. grandis*	Plantation	Mtubatuba, KwaZulu-Natal
24197	*E. grandis*	Plantation	Mtubatuba, KwaZulu-Natal
24340	*E. grandis*	Plantation	Barberton, Mpumalanga
24341	*E. grandis* x *E. urophylla*	Plantation	Whiteriver, Mpumalanga
24421	*E. grandis*	Plantation	Mkhondo, Mpumalanga
24445	*E. grandis*	Plantation	Mkhondo, Mpumalanga

Table 2. Primers used per gene region for DNA amplification and sequencing

Genus name	Gene	Primer sequence	Primers	Annealing temperature (˚C)	Reference
Calonectria	Histone H3 (HIS3)	AGGTCCACTGGTGGCAAG AGCTGGATGTCCTTGGACTG	CYLH3F CYLH3R	55	Crous *et al*. (2004) Crous *et al*. (2004)
	Calmodulin (CAL)	GAGTTC AAGGAGGCC TTC TCCC TGRTCNGCCTCDCGGATCATCTC	CAL228 CAL2RD	55	Carbone and Kohn (1999) Groenewald *et al*. (2013)
	B-tubulin (BTUB)	AACATGCGTGAGATTGTAAGT CCTGGTACTGCTGGTACTCAG	T1 CYL TUB 1R	52	O'Donnell and Cigelnik (1997) Kroon *et al*. (2004)
	Translation elongation factor (TEF-1a)	CATCGAGAAGTTCGAGAAGG GGARGTACCAGTSATCATGTT	EF1-278F EF2	52	Carbone and Kohn (1999) O'Donnell *et al*. (1998)
Coniella	Internal transcribed spacer ITS	CTT GGTCAT TTAGAG GAA GTAA TCCTCCGCTTATTGATATGC	ITS 1F ITS 4	54	Gardes and Bruns (1993) White *et al*. (1990)
	Ribosomal large sub-unit	ACCCGCTGAACTTAAGC TACTACCACCAAGATCT	LR0R LR7	54	Rehner and Samuels (1994) Vilgalys and Hester (1990)
	Translation elongation factor (TEF-1a)	CATCGAGAAGTTCGAGAAGG TACTTGAAGGAACCCTTACC GGARGTACCAGTSATCATGTT	EF1-728F EF1-986R EF2	54	Carbone and Kohn 1999 O'Donnell *et al*. (1998) O'Donnell *et al*. (1998)
Cylindrocladiella	Histone H3 (HIS3)	AGGTCCACTGGTGGCAAG AGCTGGATGTCCTTGGACTG	CYLH3F CYLH3R	55	Crous *et al*. (2004) Crous *et al*. (2004)
	B-tubulin (BTUB)	AACATGCGTGAGATTGTAAGT CCTGGTACTGCTGGTACTCAG	T1 CYL TUB 1R	52	O'Donnell and Cigelnik (1997) Kroon *et al*.(2004)
	Translation elongation factor (TEF-1a)	CATCGAGAAGTTCGAGAAGG GGARGTACCAGTSATCATGTT	EF1-278F EF2	52	Carbone and Kohn (1999) O'Donnell *et al*. (1998)
	Internal transcribed spacer	CTT GGTCAT TTAGAG GAA GTAA	ITS 1F ITS4	56	Gardes and Bruns (1993) White *et al*. (1990)

Genus name	Gene	Primer sequence	Primers	Annealing temperature (°C)	Reference
	ITS	TCCTCCGCTTATTGATATGC			
Quambalaria	Internal transcribed spacer ITS	CTTGGTCATTTAGAGGAAGTAA TCCTCCGCTTATTGATATGC	ITS 1F ITS 4	52	Gardes and Bruns (1993) White *et al*. (1990)
Teratosphaeria	Internal transcribed spacer ITS	TCCGTAGGTGAACCTGCGG TCCTCCGCTTATTGATATGC	ITS 1 ITS 4	50	White *et al*. (1990) White *et al*.(1990)
	B-tubulin (BTUB)	AACATGCGTGAGATTGTAAGT ACCCTCAGTGTAGTGACCCTT GGC	T1 Bt2b	54	O'Donnell and Cigelnik (1997) Glass and Donaldson (1995)
	Translation elongation factor (TEF-1a)	CATCGAGAAGTTCGAGAAGG TACTTGAAGGAACCCTTACC	EF1-728F EF1-986R	52	Carbone and Kohn (1999) Carbone and Kohn (1999)

Table 3. BLASTN results of all isolates

Isolate	BLASTN result	Query cover	E-value	Percent identity	Gene regions
Isolates resembling species of Calonectria					
2425	*Calonectria pauciramosa*	100 %	0.0	99.76 %	***tub2***
	Calonectria pauciramosa	100 %	0.0	100 %	***cmdA***
	Calonectria pauciramosa	100 %	0.0	100 %	***his3***
	Calonectria pauciramosa	100 %	0.0	100 %	***tef1***
2433	*Calonectria pauciramosa*	100 %	0.0	100 %	***tub2***
	Calonectria pauciramosa	100 %	0.0	100 %	***cmdA***
	Calonectria pauciramosa	100 %	0.0	100 %	***his3***
	Calonectria pauciramosa	100 %	0.0	100 %	***tef1***
2435	*Calonectria pauciramosa*	100 %	0.0	100 %	***tub2***
	Calonectria pauciramosa	100 %	0.0	100 %	***cmdA***
	Calonectria pauciramosa	100 %	0.0	100 %	***his3***
	Calonectria pauciramosa	100 %	0.0	100 %	***tef1***
2440	*Calonectria pauciramosa*	100 %	0.0	100 %	***tub2***
	Calonectria pauciramosa	100 %	0.0	100 %	***cmdA***
	Calonectria pauciramosa	100 %	0.0	100 %	***his3***
	Calonectria pauciramosa	100 %	0.0	100 %	***tef1***
2445	*Calonectria pauciramosa*	100 %	0.0	100 %	***tub2***
	Calonectria pauciramosa	100 %	0.0	100 %	***cmdA***
	Calonectria pauciramosa	100 %	0.0	100 %	***his3***
	Calonectria pauciramosa	100 %	0.0	100 %	***tef1***
2479	*Calonectria pauciramosa*	100 %	0.0	100 %	***tub2***
	Calonectria pauciramosa	100 %	0.0	100 %	***cmdA***
	Calonectria pauciramosa	100 %	0.0	100 %	***his3***

	Calonectria pauciramosa	100 %	0.0	100 %	***tef1***
2481	*Calonectria pauciramosa*	100 %	0.0	100 %	***tub2***
	Calonectria pauciramosa	100 %	0.0	100 %	***cmdA***
	Calonectria pauciramosa	100 %	0.0	100 %	***his3***
	Calonectria pauciramosa	100 %	0.0	100 %	***tef1***
2436	*Clonostachys* sp.	100 %	4e-167	100 %	**ITS**
	Clonostachys pseudochroleuca	100 %	3e-164	99.38 %	***tub2***
	Clonostachys sp.	100 %	4e-167	100 %	***cmdA***
	Cylindrocladiella arbusta	98 %	2e-88	90.23	***his3***
	Cylindrocladiella pseudohawaiiensis *Clonostachys* sp.	100 %	0.0	100 %	***tef1***
2453	*Clonostachys* sp.	100 %	4e-167	100 %	**ITS**
	Clonostachys pseudochroleuca	100 %	3e-164	99.38 %	***tub2***
	Clonostachys sp.	100 %	4e-167	100 %	***cmdA***
	Cylindrocladiella arbusta	98 %	2e-84	90.23	***his3***
	Cylindrocladiella pseudohawaiiensis *Clonostachys* sp.	100 %	2e-180	99.92 %	***tef1***
24106	*Clonostachys* sp.	100 %	4e-167	100 %	**ITS**
	Clonostachys pseudochroleuca	100 %	3e-164	99.38 %	***tub2***
	Clonostachys sp.	100 %	4e-167	100 %	***cmdA***
	Cylindrocladiella arbusta	98 %	2e-84	90.23	***his3***
	Cylindrocladiella pseudohawaiiensis *Clonostachys* sp.	100 %	0.0	100 %	***tef1***
24141	*Clonostachys* sp.	100 %	4e-167	100 %	**ITS**
	Clonostachys pseudochroleuca	100 %	3e-164	99.38 %	***tub2***
	Clonostachys sp.	100 %	4e-167	100 %	***cmdA***
	Cylindrocladiella arbusta	98 %	1e-82	89.84 %	***his3***
	Cylindrocladiella pseudohawaiiensis *Clonostachys* sp.	100 %	0.0	99.92 %	***tef1***
24174	*Clonostachys* sp.	100 %	4e-167	100 %	**ITS**
	Clonostachys pseudochroleuca	100 %	3e-164	99.38 %	***tub2***
	Clonostachys sp.	100 %	0.0	100 %	***cmdA***

	Cylindrocladiella arbusta	98 %	2e-84	90.23	***his3***
	Cylindrocladiella pseudohawaiiensis *Clonostachys* sp.	100 %	0.0	100 %	***tef1***
24277	*Clonostachys* sp.	100 %	4e-167	100 %	**ITS**
	Clonostachys pseudochroleuca	100 %	3e-164	99.38 %	***tub2***
	Clonostachys sp.	100 %	4e-167	100 %	***cmdA***
	Cylindrocladiella arbusta	98 %	2e-84	90.23 %	***his3***
	Cylindrocladiella pseudohawaiiensis *Clonostachys* sp.	100 %	0.0	100 %	***tef1***
24372	*Clonostachys* sp.	100 %	4e-167	100 %	**ITS**
	Clonostachys sp.	100 %	3e-164	99.38 %	***tub2***
	Clonostachys sp.	100 %	4e-167	100 %	***cmdA***
	Cylindrocladiella arbusta	98 %	1e-81	89.41 %	***his3***
	Cylindrocladiella pseudohawaiiensis *Clonostachys* sp.	100 %	2e-180	99.92 %	***tef1***
24369	*Clonostachys* sp.	100 %	4e-167	100 %	**ITS**
	Clonostachys sp.	100 %	3e-164	99.08 %	***tub2***
	Clonostachys sp.	100 %	4e-167	100 %	***cmdA***
	Cylindrocladiella pseudohawaiiensis	98 %	2e-88	90.23	***his3***
	Clonostachys sp.	100 %	0.0	100 %	***tef1***
24451	*Clonostachys* sp.	100 %	4e-167	100 %	**ITS**
	Clonostachys pseudochroleuca	100 %	2e-161	98.77 %	***tub2***
	Clonostachys sp.	100 %	4e-167	100 %	***cmdA***
	Cylindrocladiella kurandica	98 %	2e-90	91.44 %	***his3***
	Clonostachys sp.	100 %	2e-176	98.97 %	**tef1**
24488	*Clonostachys* sp.	100 %	4e-167	100 %	**ITS**
	Clonostachys pseudochroleuca	100 %	0.0	99.38 %	***tub2***
	Clonostachys sp.	100 %	4e-167	100 %	***cmdA***
	Cylindrocladiella pseudohawaiiensis	98 %	5e-81	89.45 %	***his3***
	Clonostachys sp.	100 %	0.0	100 %	***tef1***
Isolates resembling species of Coniella					
2421	*Pestalotiopsis* sp.	100 %	0.0	100 %	**ITS**
	Pestalotiopsis sp.	100 %	0.0	100 %	**LSU**

	Pestalotiopsis sp.	98 %	0.0	98.80 %	***tef1***
2429	*Pestalotiopsis* sp.	100 %	0.0	100 %	**ITS**
	Pestalotiopsis sp.	100 %	0.0	100 %	**LSU**
	Pestalotiopsis sp.	98 %	2e-92	98.89 %	***tef1***
2441	*Pestalotiopsis* sp.	100 %	0.0	100 %	**ITS**
	Pestalotiopsis sp.	100 %	0.0	100 %	**LSU**
	Pestalotiopsis sp.	98 %	2e-90	100 %	***tef1***
24106	*Pestalotiopsis* sp.	100 %	0.0	100 %	**ITS**
	Pestalotiopsis sp.	100 %	0.0	100 %	**LSU**
	Pestalotiopsis sp.	98 %	0.0	100 %	***tef1***
24116	*Pestalotiopsis* sp.	100 %	0.0	100 %	**ITS**
	Pestalotiopsis sp.	100 %	0.0	100 %	**LSU**
	Pestalotiopsis sp.	100 %	0.0	100 %	***tef1***
24117	*Pestalotiopsis* sp.	100 %	0.0	100 %	**ITS**
	Pestalotiopsis sp.	98 %	0.0	98.99 %	**LSU**
	Pestalotiopsis sp.	98 %	0.0	98.89	***tef1***
24338	*Pestalotiopsis* sp.	100 %	0.0	100 %	**ITS**
	Pestalotiopsis sp.	98 %	0.0	98.93 %	**LSU**
	Pestalotiopsis sp.	98 %	2e-88	98.89 %	***tef1***
Isolates resembling species of Quambalaria					
241	*Quambalaria eucalypti*	100 %	0.0	100 %	**ITS**
2412	*Quambalaria eucalypti*	98 %	0.0	99.74 %	**ITS**
2415	*Quambalaria eucalypti*	100 %	0.0	99.74	**ITS**
2484	*Quambalaria eucalypti*	100 %	0.0	100 %	**ITS**
2485	*Quambalaria eucalypti*	100 %	0.0	100 %	**ITS**
Isolates resembling species of Teratosphaeria					
2413	*Teratosphaeria destructans*	100 %	0.0	100 %	**ITS**
	Teratosphaeria destructans	98 %	0.0	99.20 %	***tub2***
	Teratosphaeria destructans	98 %	0.0	99.80 %	***tef1***
2447	*Teratosphaeria destructans*	100 %	0.0	100 %	**ITS**
	Teratosphaeria destructans	98 %	0.0	99.80 %	***tub2***
	Teratosphaeria destructans	98 %	0.0	99.89 %	***tef1***
2488	*Teratosphaeria destructans*	100 %	0.0	100 %	**ITS**
	Teratosphaeria destructans	98 %	0.0	99.82 %	***tub2***
	Teratosphaeria destructans	100 %	0.0	99.92	***tef1***
2496	*Teratosphaeria destructans*	100 %	0.0	100 %	**ITS**
	Teratosphaeria destructans	98 %	0.0	99.82 %	***tub2***

	Teratosphaeria destructans	100 %	2e-99	100 %	***tef1***
24163	*Teratosphaeria destructans*	100 %	0.0	100 %	**ITS**
	Teratosphaeria destructans	98 %	0.0	99.81 %	***tub2***
	Teratosphaeria destructans	100 %	2e-90	100 %	***tef1***
24193	*Teratosphaeria destructans*	98 %	0.0	99.60 %	**ITS**
	Teratosphaeria destructans	98 %	0.0	99.82 %	***tub2***
	Teratosphaeria destructans	98 %	0.0	99.82 %	***tef1***
24194	*Teratosphaeria destructans*	100 %	0.0	100 %	**ITS**
	Teratosphaeria destructans	98 %	0.0	100 %	***tub2***
	Teratosphaeria destructans	98 %	0.0	100 %	***tef1***
24197	*Teratosphaeria destructans*	98 %	0.0	99.40 %	**ITS**
	Teratosphaeria destructans	98 %	0.0	99.82 %	***tub2***
	Teratosphaeria destructans	100 %	2e-92	100 %	***tef1***
24340	*Teratosphaeria destructans*	100 %	0.0	100 %	**ITS**
	Teratosphaeria destructans	98 %	0.0	100 %	***tub2***
	Teratosphaeria destructans	100 %	2e-99	100 %	***tef1***
24341	*Teratosphaeria destructans*	100 %	0.0	99.79 %	**ITS**
	Teratosphaeria destructans	98 %	0.0	99.81 %	***tub2***
	Teratosphaeria destructans	100 %	2e-90	100 %	***tef1***
24421	*Teratosphaeria destructans*	100 %	0.0	99.58 %	**ITS**
	Teratosphaeria destructans	98 %	0.0	99.80	***tub2***
	Teratosphaeria destructans	100 %	2e-99	99.51 %	***tef1***
24445	*Teratosphaeria destructans*	100 %	0.0	100 %	**ITS**
	Teratosphaeria destructans	97 %	0.0	99.82 %	***tub2***
	Teratosphaeria destructans	100 %	1e-101	99.52 %	***tef1***

Table 4. Collection details of *Calonectria* isolates included in the phylogenetic analyses including GenBank accession numbers for the four gene regions, β-tubulin (*tub2*), calmodulin (*cmdA*), histone (*his3*) and translation elongation factor 1α (*tef1*).

Species	Culture no. [a]	Substrate	Collector	Collected from	GenBank accession number			
					tub2	*cmdA*	*his3*	*tef1*
Ca. brassiana	CBS 134855[T]	Soil	AC Alfenas	Teresina, Piaui, Brazil	KM395969	KM396056	KM396139	KM395882
	CBS 134856	Soil	AC Alfenas	Teresina, Piaui, Brazil	KM395970	KM396057	KM396140	KM395883
Ca. candelabrum	CMW 31001[T]	*Eucalyptus* sp.	L Lombard	Amazonas, Brazil	FJ972427	GQ267368	GQ267246	GQ267246
	CPC 1675 CMW 31000	*Eucalyptus* sp.	L Lombard	Amazonas, Brazil	FJ972426	GQ267367	FJ972476	FJ972525
	CBS 125256[T] CMW 15216	*E. grandis*	L Lombard	Pichincha, Ecuador	GQ267228	GQ267440	GQ267277	GQ267348
	CBS 125257 CMW 15218	*E. grandis*	L Lombard	Pichincha, Ecuador	GQ267227	GQ267439	GQ267349	GQ267347
Ca. colhounii	CBS 29379[T]	*Camellia sinensis*	-	Bandung, Indonesia	DQ190564	GQ267373	DQ190639	GQ267301
	CBS 114704	*Arachis pintoi*	D Hutton	Australia	DQ190563	GQ267372	DQ190638	GQ267300
Ca. colombiana	CBS 115127[T]	Soil	PW Crous	La Selva, Colombia	FJ972423	GQ267455	FJ972442	FJ972492
	CBS 115638	Soil	PW Crous	La Selva, Colombia	FJ972422	GQ267456	FJ972441	FJ972491
Ca. eucalypti	CBS 125275[T] CMW 18444	*E. grandis*	MJ Wingfield	Sumatra, Indonesia	GQ267218	GQ267430	GQ267267	GQ267338
	CBS 125273 CMW 14890	*E. grandis*	MJ Wingfield	Sumatra, Indonesia	GQ267217	GQ267429	GQ267266	GQ267337
Ca. eucalypticola	CBS 134847[T]	*Eucalyptus* sp.	AC Alfenas	Minas Gerais, Brazil	KM395964	KM369051	KM396134	KM395877
	CBS 134846	*Eucalyptus* sp.	AC Alfenas	Bahia, Brazil	KM395963	KM396050	KM396133	KM395876
Ca. fujianensis	CBS 127201[T] CMW 27257	*E. grandis*	MJ Wingfield	Fujian, China	HQ285792	-	HQ285806	HQ285820
	CBS 127200 CMW 27254	*E. grandis*	MJ Wingfield	Fujian, China	HQ285791	-	HQ285805	HQ285819
Ca. glaebicola	CBS 134852[T]	Soil	AC Alfenas	Minas Gerais, Brazil	KM395966	KM396053	KM396138	KM395879
	CBS 134853	Soil	AC Alfenas	Tocantins, Brazil	KM395967	KM396054	KM396137	KM395880
Ca. indusiata	CBS 14436[T]	*Camellia sinensis*	-	Sri lanka	GQ267239	GQ267453	GQ267262	GQ267332
	CBS 114684	*Rhododendron* sp.	NE El-Gholl	USA	AF232862	GQ267454	DQ190653	GQ267333
Ca. macroconidialis	CBS 114880[T] CPC 307	*E. grandis*	PW Crous	South Africa	AF232855	GQ267393	-	GQ267313
	CBS 110798[T]	*E. grandis* roots	PW Crous	South Africa	KX784646	KX784583	-	KX784716

	CPC 410							
Ca. metrosideri	CBS 133603^{T} LPF 101	*Metrosideros polymorpha*	AC Alfenas	Viçosa, Brazil	KC94313	KC294304	KC294307	KC294310
	CBS 133604 LPF 103	*M. polymorpha*	AC Alfenas	Viçosa, Brazil	KC94314	KC294305	KC294308	KC294311
Ca. nemuricola	CBS 134837^{T}	Soil	AC Alfenas	Minas Gerais, Brazil	KM395979	KM396066	KM396149	KM395892
	CBS 134838	Soil	AC Alfenas	Minas Gerais, Brazil	KM395980	KM396067	KM396150	KM395893
Ca. paracolhounii	CBS 114679^{T} CPC 2445	-	AY Rossman	USA	KX784644	KX784582	-	KX784714
	CBS 114705 CPC 2423	-	AY Rossman	USA	KX784645	-	-	KX784715
Ca. pauciramosa	CMW 5683^{T} CPC 971	*E. grandis*	PW Crous	South Africa	FJ918514	GQ267405	FJ918531	FJ918565
	CMW 30823 CPC 416	*E. grandis*	PW Crous	South Africa	FJ918515	GQ267404	FJ918532	FJ918566
Ca. pauciramosa	CBS 111812^{T}	*Cliffordia feruginea*	L Lombard	South Africa	KX784624	KX784566	-	KX784694
	CBS 111814	*Prunus avium*	L Lombard	South Africa	KX784625	KX784567	-	KX784695
	CBS 114458^{T}	*Erica* sp.	L Lombard	USA	KX784629	KX784571	-	KX784699
	CBS 114457	*Erica* sp.	L Lombard	USA	KX784628	KX784570	-	KX784698
	CMW 36327^{T} CBS 137243	*E. grandis* x *E. camaldulensis*	S Mausse	Mozambique	-	JX570722	JX570726	JX570718
	CMW 36329	*E. grandis* x *E. camaldulensis*	S Mausse	Mozambique	-	JX570721	JX570725	JX570717
	CBS 125270^{T} CMW 7804	*Callistemon citrinus*	G Polizzi	Sicily, Italy	FJ972417	GQ267461	FJ972436	FJ972486
	CBS 125271 CMW 10151	*Arbustus unedo*	G Polizzi	Sicily, Italy	FJ972418	GQ267462	FJ972437	FJ972487
	CBS 136635^{T} CMW 31474 CERC 1809	*E. urophylla* x *E. grandis*	L Lombard	Guangdong, China	KJ463011	KJ463128	KJ463244	KJ462898
	CBS 136637 CMW 31476 CERC 1811	*E. urophylla* x *E. grandis*	L Lombard	Guangdong, China	KJ463012	KJ463129	KJ463245	KJ462899
	CBS 125268^{T} CMW 9188	*E. grandis x E. urophylla*	L Lombard	South Africa	FJ972414	GQ267459	FJ972433	FJ972483
	CBS 125272 CMW 9896	*E. grandis x E. urophylla*	L Lombard	South Africa	FJ972415	GQ267460	FJ972434	FJ972484
	2425	*E. grandis x E. urophylla*	J Roux, I Greyling, S	Plantation- Cramond, KwaZulu-Natal, South	-	-	-	-

			Fraser, EI Rikhotso	Africa				
	2433	*E. grandis x E. urophylla*	J Roux, I Greyling, S Fraser, EI Rikhotso	Plantation- Cramond, KwaZulu-Natal, South Africa	-	-	-	-
	2435	*E. grandis x E. urophylla*	J Roux, I Greyling, S Fraser, EI Rikhotso	Plantation- Cramond, KwaZulu-Natal, South Africa	-	-	-	-
	2440	*E. dunnii*	J Roux, I Greyling, S Fraser, EI Rikhotso	Plantation-Cramond, KwaZulu-Natal, South Africa	-	-	-	-
	2445	*E. dunnii*	J Roux, I Greyling, S Fraser, EI Rikhotso	Plantation-Cramond, KwaZulu-Natal, South Africa	-	-	-	-
	2479	GN 1587	J Roux, I Greyling, S Fraser, EI Rikhotso	Nursery- Hilton, KwaZulu-Natal, South Africa	-	-	-	-
	2481	GN 1587	J Roux, I Greyling, S Fraser, EI Rikhotso	Nursery- Pietermaritzburg, KwaZulu-Natal, South Africa	-	-	-	-
Ca. piauiensis	CBS 134850^{T}	Soil	RF Alfenas	Piauí, Brazil	KM395973	KM396060	KM396143	KM395886
	CBS 134849	Soil	RF Alfenas	Piaui, Brazil	KM395972	KM396059	KM396142	KM395885
Ca. pseudocolhounii	CBS 127195^{T} CMW 27209	*E. dunni*	MJ Wingfield	Fujian, China	HQ285788	MF527091	HQ285802	HQ285816
	CBS 127196 CMW 27213	*E. dunni*	MJ Wingfield	Fujian, China	HQ285789	MF527092	HQ285803	HQ285817
Ca. pseudometrosideri	CBS 134845^{T}	*Soil*	-	Alagos, Brazil	KM395909	KM395995	KM396083	KM395821
	CBS 134844	*Eucalyptus* sp.	AC Alfenas	Maranhao, Brazil	KM395908	KM395994	KM396082	KM395820
Ca. pseudospathulata	CBS 134841^{T}	Soil	AC Alfenas	Minas Gerais, Brazil	KM395983	KM396070	KM396153	KM395896
	CBS 134840	Soil	AC Alfenas	Minas Gerais, Brazil	KM395982	KM396069	KM396152	KM395895
Ca. putriramosa	CBS 111449^{T}	*Eucalyptus* cutting	L Lombard	Brazil	KX784656	KX784591	-	KX784728

	CPC 1951							
	CBS 111470	Soil	L Lombard	Brazil	KX784657	KX784593	-	KX784729
	CPC 1940							
Ca. silvicola	CBS 135237^{T}	Soil	E Zauza	Minas Gerais, Brazil	KM395978	KM396065	KM396148	KM395891
	CBS 134836	Soil	E Zauza	Minas Gerais, Brazil	KM39575	KM396062	KM396145	KM395888
Ca. spathulata	CBS 55592^{T}	*Araucaria angustifolia*	C Hodges	Brazil	GQ267215	GQ267426	GQ267261	GQ267331
	CBS 112689	*E. viminalis*	NE Ell-Ghol	Brazil	AF308463	GQ267426	FJ918522	FJ918554
Ca. venezuelana	CBS 111052^{T}	-	L Lombard	Venezuela	KX784671	KX784601	-	KX784744
	CPC 1183							
Curvicladiella cignea	CBS 101411	Decaying seed	C Decock	French Guiana	KM232001	KM231285	KM231459	KM231866
	CBS 109167	Leaf litter	C Decock	French Guiana	KM232002	KM231287	KM231461	KM231867

[a]Designation of isolates and culture collections: CBS= The culture collection of Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CERC = China Eucalypt Research Centre, Zhanjiang, GuangDong Province, China; CMW = Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; CPC = Pedro Crous working collection housed at Westerdijk Fungal Biodiversity Institute; LPF = Labóritorio de Patologia Florestal. Universidade Federal de Viçosa, Viçosa, Brazil. Isolates obtained during the survey in this study are indicated in **bold**. "^{T}" represents type cultures.

Table 5. Collection details of *Cylindrocladiella* isolates included in the phylogenetic analyses including GenBank accession numbers for the histone (*his3*) gene region.

Species	Culture no. [a]	Host	Collector	Collected from	GenBank accession number
					his3
Cylindrocladiella arbusta	CMW 47295[T] CBS 143546	Soil in *Acacia mangium* plantation	NQ Pham	Tan Ky, Nghe An, Vietnam	MH016996
	CMW 47296 CBS 143547	*Acacia mangium* plantation soil	NQ Pham	Tan Ky, Nghe An, Vietnam	MH016997
Cy. camelliae	CPC 234 PPRI 3990	*E. grandis*	PW Crous	South Africa	AY793509
	CPC237	*E. grandis*	PW Crous	South Africa	JN098858
Cy. clavata	CBS 129563 CPC 17591	Soil	PW Crous	Australia	JN098859
	CBS 129564[T] CPC 17592	Soil	PW Crous	Australia	JN098858
Cy. cymbiformis	CBS 129553[T] CPC 17393	Soil	PW Crous	Australia	JN098866
Cy. elegans	CBS 33892[T] PPRI 4050	Leaf litter	I Rong	South Africa	AY793512
	CBS110801 CPC 525	Leaf litter	PW Crous	South Africa	JN098916
Cy. lageniformis	CBS 340.92[T] PPRI 4449	*Eucalyptus* sp.	AC Alfenas	Brazil	AY793520
	CBS 111060 CPC 1240	*Eucalyptus* sp.	PW Crous	South Africa	JN098981
	CMW 47419	Soil in *E. camaldulensis* plantation	NQ Pham	Hoang Mai, Nghe An, Vietnam	MH017010
Cy. lanceolata	CBS 129565 CPC 17566	Soil	PW Crous	Australia	JN098939
	CBS 129566[T] CPC 17567	Soil	PW Crous	Australia	JN098862
Cy. longiphialidica	CBS 129557[T] CPC 18839	Soil	PW Crous	Thailand	JN098851

	CBS 129558	Soil	PW Crous	Thailand	JN098852
Cy. malesiana	CMW 48276 CBS 143549	soil in *A. mangium* plantation	NQ Pham	Tawau, Sabah, Malaysia	MH016998
	CMW 48277 CBS 143550	soil in *A. mangium* plantation	NQ Pham	Tawau, Sabah, Malaysia	MH016999
	CMW 48278^{T} CBS 143548	soil in *A. mangium* plantation	NQ Pham	Tawau, Sabah, Malaysia	MH017000
	CMW 48279	soil in *A. mangium* plantation	NQ Pham	Tawau, Sabah, Malaysia	MH017001
Cy. natalensis	CBS 110800 CPC 529	Soil	PW Crous	South Africa	JN098915
	CBS 114943^{T} CPC 456	*Arachis hypogaea*	MJ Wingfield	South Africa	JN098895
Cy. nederlandica	CBS 143.95	*Kalanchoe* sp.	JW Veenbaas-Rijks	The Netherlands	JN098891
	CBS 152.91^{T}	*Pelargonium* sp.	JW Veenbaas-Rijks	The Netherlands	JN098910
Cy. novaezelandiae	CBS 48677^{T} CPC 2397	*Rhododendron indicum*	HJ Boesewinkel	New Zealand	AY793525
Cy. obpyriformis	CMW 47194^{T} CBS 143552	Soil in *Acacia* hybrid plantation	NQ Pham	Tuyen Quang, Vietnam	MH017003
	CMW 49940 CBS 143553	Soil in *Camellia chrysantha* nursery	NQ Pham	Tam Dao, Vinh Phuc, Vietnam	MH017004
Cy. parvispora	CMW 47193	Soil in *Acacia hybrid* plantation	NQ Pham	Tuyen Quang, Vietnam	MH017005
	CMW 47197^{T} CBS 143554	Soil in *Acacia hybrid* plantation	NQ Pham	Tuyen Quang, Vietnam	MH017006
	CMW 47207 CBS 143555	Soil in *Acacia hybrid* plantation	NQ Pham	Tuyen Quang, Vietnam	MH017007
	CMW 47208 CBS 143556	Soil in *Acacia hybrid* plantation	NQ Pham	Tuyen Quang, Vietnam	MH017008
Cy. peruviana	CBS 113022 CPC 4291	*Eucalyptus* sp.	PW Crous	South Africa	JN098906
	CMW 47297	Soil in *A. mangium* plantation	NQ Pham	Tan Ky, Nghe An, Vietnam	MH017011
	CMW 47304	Soil in *A. mangium* plantation	NQ Pham	Son Duong, Tuyen Quang, Vietnam	MH017012
	CMW 47416	Soil	NQ Pham	Bac Tu Liem, Hanoi, Vietnam	MH017014
Cy. pseudocamelliae	CBS 129555^{T}	Soil	PW Crous	Thailand	JN098843

	CPC 18825				
	CBS 129556	Soil	PW Crous	Thailand	JN098846
	CPC 18832				
Cy. solicola	CMW 47198	soil in *Acacia* hybrid plantation	PQ Pham	Tuyen Quang, Vietnam	MH017002
	CBS 143551				
Cy. variabilis	CBS 375.93	*Mangifera indica*	P.N. Chowdhry	India	JN098881
	IMI 317057				
	CBS 129561^{T}	Soil	PW Crous	Australia	JN098950
	CPC 17505				
Unknown	**2436**	*E. dunni*	EI Rikhotso, J Roux, S Fraser, I Greyling	Cramond, KZN	-
	2453	*E. grandis* x *E. nitens*	EI Rikhotso, J Roux, S Fraser, I Greyling	Cramond, KZN	-
	24141	*E. cloeziana*	EI Rikhotso, J Roux	Louis Trichardt, Limpopo	-
	24174	*E. grandis* x *E. urophylla*	I Greyling	Mtubatuba Zululand, KZN	-
	24277	*E. grandis* x *E. urophylla*	I Greyling	Mtubatuba, Zululand, KZN	-
	24369	*E. grandis*	EI Rikhotso	Magoebaskloof, Limpopo	-
	24372	*E. grandis*	EI Rikhotso, J Roux, S Fraser, I Greyling	Howick, KZN	-
	24451	*E. grandis*	EI Rikhotso, J Roux	Mkhondo, Mpumalanga	-
	24488	*E. grandis*	EI Rikhotso, J Roux	Barberton, Mpumalanga	-
	24106	*E. grandis* x *E. camaldulensis*	EI Rikhotso, J Roux	Louis Trichardt, Limpopo	-
Curvicladiella cignea	CBS 101411	Decaying seed	C Decock	French Guiana	KM231459
	CBS 109168	Decaying seed	C Decock	French Guiana	KM231460

[a]Designation of isolates and culture collections: CBS= The culture collection of Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CMW = Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; CPC = Pedro Crous working collection housed at Westerdijk Fungal Biodiversity Institute; IMI = International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; PPRI = Plant Protection Research Institute, Agricultural Research Council, Pretoria, South Africa. Isolates obtained during the survey in this study are indicated in **bold**. "^{T}" represents type cultures.

Table 6. Collection details of *Quambalaria* isolates included in the phylogenetic analyses including GenBank accession numbers for the three gene regions, ITS and 5.8S and translation elongation factor 1α (*tef1*).

Species	Culture no. [a]	Host	Collector	Collected from	GenBank accession number
					ITS
Q. coyrecup	CMW 35361	-	T Duong	New South Wales	Pending
	BRIP 48338	*Corymbia polycarpa*	R Pitkethley	Darwin, Northern Territory, Australia	EF444877
	BRIP 48339	*C. polycarpa*	R Pitkethley	Darwin, Northern Territory, Australia	EF444878
	WAC 12950	*C. calophylla*	T Paap	Western Australia, Australia	DQ823429
	WAC 12947	*C. calophylla*	T Paap	Western Australia, Australia	DQ823431
	WAC 12949	*C. calophylla*	T Paap	Western Australia, Australia	DQ823432
Q. cyanescens	WAC 12952	*C. calophylla*	T Paap	Western Australia, Australia	DQ823419
		C. citriodora	GS Pegg	Queensland, Australia	EF444873
	BRIP 48403	*C. citrodora*	GS Pegg	Queensland, Australia	EF444876
	MK 742	*Chaetoptelius vestitus* on *Pistacia vera*	M Kolarik	Icel, Turkey	AM261920
	CBS 876.73 CMW 5584	*E. pauciflora*	VF Brown	New South Wales, Australia	DQ317623
	BRIP 48398	C. citriodora	GS Pegg	Queensland, Australia	EF444875
	QY 229	Red kojic rice	Z Zhang	China	HM013823
	CBS 35773	-	M Kolarik	The Netherlands	DQ119135
	BRIP 48396	*C. citriodora*	GS Pegg	Queensland, Australia	EF444874
	WAC 12955	*C. calophylla*	T Paap	Western Australia, Australia	DQ823421
	WAC 12953	*C. ficifolia*	T Paap	Western Australia, Australia	DQ823422

Species	Culture no. [a]	Host	Collector	Collected from	GenBank accession number
					ITS
	MK 808	*Phleotribus scarabeoides* on *Olea europea*	M Kolarik	Krak des Chevaliers, Syria	AM261921
	MK 1710	*Scolytus intricatus* on *Quercus* sp.	M Kolarik	Bachkovo, Bulgaria	AM261922
	IMI 178848	*E. pauciflora*	SRH Langrell	New South Wales, Australia	AJ536610
	CF 3526	-	M Kolarik	Turkey	DQ119134
	MK 1617	*Phleotribus scarabeoides* on *Olea europea*	M Kolarik	Huelva, Spain	AM261924
Q. eucalypti	CBS 118844 CMW 1101	*E. grandis*	MJ Wingfield	Kwambonambi, KwaZulu-Natal, South Africa	DQ317625
	CMW 14329	*E. grandis* x *E. camaldulensis*	J Roux	Kwambonambi, KwaZulu-Natal, South Africa	DQ317614
	CMW 17253	*E. nitens*	ZL Mthalane & J Roux	Rooihoogte, Mpumalanga, South Africa	DQ317610
	CMW 17255	*E. nitens*	ZL Mthalane & J Roux	Rooihoogte, Mpumalanga, South Africa	DQ317612
	CBS 118616 CMW 17771	*E. grandis*	J Roux	Kwambonambi, KwaZulu-Natal, South Africa	DQ317613
	BRIP 48367	*C. torelliana* x *C. citriodora* subsp. *variegata*	GS Pegg	Queensland, Australia	EF444823
	BRIP 48416	*E. dunnii*	GS Pegg	New South Wales, Australia	EF444824
	UYI 1036	*Myrceugenia glaucescens*	CA Pérez	Uruguay	EU439922
	BRIP 48422	*E. dunnii*	AJ Carnegie	New South Wales, Australia	EF444832
	BRIP 48498	*E. grandis*	AJ Carnegie	New South Wales, Australia	EF444844
	BRIP 48490	*E. grandis*	GS Pegg	New South Wales	EF444834
	BRIP 48414	*E. grandis*	GS Pegg	Queensland, Australia	EF444821
	PE3 MEAN 996	*E. globulus*	-	Portugal	JX297605
	PE29 MEAN 999	*E. globulus*	-	Portugal	JX297602

Species	Culture no. [a]	Host	Collector	Collected from	GenBank accession number
					ITS
	PE30 MEAN 1001	*E. globulus*	-	Portugal	JX297601
	PE52 MEAN 1002	*E. globulus*	-	Portugal	JX297606
	CERC 8476	*E. grandis*	SF Chen & JQ Li	Guangdong, China	KY615009
	CERC 8479	*E. grandis*	SF Chen & JQ Li	Guangdong, China	KY615012
	CERC 8482	*E. grandis*	SF Chen & JQ Li	Guangdong, China	KY615015
	241	*E. dunnii*	J Roux, I Greyling, S Fraser, EI Rikhotso	Nursery- Cramond, KwaZulu-Natal, South Africa	-
	2410	*E. grandis* x *E. nitens*	J Roux, I Greyling, S Fraser, EI Rikhotso	Nursery-Wartburg, KwaZulu-Natal, South Africa	-
	2412	*E. dunnii*	J Roux, I Greyling, S Fraser, EI Rikhotso	Nursery-Wartburg, KwaZulu-Natal, South Africa	-
	2484	*E. grandis* x *E. nitens*	J Roux, I Greyling, S Fraser, EI Rikhotso	Plantation- Pietermaritzburg, KwaZulu- Natal, South Africa	-
	2485	*E. grandis* x *E. nitens*	J Roux, I Greyling, S Fraser, EI Rikhotso	Plantation- Pietermaritzburg, KwaZulu- Natal, South Africa	-
Q. pitereka	DAR 19773	*C. exima*	AL Bertus & J Walker	New South Wales, Australia	EF427376
	QP 26	*C. citriodora* subsp. *variegata*	GS Pegg	Queensland, Australia	DQ823424
	CBS 118828 CMW 5318	*C. citriodora* subsp. *variegata*	M Ivory	Queensland, Australia	DQ317628
	BRIP 48361	*C. citriodora* subsp. *variegata*	GS Pegg	Queensland, Australia	EF427367
	BRIP 48383	*C. citriodora* subsp. *variegata*	GS Pegg	Queensland, Australia	EF444859
	WAC 12957	*C. ficifolia*	T Paap	Western Australia, Australia	DQ823426

Species	Culture no. [a]	Host	Collector	Collected from	GenBank accession number
					ITS
	BRIP 48370	*C. citriodora* subsp. *variegata*	GS Pegg	Queensland, Australia	EF427368
	BRIP 48531	*C. citriodora* subsp. *variegata*	GS Pegg	Queensland, Australia	EF427371
	BRIP 48512	*C. henryi*	GS Pegg	New South Wales, Australia	EF444856
	BRIP 48384	*C. citriodora* subsp. *variegata*	GS Pegg	Queensland, Australia	EF427369
	BRIP 48328	*C. citriodora* subsp. *variegata*	GS Pegg	New South Wales, Australia	EF444872
	CMW 23610	*C. citriodora*	YJ Xie	Guangdong, China	EF427372
	CERC 8486	*C. citriodora* provenance	SF Chen & GQ Li	Guangdong, China	KY615017
	CERC 9093	*C. citriodora* provenance	SF Chen & Y Lin	Guangdong, China	KY615022
	CERC 9102	*C. citriodora* provenance	SF Chen & Y Lin	Guangdong, China	KY615031
	BRIP 48325	*C. citriodora* subsp. *variegata*	GS Pegg	Queensland , Australia	EF427366
	BRIP 48424	*C. citriodora* subsp. *variegata*	GS Pegg	New South Wales, Australia	EF444868
	BRIP 48432	*C. citriodora* subsp. *variegata*	GS Pegg	New South Wales, Australia	EF444873
	BRIP 48321	*C. citriodora* subsp. *variegata*	GS Pegg	Queensland, Australia	EF444855
Q. purpurascens	CMW 35351	-	T Duong	New South Wales	Pending
	CMW 35356	-	T Duong	New South Wales	Pending
	CMW 35360	-	T Duong	New South Wales	Pending
	CMW 35354	-	T Duong	New South Wales	Pending
	CMW 35352	-	T Duong	New South Wales	Pending
	CMW 35358	-	T Duong	New South Wales	Pending

Species	Culture no. [a]	Host	Collector	Collected from	GenBank accession number
					ITS
	CMW 35357	-	T Duong	New South Wales	Pending
Q. pusilla	Quam7	-	T Duong	Laos	Pending
	Quam8	-	T Duong	Laos	Pending
	Quam3b	-	T Duong	Laos	Pending
	Quam4b	-	T Duong	Laos	Pending
	Quang3a	-	T Duong	Laos	Pending
	T1	-	T Duong	Thailand	Pending
Q. simpsonii	CBS 124773	*Eucalyptus* sp.	R Cheewangkoon	Lamphoon, Thailand	GQ303291
	CBS 124772	*Eucalyptus tintinnans*	BA Summerell	Edith Falls, Australia	GQ303290
	CERC 8496	*E. urophylla* x *E. grandis*	SF Chen & QL Liu	Hainan, China	KY615034
	CERC 8499	*E. urophylla* x *E. grandis*	SF Chen & QL Liu	Hainan, China	KY615035
	CERC 8505	*E. urophylla* x *E. grandis*	SF Chen & QL Liu	Hainan, China	KY615036
	CERC 8519	*E. urophylla* x *E. grandis*	SF Chen & QL Liu	Hainan, China	KY615042
Sympodiomycopsis kandeliae	BCRC 23165 FIRDI 007	Kandelia candel	YH Wei	Hsinchu, Taiwan	GQ465043
S. kandeliae	BCRC 07F0494	*Kandelia candel*	YH Wei	Hsinchu, Taiwan	GQ465045

[a]Designation of isolates and culture collections: CBS = The culture collection of Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CMW = Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; BRIP = Biosecurity Queensland Plant Pathology Herbarium, Brisbane, Queensland, Australia; IMI = International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K; WAC = Western Australian Plant Pathology Reference Culture Collection, Australia. Isolates obtained during the survey in this study are indicated in **bold**. "[T]" represents type cultures.

Table 7. Collection details of *Teratosphaeria* isolates included in the phylogenetic analyses including GenBank accession numbers for the three gene regions, ITS and 5.8S, β-tubulin (*tub2*) and translation elongation factor 1α (*tef1*).

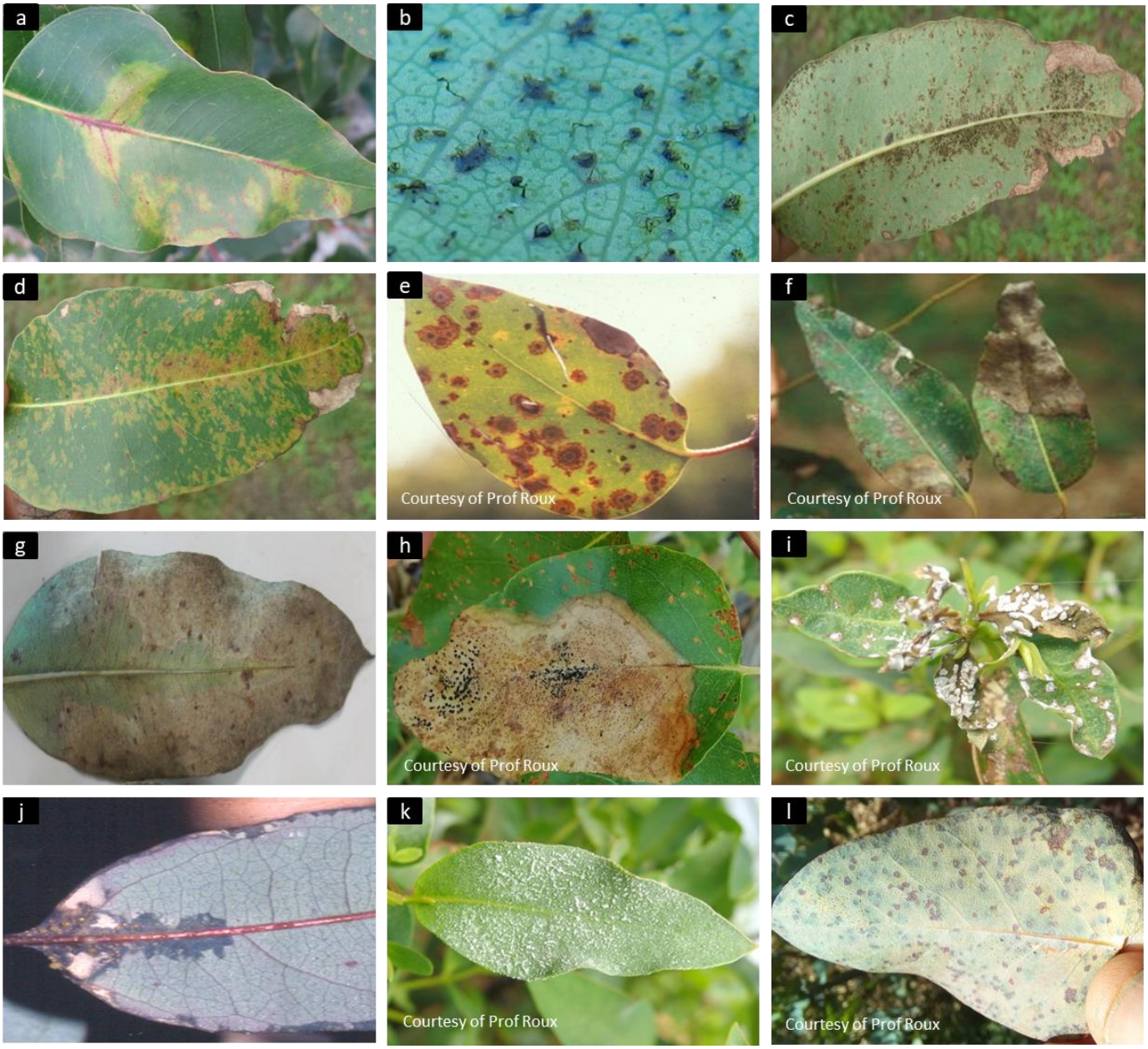
Species	Culture no. [a]	Host	Collector	Collected from	GenBank accession number		
					ITS	*tub2*	*tef1*
T. destructans	CMW 44962	*Eucalyptus grandis* x *E. urophylla*	I Greyling	Zululand, South Africa	KT343574	KT343568	KT343580
	DRF 1168[T]	*E. grandis*	MJ Wingfield	Sumatra, Indonesia	KT972278	KT972310	KT972342
	DRF 1169	*E. grandis*	MJ Wingfield	Sumatra, Indonesia	KT972279	KT972311	KT972343
	CMW 44957	*E. grandis* x *E. urophylla*	I Greyling	Zululand, South Africa	KT343569	KT343563	KT343575
	CMW 44959	*E. grandis* x *E. urophylla*	I Greyling	Zululand, South Africa	KT343571	KT343565	KT343577
	CBS 111370	*E. grandis*	W Quaedvlieg	Indonesia	KF901574	KF903000	KF903301
	CMW 19922	*E.urophylla*	TI Burgess	Goungdong, China	KT972280	KT972312	KT972344
	CMW 19911	*E. urophylla*	TI Burgess	Goungdong, China	EU046369	EU046365	EF686474
	CMW 13710	*E. camaldulensis*	MJ Wingfield	Tatoom, Thailand	EF686505	EF686274	EF686464
	CMW 15090	*E. camaldulensis*	TI Burgess	MinhDuc, S-E Vietnam	EF031466	EF031478	EF031490
	24445	*E. grandis*	J Roux, EI Rikhotso	Plantation- Mkhondo, Mpumalanga, South Africa	-	-	-
	24421	*E. grandis*	J Roux, EI Rikhotso	Plantation- Whiteriver, Mpumalanga, South Africa	-	-	-
	24194	Clone *PRB3*	I Greyling	Plantation- Mtubatuba, KwaZulu -Natal, South Africa	-	-	-
	2488	*E. grandis* x *E. nitens*	J Roux, I Greyling, S Fraser, EI Rikhotso	Nursery- Hilton KwaZulu-Natal, South Africa	-	-	-

Species	Culture no. [a]	Host	Collector	Collected from	GenBank accession number		
					ITS	*tub2*	*tef1*
	24341	*E. grandis* x *E. urophylla*	J Roux, EI Rikhotso	Plantation- Whiteriver, South Africa	-	-	-
	2496	*E. grandis* x *E. urophylla*	J Roux, I Greyling, S Fraser, EI Rikhotso	Plantation- Pietermaritzburg, KwaZulu-Natal, South Africa	-	-	-
	2413	*E. grandis* x *E. urophylla*	J Roux, I Greyling, S Fraser, EI Rikhotso	Nursery- Wartburg, KwaZulu-Natal, South Africa	-	-	-
	2447	*E. grandis* x *E. nitens*	J Roux, I Greyling, S Fraser, EI Rikhotso	Plantation- Cramond, KwaZulu-Natal, South Africa	-	-	-
	24163	*E. grandis* x *E. urophylla*	I Greyling	Plantation- Mtubatuba, KwaZulu-Natal, South Africa	-	-	-
	24197	Clone *PRB3*	I Greyling	Plantation- Mtubatuba, KwaZulu-Natal, South Africa	-	-	-
	24193	*E. grandis*	I Greyling	Plantation- Mtubatuba, KwaZulu-Natal, South Africa	-	-	-
	24340	*E. grandis*	J Roux, EI Rikhotso	Plantation- Barberton, Mpumalanga, South Africa	-	-	-
T. eucalypti	CMW 19453	*E. nitens*	M Dick	Settlement Rd, New Zealand	FJ793234	EU101529	EU101585
	CMW 19455	*E. nitens*	V Andjic	Coxs, New Zealand	FJ793260	EU101571	EU101628
	CMW 19461	*E. nitens*	M Dick	Sun Valley, New Zealand	FJ793232	EU101527	EU101583
	MUCC 623	*E. nitens*	AJ Carnegie	Dorrigo, Australia	FJ793255	EU101566	EU101623
T. novaehollandiae	AQISWA 201513	*Eucalyptus sp.*	MJ Wingfield	Derby, WA, Australia	KT972291	KT972323	KT972355
	AQISWA 201514	*Eucalyptus sp.*	MJ Wingfield	Derby, WA, Australia	KT972292	KT972324	KT972356
	AQISWA	*Eucalyptus sp.*	MJ Wingfield	Derby, WA, Australia	KT972293	KT972325	KT972357

Species	Culture no. [a]	Host	Collector	Collected from	GenBank accession number		
					ITS	*tub2*	*tef1*
	201515						
	AQISWA 201402	*E. victrix*	G Hardy	Pibara, WA, Australia	KT972288	KT972320	KT972352
	AQISWA 201401 BRIP 63523 CBS 141554	*E. victrix*	G Hardy	Pibara, WA, Australia	KT972287	KT972319	KT972351
	AQISWA 201403	*E. victrix*	G Hardy	Pibara, WA, Australia	KT972289	KT972321	KT972353
	AQISWA 201302[T] BRIP 59486	*E. camaldulensis*	A Maxwell	Kununurra, Australia	KT972281	KT972313	KT972345
	AQISWA 201304 BRIP 59488 CBS 141552	*E. camaldulensis*	V Andjic	Nothern Territory, Australia	KT972283	KT972315	KT972347
	AQISWA 201303 BRIP 59487	*E. camaldulensis*	A Maxwell	Kununurra, Australia	KT972282	KT972314	KT972346
	AQISWA 201307 BRIP 59481	*E. camaldulensis*	A Maxwell	Northern Territory, Australia	KT972286	KT972318	KT972350
T. pseudoeucalypti	MUCC 607	*E. grandis* x *E. camaldulensis*	V Andjic	Queensland, Australia	FJ793220	EU101542	EU101598
	MUCC 615	*Eucalyptus sp.*	V Andjic	Queensland, Australia	FJ793231	EU101556	EU101613
	MUCC 599	*E. globulus* x *E. camaldulensis*	V Andjic	Queensland, Australia	FJ793216	EU101537	EU101593
	MUCC 605	*E. globulus* x *E. camaldulensis*	V Andjic	Queensland, Australia	FJ793225	EU101559	EU101616
	MUCC 612	*E. grandis x E. camaldulensis*	V Andjic	Queensland, Australia	FJ793223	EU101545	EU101601
T. tiwiana	AQISWA 201501 CBS 141547	*E. grandis* x *E. urophylla*	TI Burgess	Tiwi Island, Australia	KT972298	KT972329	KT972361

Species	Culture no. [a]	Host	Collector	Collected from	GenBank accession number		
					ITS	*tub2*	*tef1*
	AQISWA 201502[T] BRIP63496 CBS 141549	*E. urophylla hybrids*	TI Burgess	Tiwi Island, Australia	KT972298	KT972330	KT972362
	AQISWA 201506 BRIP 63491 CBS 141551	*E. grandis* x *E. urophylla*	TI Burgess	Tiwi Island, Australia	KT972202	KT972334	KT972366
	AQISWA 201508 BRIP63493	*E. grandis* x *E. urophylla*	TI Burgess	Tiwi Island, Australia	KT972204	KT972336	KT972368
	AQISWA 201507 BRIP 63494	*E. grandis* x *E. urophylla*	TI Burgess	Tiwi Island, Australia	KT972203	KT972335	KT972367
T. viscidus	CBS 121156 MUCC 452	*E. grandis*	TI Burgess	Mareeba, Australia	EF031471	EF031483	EF031495
	CBS 121157[T] MUCC 453	*E. grandis*	TI Burgess	Mareeba, Australia	EF031472	EF031484	EF031496
	FNQ 148 MUCC 454	*E. grandis*	TI Burgess	Mareeba, Australia	EF031473	EF031485	EF031497
	FNQ 149 MUCC 455	*E. grandis*	TI Burgess	Mareeba, Australia	EF031474	EF031486	EF031498
Readeriella angusta	CBS 124998	*E. delegatensis*	BA Summerell	Tasmania, Australia	MH863445	KF902949	KF903245
	CBS 124997	*E. delegatensis*	BA Summerell	Tasmania, Australia	KF901759	KF902950	KF903246

[a]Designation of isolates and culture collections: AQISWA = Biosecurity Australia Fungal Culture Collection, Perth, Western Australia, Australia; CBS= The culture collection of Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CMW = Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; BRIP = Biosecurity Queensland Plant Pathology Herbarium, Brisbane, Queensland, Australia; MUCC = Murdoch University Culture Collection, Perth, Western Australia, Australia. Isolates obtained during the survey in this study are indicated in **bold**. "[T]" represents type cultures



Photos E, F, H, I, K, L provided by Prof J. Roux.

Fig. 1. Leaf disease symptoms observed in the field and nursery. (a) pale green lesions with purple margins caused by *T. destructans*, (b) black pycnidia of *T. destructans* exuding spores in black cirri on the abaxial leaf surfaces, (c) brown to black spores of *T. epicoccoides* covering the abaxial leaf surface,(d) purple leaf spots of *T. suttonii* which expand and coalesce on the adaxial surface of older leaves, (e) raised, crusty leaf spots of *Aulographina* sp. scattered over the leaf surface, (f) large brown lesions of *Calonectria* sp. with microscopic white spore masses (g) leaf blight of *Calonectria* sp. covering almost the entire leaf in severe conditions, (h) leaf spots of *Coniella* sp. with pale brown to black fruiting bodies, (i) white masses of conidia caused by *Quambalaria* sp., (j) water soaked lesions of bacterial blight, (k) powdery white patches caused by powdery mildew, (l) leaf spots of *Phakopsora* sp. that have coalesced to form discolouration and necrotic lesions with uredinia.

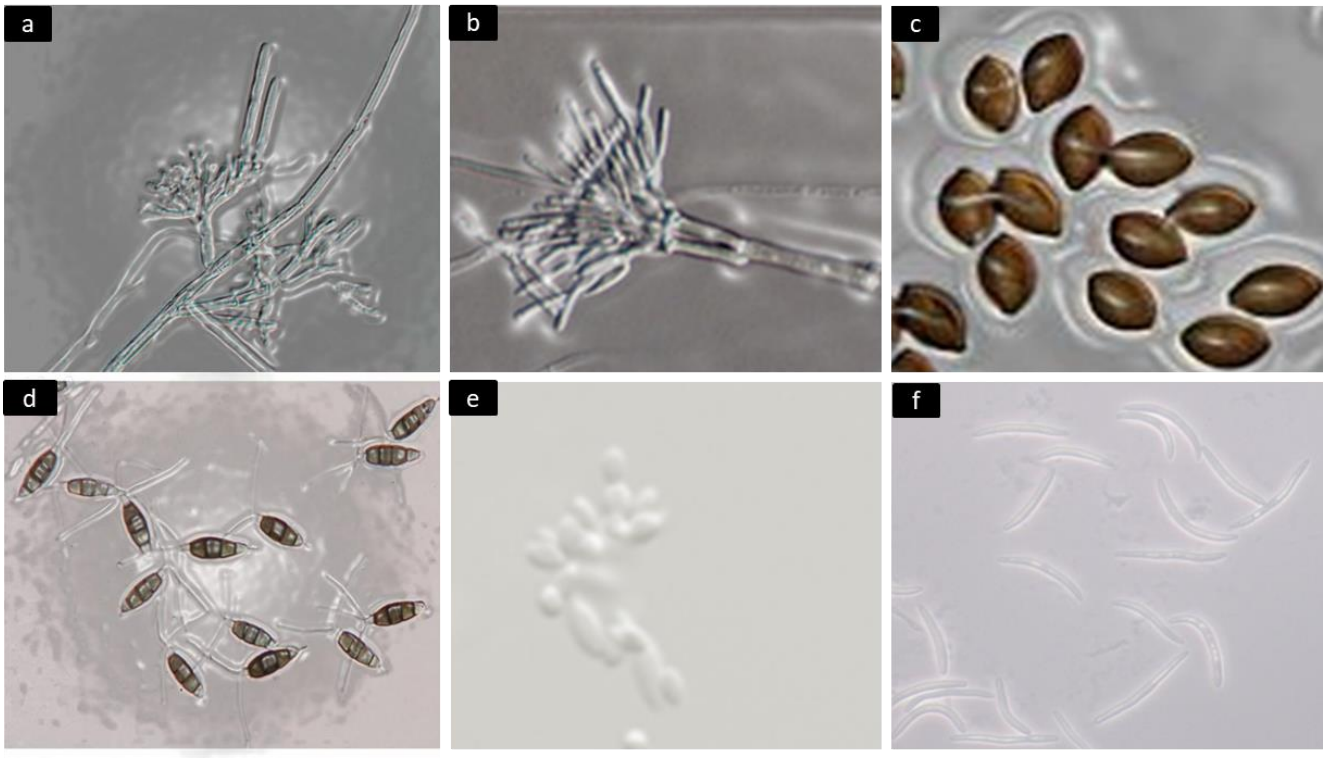


Fig. 2. Spore morphology of leaf spot and leaf blight fungi as observed on leaf samples. (a-b) conidiophore branches of *Calonectria* sp.,(c) conidia of *Coniella* sp., (d) conidia of *Pestalotiopsis* sp., (e) conidia of *Quambalaria* sp., (f) conidia of *T. destructans*.

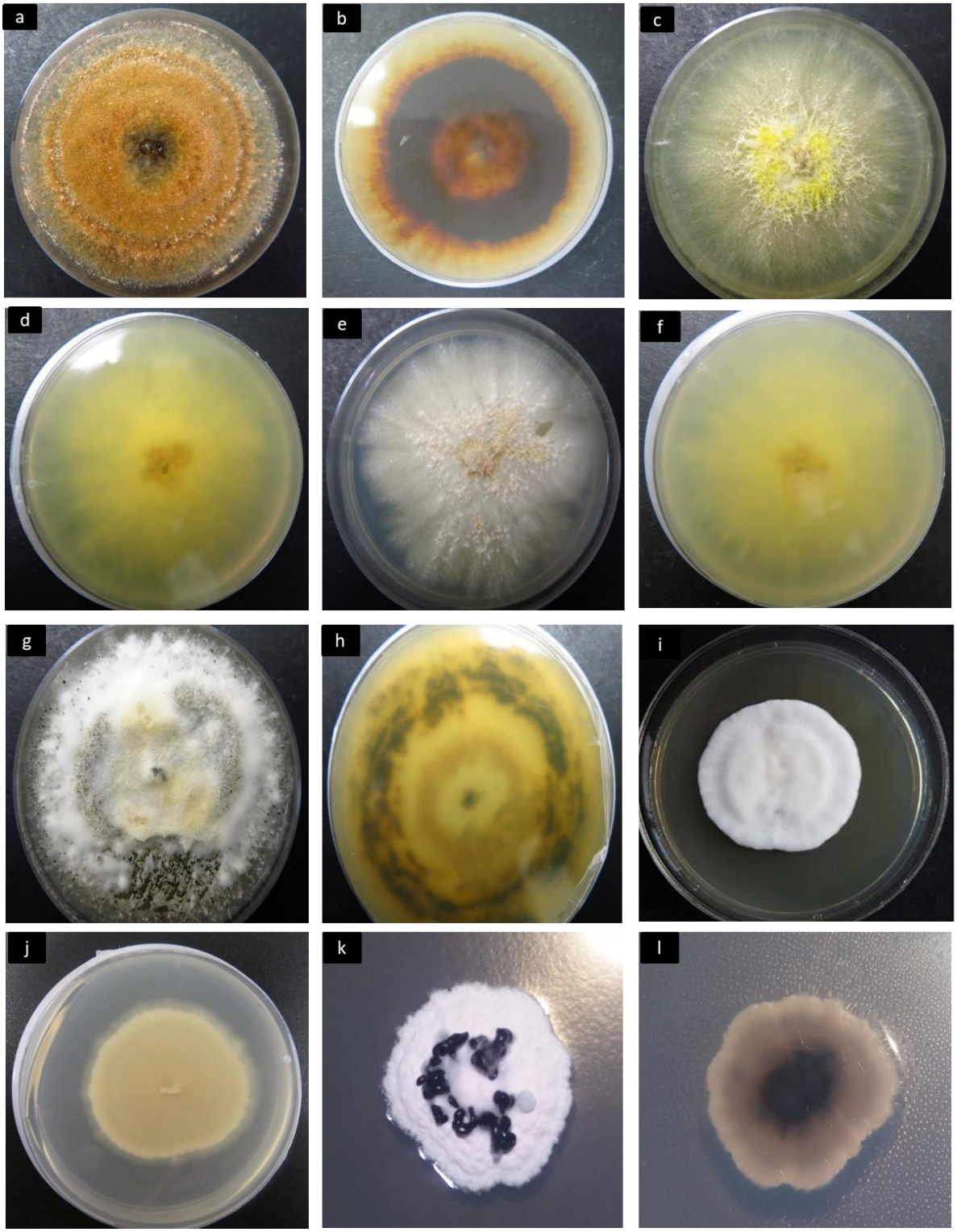


Fig. 3. Cultural characteristics. (a) culture of *Calonectria* sp. characterized by white to brown mycelium with feathery irregular brown margins and white spore masses on the upper surface, (b) reversed culture plate of *Calonectria* sp. characterized by a brown colour in the center, (c-e) culture of *Calonectria* sp. characterized by feathery irregular mycelium at the edges and yellow/white spore masses on the upper surface, (d- f) reversed culture plate of *Calonectria* sp. Characterized by a yellow/white colour, (g) culture of *Coniella* sp. characterized by fluffy white aerial mycelium that was spreading from the centre with black spore masses, (h) reversed plate of *Coniella* sp. characterized by the colour white with black zones, (i) culture of *Quambalaria* sp. characterized by white mycelium on the upper surface, (j) reversed culture plate of *Quambalaria* sp. Characterized by a darker appearance, (j) culture of *T. destructans* characterized by Pink cultures with black spore masses on the upper surface, (l) reversed culture plate of *T. destructans* characterized by an olive-black appearance on the reverse side.

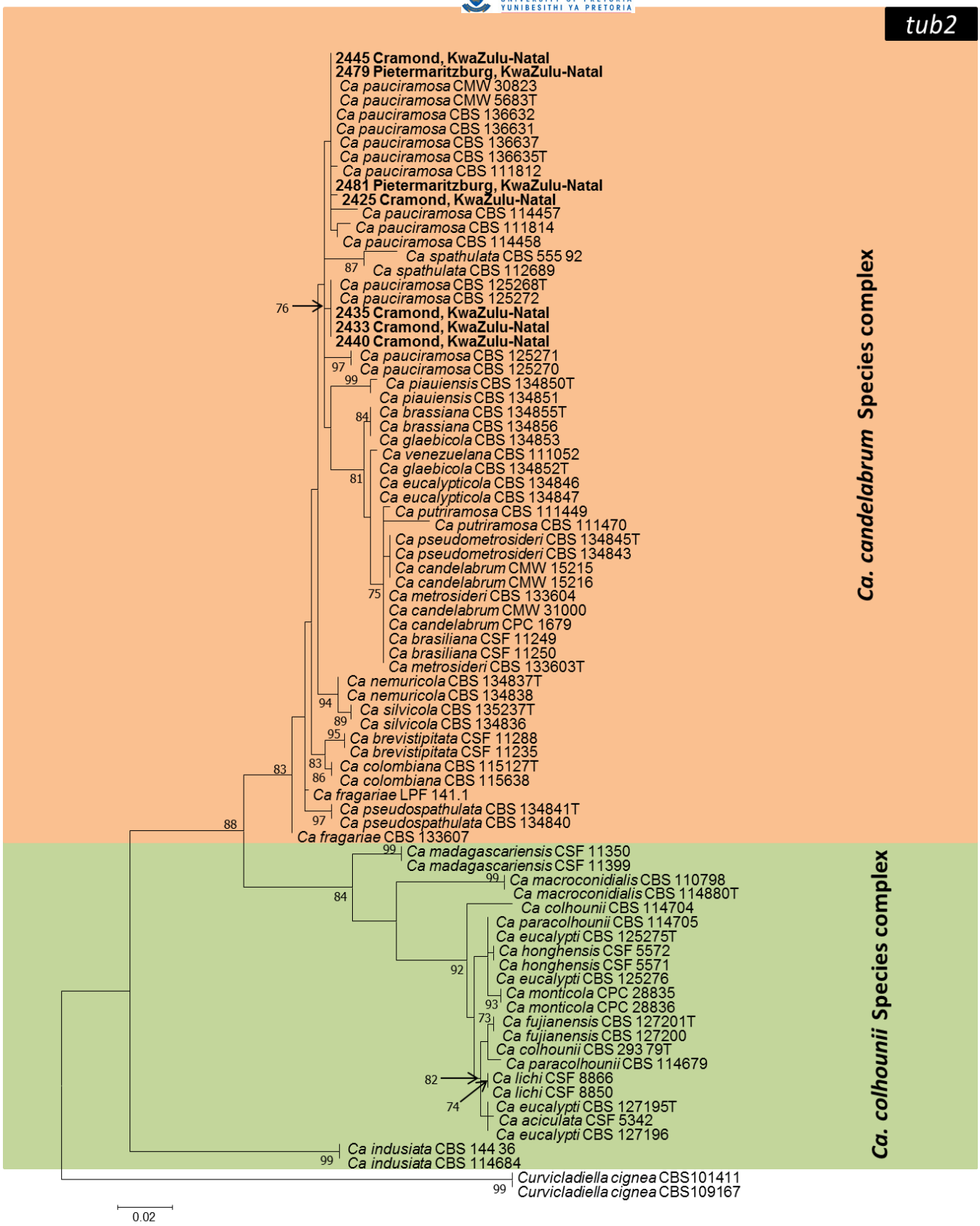


Fig. 4

Phylogenetic tree based on maximum likelihood (ML) analysis of *tub2* sequences for *Calonectria* spp. in the *Ca. candelabrum* and *Ca. colhounii* species complex. Bootstrap value ≥ 70% indicated at the nodes. Bootstrap values lower than 70% were removed. Isolates collected in this study are highlighted in **bold**. *Curvicladiella cignea* (CBS 101411 and CBS 109167) represent the outgroup.

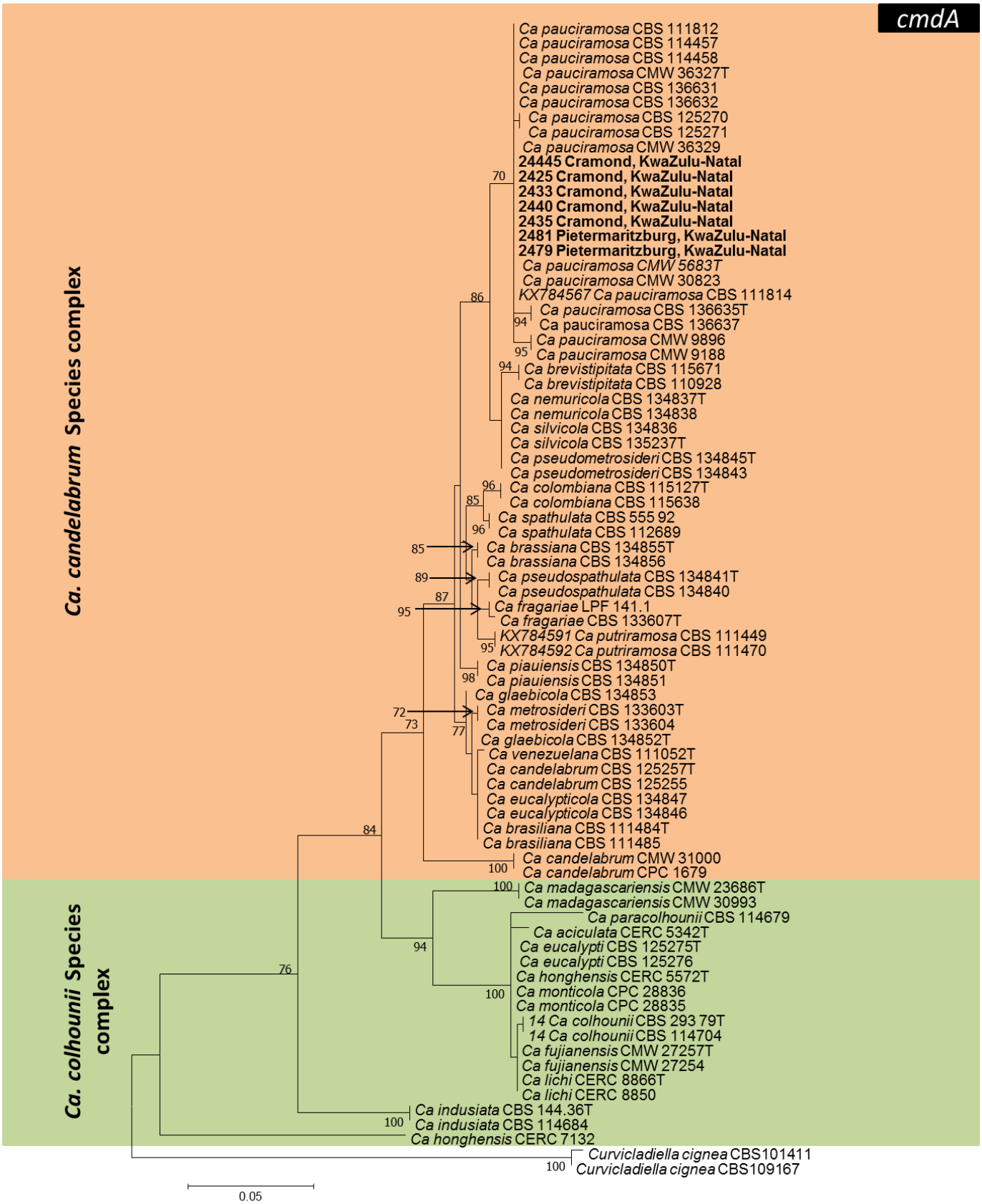


Fig. 5.

Phylogenetic tree based on maximum likelihood (ML) analysis of *cmdA* sequences for *Calonectria* spp. in the *Ca. candelabrum* and *Ca. colhounii* species complex. Bootstrap value ≥ 70% indicated at the nodes. Bootstrap values lower than 70% were removed. Isolates collected in this study are highlighted in **bold**. *Curvicladiella cignea* (CBS 101411 and CBS 109167) represent the outgroup.

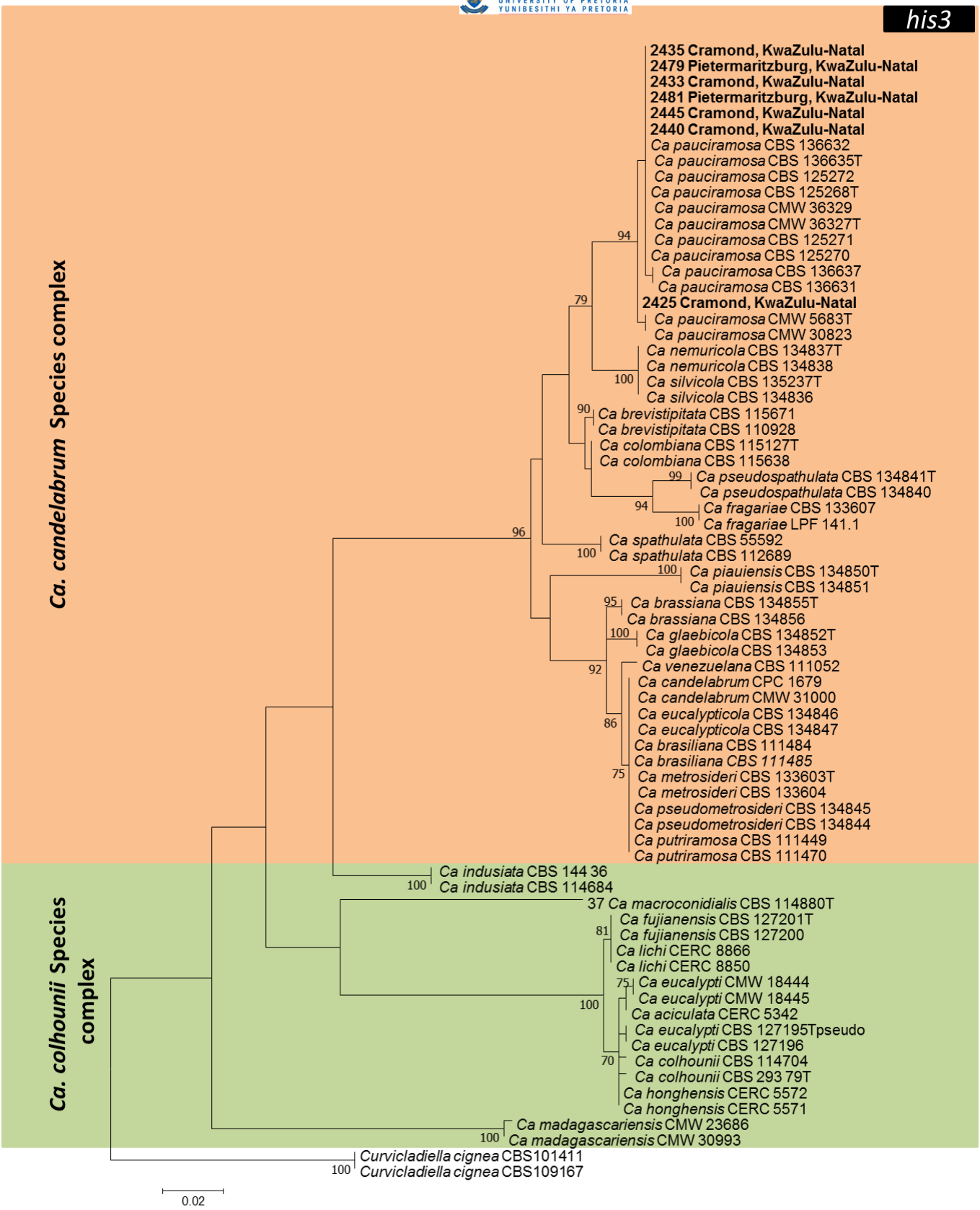


Fig. 6.

Phylogenetic tree based on maximum likelihood (ML) analysis of *his3* sequences for *Calonectria* spp. in the *Ca. candelabrum* and *Ca. colhounii* species complex. Bootstrap value ≥ 70% indicated at the nodes. Bootstrap values lower than 70% were removed. Isolates collected in this study are highlighted in **bold**. *Curvicladiella cignea* (CBS 101411 and CBS 109167) represent the outgroup.

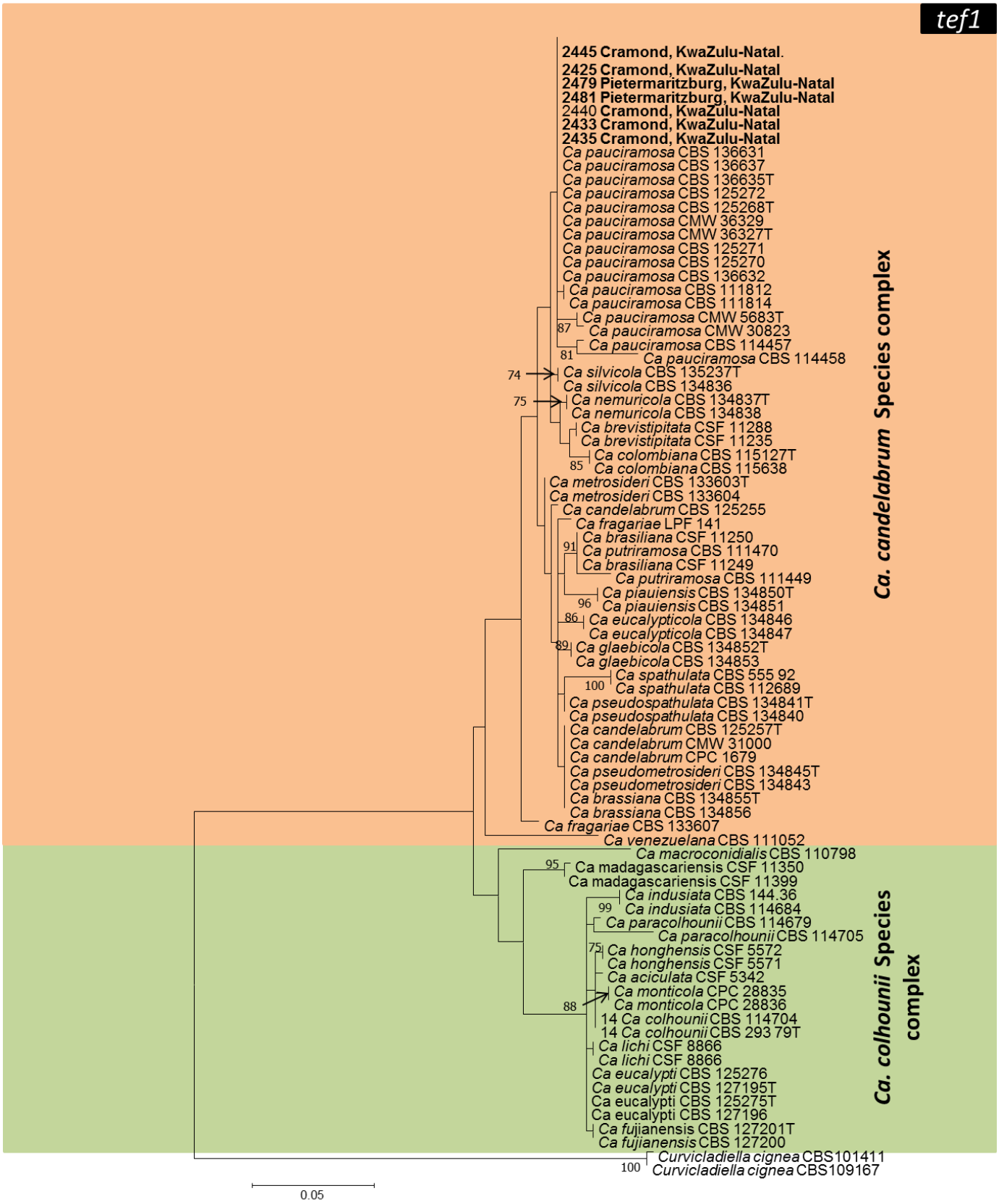


Fig. 7.

Phylogenetic tree based on maximum likelihood (ML) analysis of *tef1* sequences for *Calonectria* spp. in the *Ca. candelabrum* and *Ca. colhounii* species complexes. Bootstrap value ≥ 70% indicated at the nodes. Bootstrap values lower than 70% were removed. Isolates collected in this study are highlighted in bold. *Curvicladiella cignea* (CBS 101411 and CBS 109167) represent the outgroup.

Fig. 8.

Phylogenetic tree based on maximum likelihood (ML) analysis of *tub2*, *cmdA*, *his3* and *tef1* sequences for *Calonectria* spp. in the *Ca. candelabrum* and *Ca. colhounii* species complexes. Bootstrap value ≥ 70% indicated at the nodes. Bootstrap values lower than 70% were removed. Isolates collected in this study are highlighted in **bold**. *Curvicladiella cignea* (CBS 101411 and CBS 109167) represent the outgroup.

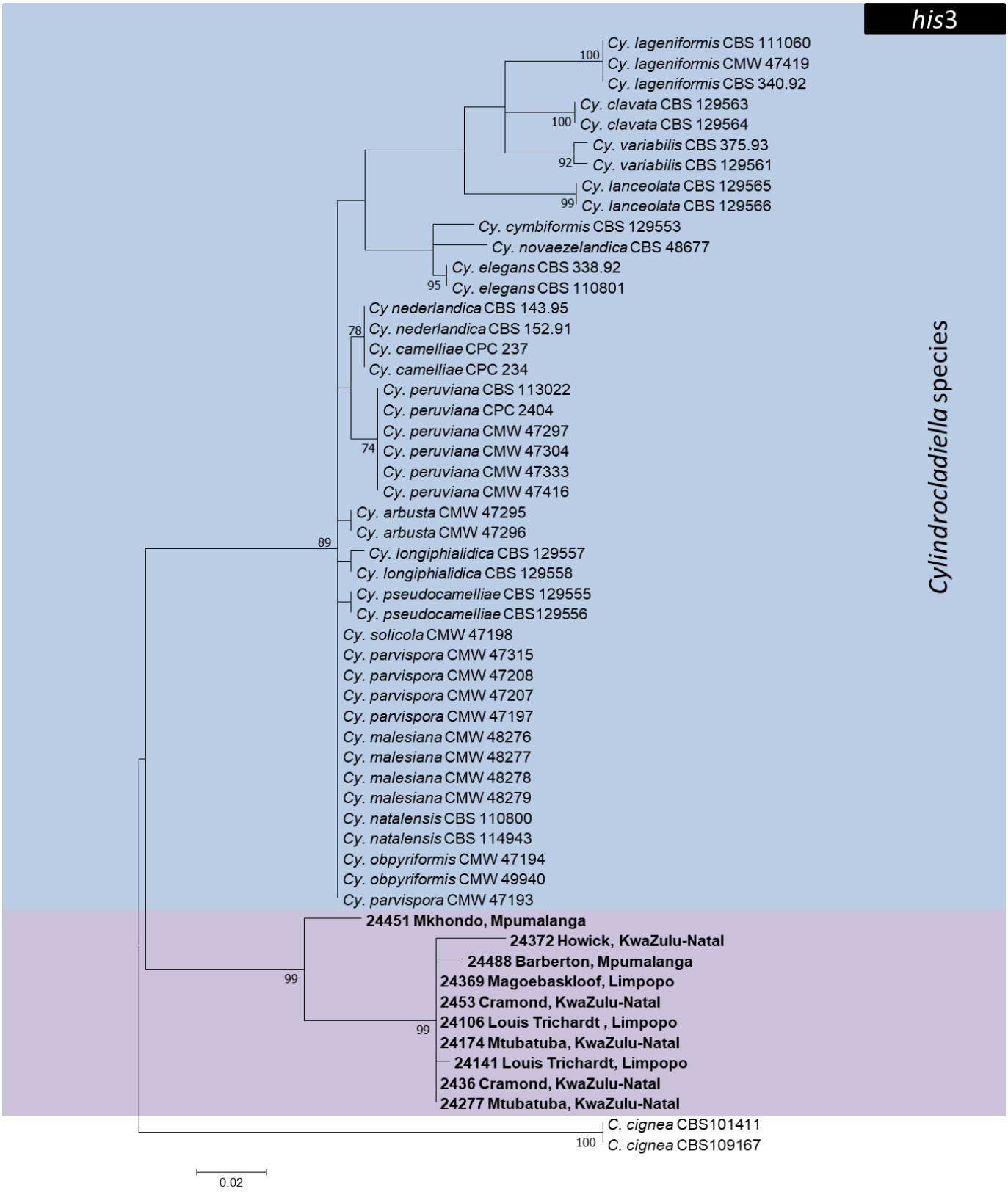


Fig. 9

Phylogenetic tree based on maximum likelihood (ML) analysis of *his3* sequences for *Cylindrocladiella* spp. Bootstrap value ≥ 70% indicated at the nodes. Bootstrap values lower than 70% were removed. Isolates collected in this study are highlighted in **bold**. *Curvicladiella cignea* (CBS 101411 and CBS 109167) represent the outgroup

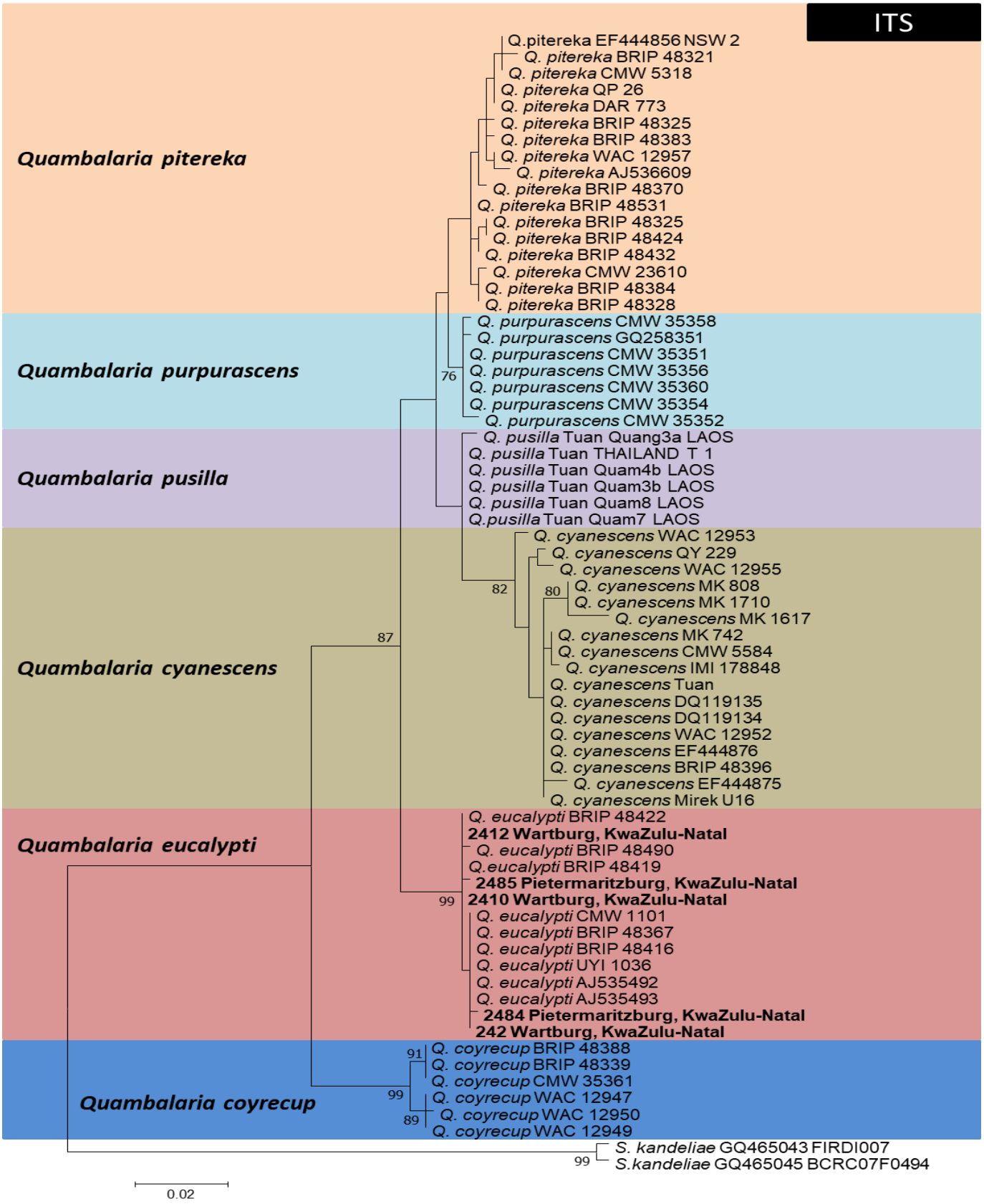


Fig. 10

Phylogenetic tree based on maximum likelihood (ML) analysis of *ITS* sequences for *Quambalaria* spp. Bootstrap value ≥ 70% indicated at the nodes. Bootstrap values lower than 70% were removed. Isolates collected in this study are highlighted in **bold**. *Sympodiomycopsis kandeliae* (FIRDI007 and BCRC07F0494) represent the outgroup

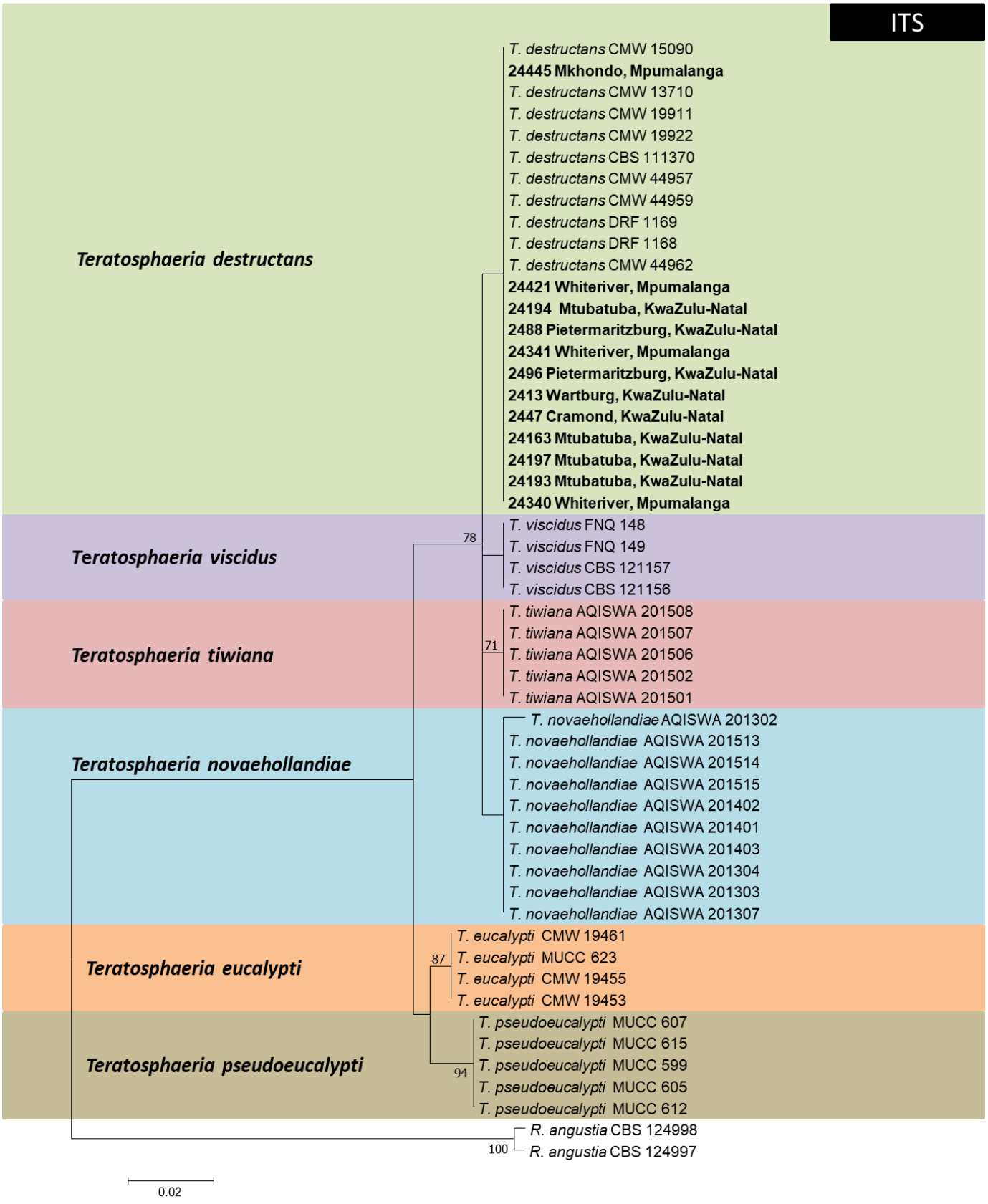


Fig. 11.

Phylogenetic tree based on maximum likelihood (ML) analysis of ITS sequences for *Teratosphaeria* spp. Bootstrap value ≥ 70% indicated at the nodes. Bootstrap values lower than 70% were removed. Isolates collected in this study are highlighted in **bold**. *Readeriella angusta* (CBS 124997 and CBS 124998) represent the outgroup

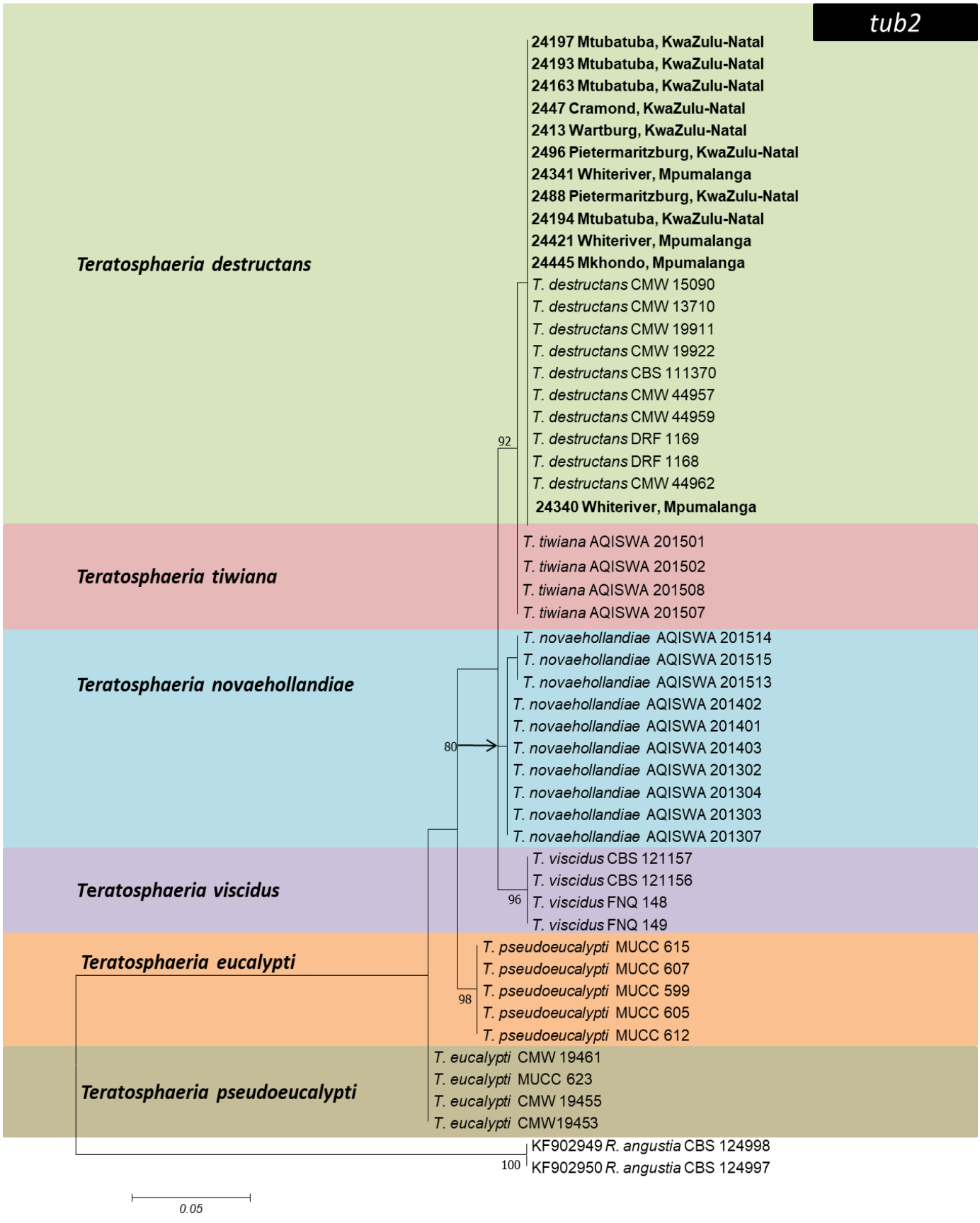


Fig. 12.

Phylogenetic tree based on maximum likelihood (ML) analysis of *tub2* sequences for *Teratosphaeria* spp. Bootstrap value ≥ 70% indicated at the nodes. Bootstrap values lower than 70% were removed. Isolates collected in this study are highlighted in **bold**. *Readeriella angusta* (CBS 124997 and CBS 124998) represent the outgroup

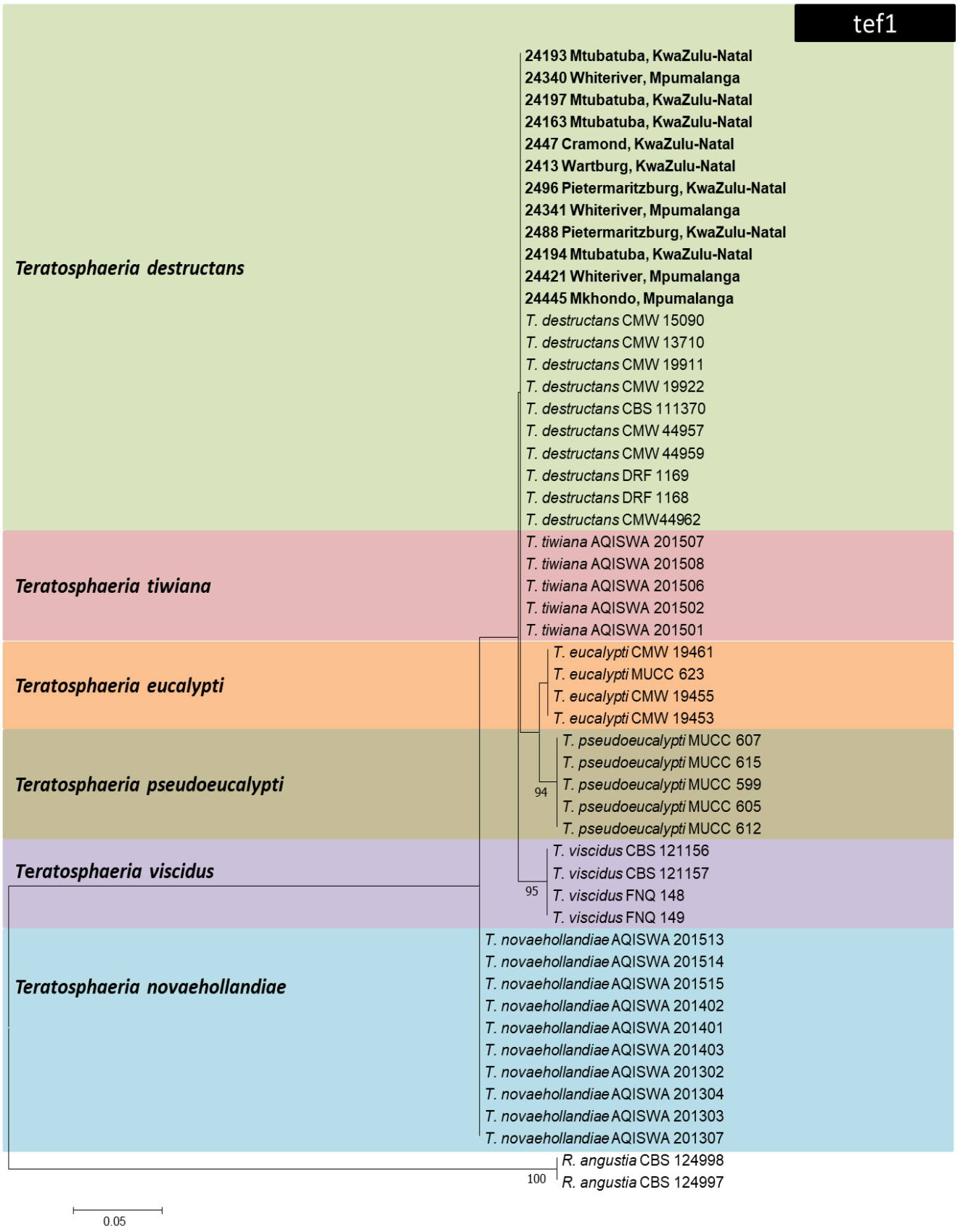


Fig. 13.

Phylogenetic tree based on maximum likelihood (ML) analysis of *tef1* sequences for *Teratosphaeria* spp. Bootstrap value ≥ 70% indicated at the nodes. Bootstrap values lower than 70% were removed. Isolates collected in this study are highlighted in **bold**. *Readeriella angusta* (CBS 124997 and CBS 124998) represent the outgroup

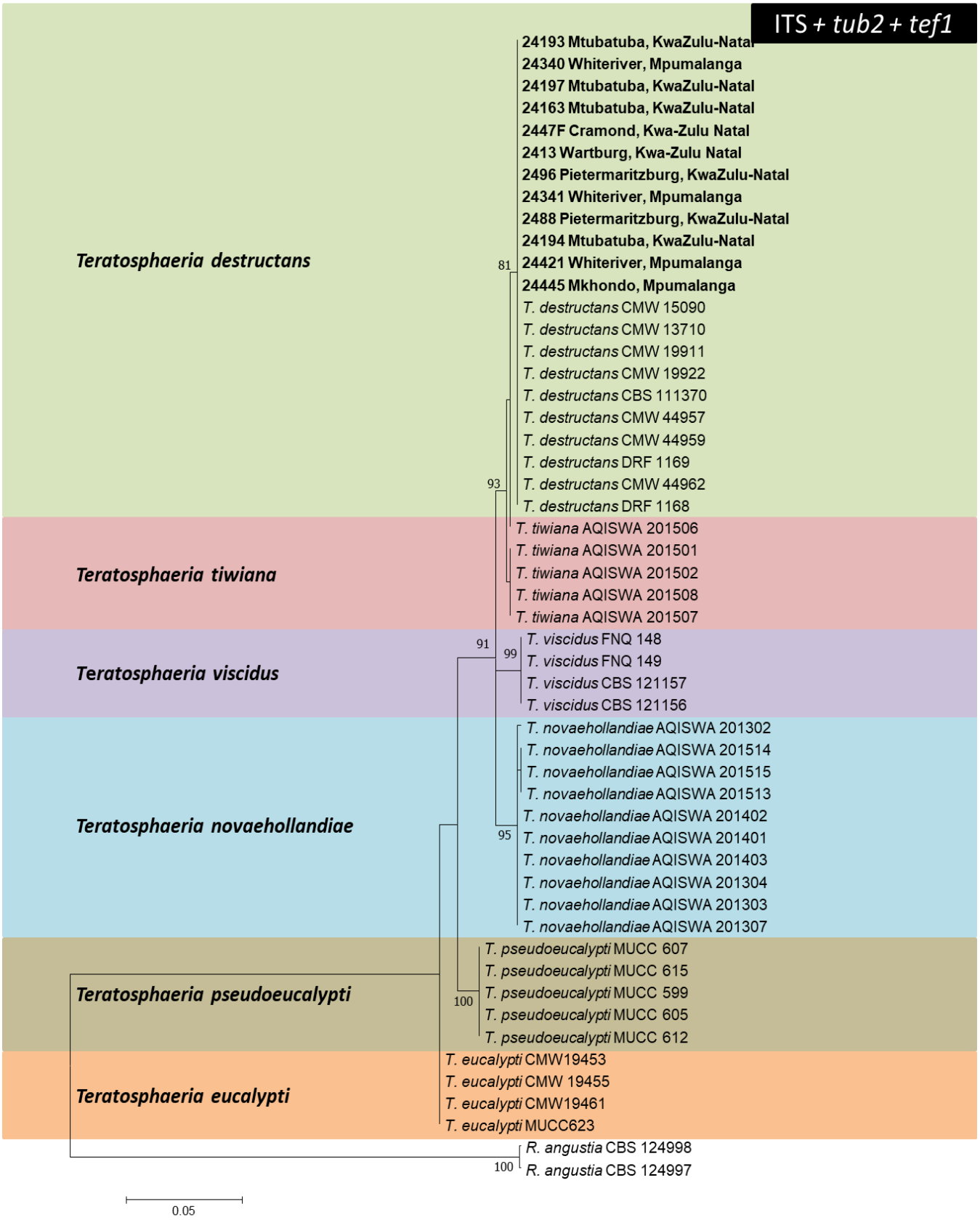


Fig. 14.

Phylogenetic tree based on maximum likelihood (ML) analysis of ITS, *tub2* and *tef1* sequences for *Teratosphaeria* spp. Bootstrap value ≥ 70% indicated at the nodes. Bootstrap values lower than 70% were removed. Isolates collected in this study are highlighted in **bold**. *Readeriella angusta* (CBS 124997 and CBS 124998) represent the outgroup

CHAPTER 3

IDENTIFICATION OF OPTIMAL ENVIRONMENTAL CONDITIONS FOR THE CULTURE GROWTH, CONIDIAL GERMINATION AND INFECTION OF *TERATOSPHAERIA DESTRUCTANS*

Abstract: *Teratosphaeria destructans* causes a serious leaf and shoot blight disease of *Eucalyptus* species and hybrids in South Africa and some countries in Asia. The pathogen was first reported from South Africa in 2016 and has now spread throughout most of the commercial plantation growing regions in the eastern parts of the country. Contrary to early predictions that it would be restricted to the sub-tropical regions of the country, the disease also affects *Eucalyptus* trees in the temperate regions of South Africa. Nothing is known of the infection biology and epidemiology of *T. destructans*. This information is important for predicting its distribution and for the development of screening techniques for the selection of disease tolerant genotypes. In this study, growth, germination and artificial inoculation studies were conducted in order to identify the optimal environmental conditions for *T. destructans*. Multiple experiments were done in order to develop the most effective protocols. Cultures of the fungus grown at temperatures between 10 and 30 ˚C with or without light, showed optimum growth at 25 ˚C (20.84 mm). Conidia obtained from cultures and naturally infected leaves were germinated at temperatures between 10 and 30 ˚C with or without light. The optimum germination temperatures for conidia from cultures were 20 and 25 ˚C; while that of conidia obtained from naturally infected leaves was 20 ˚C. The overall germination of spores obtained directly from naturally infected leaves was higher (88 %) than that of spores obtained from cultures (12 %). Attempts were made to get successful infection while simultaneously assessing and testing the effect of temperature and leaf wetness period in six separate experiments. In three experiments, no symptoms of infection were observed. Two experiments where symptoms developed were conducted using spores from cultures and one with spores obtained directly from naturally infected leaves. In all three experiments, symptoms of leaf blight were observed after incubation at optimum temperatures of 20 and 25 ˚C approximately four weeks after inoculation with leaf wetness periods ranging from two to eight days for spores from cultures and seven days for spores from infected leaves. The development of fruiting bodies took place between four and eight weeks after inoculation at both temperatures, although more abundantly at 25 ˚C. The results from this study can be used to inform the development of artificial inoculation protocols that can be used to screen *Eucalyptus* species.

INTRODUCTION

Species of *Teratosphaeria* are the most common pathogens associated with leaf diseases of *Eucalyptus* (Crous et al. 1998, Crous et al. 2004). These pathogens cause diseases known as Mycosphaerella leaf disease (MLD) and Teratosphaeria leaf disease (TLD) (Hunter *et al.* 2011). *Teratosphaeria* species that are regarded as the most important causal agents of leaf and shoot diseases in temperate regions are *T. cryptica* and *T. nubilosa* (Hunter et al. 2009, Burgess and Wingfield 2017). In addition, a number of other, mostly cryptic *Teratosphaeria* species are considered to be significant foliar pathogens causing leaf and shoot diseases in tropical and subtropical regions. These species have very similar morphological features and possibly similar biology and include *T. eucalypti, T. novaehollandiae, T. pseudoeucalypti, T. tiwiana, T. viscidus* and *T. destructans* (Andjic et al. 2007, Andjic et al. 2010a, Andjic et al. 2011, Andjic et al. 2016, Andjic et al. 2019). Of these species, *T. destructans* is of significant importance in South Africa where it causes considerable damage to especially *Eucalyptus* hybrids in the sub-tropical regions of the country (Greyling *et al.* 2016).

Teratosphaeria destructans causes a leaf, bud and shoot blight disease on *Eucalyptus* species that are planted in tropical and sub-tropical regions of South-East Asia and Africa (Park *et al.* 2000, Old *et al.* 2003). This pathogen was first discovered in Sumatra, Indonesia causing serious leaf disease on 1-3 year old *E. grandis* in 1996 (Wingfield *et al.* 1996). It has since been reported from China, East Timor, Lao, Malaysia, Thailand, Vietnam and South Africa (Wingfield *et al.* 1996, Old *et al.* 2003, Burgess *et al.* 2006, Greyling *et al.* 2016). Symptoms of *T. destructans* are characterized by large brown to purple sub-circular leaf spots with diffuse borders associated with a purple discolouration on the lesion margins of younger leaves, buds and shoots (Wingfield *et al.* 1996, Andjic *et al.* 2007, Burgess *et al.* 2007, Andjic *et al.* 2010b). The disease may result in severe defoliation, die-back and possibly tree death (Wingfield *et al.* 1996, Burgess *et al.* 2006, Burgess *et al.* 2007).

The biology of *T. destructans* has not been well studied. However, research on the closely related temperate species, *T. cryptica*, which is ranked with *T. destructans* in terms of its ability to cause devastating leaf diseases (Park 1988a, Andjic *et al.* 2007), may offer some clues to the biology of *T. destructans*. Infection of *T. cryptica* takes place by conidia and ascospores (Beresford 1978, Ganapathi 1979, Park and Keane 1982b). For *T. destructans*, only the asexual state (conidia) has ever been found (Wingfield *et al.* 1996, Burgess *et al.* 2006) although data from whole genome sequences (Havenga *et al.* 2020) shows that it has a

heterothallic mating system. Both *T. cryptica* and *T. destructans* infect the leaves by penetrating through the stomata on the abaxial leaf surface (Park and Keane 1982b, Havenga *et al.* 2021)although *T. cryptica* has also been shown to penetrate through the cuticle on the adaxial surface (Park and Keane 1982b, Park 1988a).

The environmental, pathogen and host factors impacting infection of *E. delegatensis*, *E. globulus* and *E. obliqua* by conidia of *T. cryptica* included temperature, inoculum concentration, leaf surface penetration, leaf age and leaf wetness period (Beresford 1978, Park and Keane 1982b, Park 1988a, Park 1988b). In artificial inoculations with conidia obtained from naturally infected leaves, infection took place at temperatures of 15, 20, 25 and 30 ˚C, with optima of 20 ˚C (Park 1988a) and 15 ˚C (Park and Keane 1982b). However, Ganapathi (1979) found that impact of temperature on infection of *E. delegatensis* and *E. regnans* by *T. cryptica* depended on light conditions, the optimum temperature in the dark was 25 ˚C, but 18 ˚C in the light. Infection took place at leaf wetness periods ranging from two to seven days, with the optimum being five to seven days (Park 1988a). The leaf wetness period of five to seven days produced severe symptoms appearing between four and eight weeks after inoculation. Increased leaf wetness period (more than seven days) resulted in increased disease severity while lower leaf wetness periods (less than two days) resulted in no symptom development (Park 1988a). Studies conducted in the field revealed that water splash dispersal of conidia was important in disease spread within a tree and between trees (Beresford 1978). *Teratosphaeria cryptica* has a polycyclic epidemic cycle which means it has repeated infection cycles that are completed within one plant growth season (Park 1988b). Inoculum concentrations of 10^4 and 10^5 spores ml^{-1} produced severe symptoms such as blight lesions, premature leaf loss and petiole and stem infections, with the latter concentration affecting more than 50 % of the leaf surface (Park 1988a). Infection took place from both the abaxial and adaxial leaf surfaces on young expanding leaves (less than 46 days old on the first four nodes) (Park and Keane 1982b, Park 1988a).

To address the current lack of knowledge of the biology and epidemiology of *T. destructans* and support the development of control strategies, the aim of this study was to: (1) study the optimum temperature and light conditions required for *T. destructans* culture growth; (2) study the optimum temperature, light conditions and spore source (spores obtained from fresh leaves and spores obtained from cultures) required for the conidial germination of *T. destructans*; (3) investigate the environmental factors required for infection by *T. destructans*

such as temperature, leaf wetness period and spore source. This was achieved by conducting detailed studies under controlled conditions in the laboratory and phytotron.

MATERIALS AND METHODS

Source of fungal material

Cultures of *T. destructans* were obtained from isolations made from cirri on foliage samples of *E. grandis* and *E. grandis* x *E. urophylla* hybrids collected from sites in KwaZulu-Natal and Mpumalanga in 2016 and 2017 (Table 1). KwaZulu-Natal collections were made from a nursery in Cramond and plantations in Pietermaritzburg. Mpumalanga collections came from plantations in Mkhondo. Isolations were made under a dissecting microscope (ZEISS Stereo Discovery.v8). Using a sterile needle, cirri were plated onto 2% Malt Extract Agar amended with streptomycin (MEAS) (20g/L Biolab malt extract, 15g/L Biolab agar, Midrand, South Africa, 100mg/L streptomycin, Biotech laboratories, Midrand). The plates were incubated at room temperature (25°C). To obtain pure cultures, single hyphal tips and/or spore drops were transferred to fresh Malt Extract Agar (MEA) using a dissecting microscope and incubated under the same conditions for approximately four weeks.

In some germination and inoculation experiments, fresh spores from leaf samples were used. For these experiments, the spores were harvested using a sterile scalpel directly from the leaf samples and suspended in 0.05 % distilled water with Tween 20 (DWT20) or just distilled water in a falcon tube. The leaf samples of *E. grandis* x *E. urophylla* from which spores were obtained were collected from Wartburg and Mtubatuba, KwaZulu-Natal in 2018 (Table 2). These leaf samples were kept in brown bags and placed at room temperature (25 ˚C) for no longer than one week before use.

Growth studies: The effect of light and temperature on culture growth

An experiment was conducted to identify the optimal light and temperature conditions for growth of *T. destructans* in culture. This experiment was initiated on 30 July 2018 using six isolates obtained from KwaZulu-Natal and Mpumalanga (Table 1). Using a cork borer, 2 mm diameter plugs were cut from 5-week-old actively growing *T. destructans* cultures and placed with mycelium facing down at the centers of 55 mm Petri dishes containing 2% MEA. The experiment was conducted using light (c. 15 µmol m^{-2} s^{-1}) and dark conditions at five temperatures (10, 15, 20, 25 and 30 °C). Plates placed under the dark treatment were wrapped

in aluminum foil. Plates were randomized and placed in incubators set to the required temperature. There were five replicate plates for each treatment combination (isolate × temperature × light; 300 plates in total). Growth was measured at five weeks by taking two measurements of the colony diameter perpendicular to each other. For analysis, the two measurements were averaged. This experiment was repeated on the 17th of September 2018 using the same design and conditions.

Germination studies: The effect of light and temperature on germination of spores from culture and infected leaves

The first experiment was initiated on the 19th of April 2018. This experiment was conducted to test protocols and techniques required to determine the optimum conditions required for the germination of spores obtained from cultures. Conidial masses from three five-week old cultures (Table 1) were separately suspended in 50 ml of distilled water, stirred and adjusted to 1 × 10^{5} spores ml^{-1} using a haemocytometer. Aliquots of 100 µl were immediately pipetted onto sterile microscope slides that were placed on 90 mm 1.5 % water agar (WA) plates (15g/L Biolab agar, Midrand, South Africa). Plates to be incubated in the dark were wrapped in aluminum foil. All the plates (light and dark) were randomized and incubated at either 20 or 25 °C. The selection of these temperatures was based on the results obtained from the growth studies. There were five replicate plates for each treatment combination (isolate × temperature × light; 60 plates in total). Germination was assessed after one day (24 hours), two days (48 hours) and three days (72 hours) at a ×10 magnification under a compound microscope. The germination status of 100 spores within randomly chosen fields of view was recorded (between 2 and 5 fields of view).

The second experiment, initiated on the 18th of July 2018, used spores obtained directly from ten leaves with abundant cirri that were collected from Wartburg and Mtubatuba, KwaZulu-Natal (Table 2). This experiment was conducted to test the above protocols and techniques on spores taken from naturally infected leaves and the effect of temperature on germination. Conidia were scraped off ten different leaf samples and separately suspended in 50 ml of distilled water and adjusted to 1 × 10^{5} spores ml^{-1} using a haemocytometer. The design of the experiment was the same as the first one with two exceptions; six temperatures were used (10, 15, 20, 25, 30 and 35 °C) and germination was assessed at a one day (24 hours) and two day (48 hours) period as it was found, in Experiment 1, that after 2 days the majority of

spores had germinated. Each leaf sample was treated as a replicate per temperature and light treatment combination (leaf spore sample × temperature × light; 120 plates in total).

For the third experiment, germination of spores from both sources was assessed; spores obtained from sporulating cultures and spores obtained directly from naturally infected *Eucalyptus* leaf material (Table 2). This experiment, initiated on the 13th of November 2018, was conducted to directly compare the viability of spores from the two sources and to identify the optimal temperature for germination. The design was the same as the second experiment with four exceptions. Only the dark condition was tested, spores from six cultures and five leaves were used, five temperatures (10, 15, 20, 25 and 30 °C) were used and germination was assessed only after a three day (72 hours) period. Under each temperature treatment, there were five replicate plates for each isolate and five plates in total for spores from leaves (isolate × temperature, 150 plates; leaf spore sample × temperature, 25 plates; 175 plates in total).

Artificial inoculation studies

Source of plant material

Eight-month old *E. grandis* x *E. urophylla* clonal (W1700) cuttings in Unigrow plugs were obtained from a forestry company in KwaZulu-Natal in March 2016. They were initially kept and maintained in a 25 ˚C phytotron at the University of Pretoria, FABI. They were re-planted into 0.75 L black plant bags on the 22nd of August 2017 and were maintained at the experimental farm at the Future Africa campus for three months until they were transported back to the phytotrons at the University of Pretoria, FABI to acclimatize. The plants were regularly watered and pruned for the development of new young leaves two months after the first experiment and before each of the experiments that followed afterwards.

Spore suspensions

Under a dissecting microscope, and using a sterile needle, cirri were picked from cultures and suspended in 40 ml of either 0.05 % distilled water with Tween 20 (DWT20) or just distilled water in a falcon tube. The spore suspensions were obtained from literature (Park 1988a), where artificial inoculation experiments were done using a different *Teratosphaeria* species. The spore concentration was estimated using a haemocytometer and adjusted to 6.5 x 10^5 spores ml^{-1} consistently for all experiments except one (experiment five) which had a slightly higher spore concentration of 9.5 x10^5 spores ml^{-1} due to a high spore load on the source leaves. Because the concentration was very similar to that of the other experiments, also in

the 10^5 range, it was not adjusted. The spores were applied immediately after determining the concentrations. High concentrations were selected as they contained abundant conidia. Spores obtained directly from leaf material were scraped off using a sterile scalpel and suspended in 120 ml of distilled water. The spore concentration was estimated using a haemocytometer and adjusted to 6.5 x 10^5 spores ml^{-1}. A LPH80 spray gun connected to a IS 875HT Smart Jet Tubular Compressor (Iwata, Portland, Oregon) was used to inoculate all the plants by applying the spore suspension to both the adaxial and abaxial leaf surfaces of all leaves.

The effect of temperature, leaf wetness period and spore source on infection by T. destructans

A total of six artificial inoculation experiments were conducted (Table 3) on the *E. grandis* x *E. urophylla* W1700 clone over three years with regular pruning between each experiment. Each experiment was done subsequently to the previous one by adjusting protocols based on experiences from the previous experiment. The aim of the experiments was to find the most appropriate inoculation protocol for *T. destructans*. Leaves of different age classes were inoculated in order to monitor which leaf stages are infected by the pathogen. At the same time the effect of temperature and leaf wetness was also investigated. Symptom development was monitored from four weeks post inoculation, with final evaluations at eight weeks. The first experiment took place on the 11th of May 2017. The aim was to test the effect of temperature (20, 25 and 28 °C) and leaf wetness period (12 hours, one, four, six and eight days) on infection by *T. destructans*. Spores from cultures of two isolates (2433 and 2488) were separately suspended in DWT20 with a concentration of 6.5 x 10^5 spores ml^{-1}. Each spore suspension was applied to 90 one-year-old seedlings. A total number of 90 plants were sprayed with a DWT20 solution without spores as negative controls. Each inoculation treatment was then divided into three groups of 30 plants each. These were independently placed in dew chambers consisting of large, clear sealed autoclave bags that had been moistened internally with distilled water. One dew chamber of each inoculum treatment was then placed in one of three phytotrons (20, 25 and 28 °C). Six plants were removed from each dew chamber at each leaf wetness period and were placed on a shelf in the same phytotron. The presence of symptoms was assessed regularly for eight weeks. To test the viability of spores and whether or not they germinated, aliquots of 100 µl from the same suspension were pipetted onto sterile microscope slides. These were placed on 90 mm water agar (WA) plates and incubated with the inoculated plants and were assessed a day later using a compound microscope. The same spore viability assessment was applied for all the experiments listed below.

The second experiment was initiated on the 5th of January 2018 using spores from cultures of a different isolate (2496) as spores from the two cultures used in experiment one did not provide infection. The main difference of this experiment compared to the first is that distilled water was used instead of DWT20. Fifty plants were inoculated and incubated as above at 20 or 25 ˚C with leaf wetness periods of six and 12 hours, one, two and eight days. A total number of 50 plants were sprayed with distilled water without spores to act as negative controls. Each inoculation treatment was then divided into two groups of 25 plants each and was placed in dew chambers. Twenty-five inoculated plants and 25 mock-inoculated negative control plants (50 plants) were incubated at each temperature. Five plants of each inoculation treatment were removed from the dew chamber at each leaf wetness period and placed under the same temperature conditions for seven weeks. The presence of symptoms was assessed regularly for seven weeks.

The third experiment was initiated on the 7th of July 2018. This experiment was conducted using spores obtained directly from naturally infected leaves, by using fresh spores obtained from ten leaves containing abundant cirri. Two temperatures (20 and 25 ˚C), one leaf wetness period (seven days) and two leaf sources (from Wartburg and Mtubatuba) were tested in this protocol. Two spore suspensions were made per leaf source using distilled water and applied to 20 pruned plants with new and freshly developed leaves. A total number of 20 plants were sprayed with distilled water without spores as negative controls. Each inoculation treatment was then divided into two groups of ten plants each and was placed in the dew chambers (five plants per bag) following the above descriptions. Dew chambers were placed in one of two phytotrons (20 and 25 °C). Each phytotron contained 10 inoculated plants and 10 mock-inoculated negative control plants (20 plants). Only one leaf wetness period was tested, thus all the plants were removed from the dew chambers after seven days and placed under the same temperature conditions. The presence of symptoms was assessed regularly for eight weeks.

The fourth experiment was initiated on the 1st of October 2018, also using spores obtained directly from four naturally infected leaves that had abundant cirri. The aim of this study was to test leaf wetness period (zero, two, four and seven days) under the temperature (20 ˚C) found to be optimal in experiment 3. Four leaf sources (two from Wartburg and two from Mtubatuba) were used in this experiment. Four spore suspensions containing distilled water were made per leaf (one per leaf) and applied to 24 pruned plants with new and freshly

developed leaves. A total number of 24 plants were sprayed with distilled water without spores to act as negative controls. The plants were placed in dew chambers (four plants per bag) following the above descriptions. Two inoculated plants per leaf source (eight plants) and eight negative control plants were not placed in dew chambers as their leaf wetness period was zero. Dew chambers were placed in one phytotron (20 ˚C). Two plants were removed from the dew chamber at each leaf wetness period and placed under the same temperature conditions. The presence of symptoms was assessed regularly for eight weeks.

The fifth experiment was initiated on the 18th of March 2019 using spores obtained directly from one leaf (from Wartburg) that had abundant cirri. The aim of this experiment was to set up and test a new protocol and technique based on lessons learned from the first four experiments. In this experiment a large 2m x 1m dew chamber structures made from a steel frame and thick plastic, instead of using the multiple dew chambers made from individual polythene bags used in the first experiments. Two temperatures (20 and 25 ˚C) and longer leaf wetness periods (one, four, five, seven and ten days) were tested. Each phytotron (20 and 25 °C) was layered with a thick plastic on the floor with 15 trays placed on top of it. A concentration of 9.5 x10^{5} spores ml^{-1} was used and was more concentrated than the suspensions used for the other experiments. The spore suspension was made using distilled water and applied to 100 plants that were pruned and had new and freshly developed leaves. A total number of 50 plants were sprayed with distilled water without spores to act as negative controls. Each inoculation treatment was then divided into two groups of 75 plants from which smaller groups of five were put in trays (each tray contained five plants). Each phytotron therefore contained 50 inoculated plants and 25 negative control plants distributed in 15 trays. Warm water (40-50 °C) was poured on the polythene sheets to build up moisture and the plants were immediately covered by the dew chamber structure. At each leaf wetness period, ten plants were removed from the chamber structure and placed under the same temperature conditions for seven weeks. Warm water was poured again on the polythene sheets before closing the dew chamber to maintain humidity. The presence of symptoms was assessed regularly for eight weeks.

Scanning electron microscope (SEM) of spores on the leaf surface

A 6th artificial inoculation experiment was initiated on the 5th of March 2018 (between the second and the third experiment). The aim of this experiment was different from the others; it was conducted specifically to see what happens to the spores when they land on the leaf

surface; whether they are alive and germinating or dead. The spores were visualized using a scanning electron microscope (SEM). For this experiment, two temperatures (20 and 25 °C), culture spores from one isolate (2496) as well as three leaf wetness periods (one, four and seven) were used. The spore suspension (6.5 x 10^5 spores ml^{-1}) was applied to four pruned plants with new and freshly developed leaves. There were no negative controls. The inoculated plants were divided into two groups of two plants for each temperature and were placed in the dew chambers following the same protocols as in experiments one to four. Two leaves were detached from the dew chambered plants at each leaf wetness period and the plants were removed on the day of the last leaf wetness period. The detached leaves were placed in open petri dishes and left in the phytotrons until the day of the last leaf wetness period. From there, the leaves were taken for assessment and visualization at the SEM facility at the University of Pretoria. The presence of symptoms from the remaining plants was assessed regularly for eight weeks.

SEM protocol

Leaf samples were cut into small 1 mm^3 pieces and placed in empty 2ml Eppendorf tubes. Glutaraldehyde/ Formaldehyde (2.5 %) (GA/FA fixative; 1 ml GA, 1 ml FA, 3 ml dH_2O) was added to the tubes and incubated for 60 minutes. After the fixative solution was removed, the leaf samples were washed with a phosphate washing buffer (50 % $NaPO_4$: 50 % H_2O) three times for 15 minutes per wash. The washing buffer was removed and discarded. In the fume hood, the leaves were suspended in Osmium Tetroxide for 60 minutes. Leaf samples were washed three times with the phosphate washing buffer for 15 minutes, with the first wash still in the fume hood. After removing and discarding the buffer, the leaf samples were then dehydrated using a graded series of ethanol (30, 50, 70, 90 and three times 100 %) for 15 minutes each and 30 minutes for the final step in 100% ethanol. In the fume hood, the leaf pieces were suspended in a 50:50 mixture of Hexamethyldislizane (HMDS) and 100 % ethanol and incubated covered for 60 minutes. Two additional drops (~ 2 µl) of HMDS were added to the leaf pieces and were left uncovered to evaporate and dry overnight. Aluminium stubs were washed with methanol and the leaf samples were mounted on them using carbon tape. The leaf samples were coated with carbon three times using two carbon rods. The samples were visualized using a high resolution JEOL 6000 with EDAX facilities.

Statistical analysis

Data visualization and statistical analyses for all data were carried out in R software (R Core Team, 2014). Different methods were used to transform data towards normality until near-normality was achieved. Each analysis started with a maximum model (including all explanatory variables and their interactions) before identification of the minimal adequate model through model simplification using the stepwise procedure.

Growth studies

Culture growth data were log transformed before analysis. Polynomial regression models (in the form, $y = a + bx + cx^2 + dx^3$) were created using the linear modelling (lm) function to assess the impact of temperature and light on culture growth. The explanatory variables were experiment (repeat), isolate, light treatment, temperature and their interactions. Experiments one and two were later analysed separately.

Germination studies

The spore germination datasets from each experiment were analysed separately. Germination data of all three experiments were arcsine transformed and experiment three was further log transformed. Polynomial regression models were also created using the linear modelling (lm) function to assess the impact of temperature, light and spore type on spore germination. Spore type, light treatment and temperature were included as explanatory variables in the analyses. Data from experiments with repeated measures on separate days were separated into datasets for each day to avoid pseudoreplication. Experiment one was therefore re-analysed separately into days one, two and three while experiment two was re-analysed separately into days one and two. Experiment three was re-analysed separately into spores obtained from cultures and those obtained from leaves.

RESULTS

Growth studies: The effect of light and temperature on culture growth

Cultures grew within a temperature range of 10-30 ˚C. Generally, there was very little growth at 10 °C, intermediate growth at 15 and 30 °C, and most growth occurred at 20 and 25 °C, with more growth at 25 °C (Fig. 1a - b; 20.84 mm). There was no statistical difference in growth of cultures under light or dark conditions. There was a significant four-way interaction between experiment, temperature, light and isolate (ANOVA, d.f = 15, F = 34.43, $P < 0.001$) showing that the relationship between temperature and growth varied with isolate, light, experiment and their interactions. Experiments were re-analyzed separately to inspect these relationships further. For the experiment one data, there were two-way significant

interactions between light and temperature (ANOVA, d.f = 3, F = 6.3877, P < 0.001; Table 4), isolate and temperature (ANOVA, d.f = 15, F = 5.6761, P < 0.001) and isolate and light treatment (ANOVA, d.f = 5, F = 5.2740, P < 0.001). In experiment two, there was a significant three-way interaction between temperature, isolate and light (ANOVA, d.f = 15, F = 5.7514, P < 0.001; Table 5). The results from both experiments showed a clear indication that 25 ˚C was the optimum temperature for growth, with or without light conditions. P-values (<0.001) also support the significant two-way interactions between temperature and isolate, temperature and light and that of isolate and light from both experiments.

Germination studies: The effect of light and temperature on germination of spores from culture and infected leaves

In the first experiment, the germination of spores obtained from cultures were investigated at temperatures of 20 and 25 ˚C (Fig. 2 - 4). These temperatures were selected based on results obtained for the culture growth studies explained above. Germination rates were low for all three isolates at both temperatures, reaching mean percentages of 8.23 % on day one, 13.08 % on day two and 15.37 % on day three from both light and dark conditions. The relationship between temperature and germination was inconsistent between isolates and light treatments. There was a significant three-way interaction between temperature, isolate and light on day one (ANOVA, d.f = 2, F = 3.6925, P < 0.05; Table 6), day two (ANOVA, d.f = 2, F = 8.7803, P < 0.001; Table 7) and day three (ANOVA, d.f = 2, F = 4.7177, P < 0.05; Table 8).

From the second experiment, germination of spores obtained from leaf material was assayed at temperatures between 10-35 °C. Low germination percentages were observed at 10 (day one = 11.7 % and day two = 18.6) and 35°C (day one = 27.2 % and day two = 36.7 %) and greater germination was observed at 20 and 25 °C on day one (75.65 %) and day two (83.6 %). The highest germination percentage was observed at 20 °C (Fig. 5 & 6). The effect of light was not significant (P > 0.05). Temperature had a highly significant effect on spore germination (ANOVA, d.f = 3, F = 32.47, P < 0.001) on both days.

In the third experiment, germination was analyzed separately for two spore sources: spores obtained from cultures (Fig. 7a) and those obtained from leaf material (Fig. 7b) at five temperatures ranging from 10 - 30 °C. Spores obtained directly from infected leaves germinated better than spores obtained from cultures. For spores obtained from cultures, germination on day three at 20 was only 12 %, compared to 88 % for spores obtained from leaves. Germination of spores for both sources were lowest at 10 and 30 °C, intermediate at

15 °C and greatest at 20 and 25 °C. There was a highly significant two-way interaction between temperature and isolate (ANOVA, d.f = 15, F = 2.8725, P < 0.001; Table 9) therefore, the relationship between temperature and germination varied between the isolates.

Artificial inoculations: The effect of temperature, leaf wetness period and spore source on infection by T. destructans

Symptoms developed on young and intermediate leaves of the inoculated *E. grandis* x *E. urophylla* plants after four to eight weeks in experiments two (Table 10), three (Table 11) and six (Table 12). No symptoms or signs of infection were observed in experiments one, four and five. Symptoms did not appear on negative control plants in any experiment.

Experiment two was conducted using spores obtained from cultures of one isolate (2496). Leaf blight symptoms developed at 20 and 25 °C with leaf wetness periods of two to eight days. Leaf blight symptoms were observed on all five plants incubated with leaf wetness period of eight days at both 20 and 25 °C. Symptoms were observed on fewer plants incubated with a leaf wetness period of two days at 20 °C (40 %, n = 5) and 25 °C (60%, n = 5). No symptoms were observed on plants exposed to leaf wetness periods of 6, 12 or 24 hours at either temperature. Fruiting bodies of *T. destructans* were observed mostly at 25 °C with a leaf wetness period of eight days (80 %, n = 5). At 20 °C, fruiting bodies developed on 40 % of plants exposed to an eight day wetting period (Fig. 8a). No fruiting bodies developed on plants exposed to leaf wetness periods less than eight days.

Experiment three was conducted using spores obtained from naturally infected leaves from Mtubatuba and Wartburg. Symptoms of leaf blight developed on plants exposed to a leaf wetness period of seven days at both 20 and 25 °C. Under this wetness period, leaf blight symptoms were observed on 80 % (Mtubatuba) and 100 % (Wartburg) of plants at 25 °C, while symptoms were observed on 40 % (Mtubatuba) and 60 % (Wartburg) of plants at 20 °C. Fruiting bodies of *T. destructans* were observed on more plants at 25 °C (60 %, n = 5) from both areas, compared to 20 °C (20 %, Mtubatuba and 40 %, Wartburg, n = 5) (Fig. 8b - c).

Scanning electron microscope (SEM) of spores on the leaf surface

In this experiment, two leaves were detached from two plants each at each leaf wetness period (one, four and seven days) and the rest of the four plants were left in the phytotrons until the last leaf wetness period was completed (seven days). Leaf blight symptoms

developed at both 20 and 25 °C with 100 % of plants infected at a leaf wetness period of seven days. Fruiting bodies of *T. destructans* were observed on both plants at 25 °C and on one of the two plants at 20 °C (Fig. 8d).

From the detached leaves, the SEM revealed the presence of *T. destructans* conidia that had been applied during the artificial inoculation experiment although no symptoms were observed at these time points. Conidia were seen on the leaf surface at a leaf wetness period of one day (Fig. 9). Germ tubes were observed on the samples harvested after four days (Fig. 10) and after seven days, germ tubes extended around the stomatal area (Fig. 11). The development of germ tubes did not vary with temperatures. Sporulation took place after infection at a leaf wetness period of seven days (Fig. 12). This confirms that spores of *T. destructans* were alive post inoculation as seen on the abaxial leaf surface. It can thus be ruled out that the unsuccessful experiments were due to spores dying upon inoculation.

DISCUSSION

This study was the first to investigate the optimum spore germination, growth and infection conditions for the important leaf pathogen *Teratosphaeria destructans*. It provides information that increases the understanding of the biology of *T. destructans* and will help in future studies to select disease tolerant planting material and predict possible disease outbreak areas. From these studies it is now known what the optimal growth and spore germination conditions are for this important pathogen. An artificial screening protocol was also developed which can be adjusted and used in screening of *Eucalyptus* genotypes against *T. destructans*.

The optimum temperature for culture growth and conidial germination of *T. destructans* were identified. Growth of *T. destructans* cultures was greatest at 25 ˚C, with light conditions and isolate having inconsistent effects. Similar to some other *Teratosphaeria* species, optimal spore germination for *T. destructans* was at a lower temperature, 20 ˚C, than for culture growth. A similar trend was reported for *T. nubilosa* and *T. parva* which had an optimum germination temperature of 20 ˚C. However, their germination took place via ascospores. For *T. cryptica* conidia, germination was most rapid at a lower temperature of 15 ˚C (Park and Keane 1982b). The average optimal spore germination and growth temperatures identified in this study for *T. destructans* are considered tropical/subtropical and are thus as expected for the pathogen which is considered a subtropical pathogen (Park *et al.* 2000, Old *et al.* 2003).

Major *Eucalyptus* growing areas in South Africa such as KwaZulu-Natal and Mpumalanga have tropical/subtropical regions and could therefore be at higher risk of damage by *T. destructans*. Susceptible *Eucalyptus* species from these areas, where *T. destructans* is more likely to be in abundance, could suffer severe loss. Impacts that can be caused by *T. destructans* in these areas include severe defoliation, shoot death and loss of apical growth (Old *et al.* 2003) which may result in reduced growth and vigour (Burgess *et al.* 2006).

Artificial inoculations revealed that *T. destructans* was able to infect young to intermediate aged leaves of *E. grandis* x *E. urophylla* at 20 and 25 ˚C and leaf wetness periods ranging from two to eight days. However, fruiting bodies of *T. destructans* only developed with a leaf wetness period of more than seven days and fruiting bodies were observed on slightly more plants inoculated at 25 ˚C than 20 ˚C. No symptoms were observed on older leaves. These results are similar to those reported for *T. cryptica* where a leaf wetness period of seven days was optimum while those of two days or less had no sign of infection (Park 1988a). Although Park (1988a) indicated that infection took place from a leaf wetness period of as little as two days, he also highlighted that the development of symptoms intensified as the leaf wetness period increased. In the case of the current study, infection took place from a leaf wetness period of as little as two days, but no fruiting bodies developed and symptoms developed on far more plants exposed to more than seven days leaf wetness. In New Zealand, infection by *T. cryptica* also occurred during flushing of new leaves (juvenile developing leaves) and at prolonged leaf wetness periods (Cheah 1977, Beresford 1978). Infection of young and intermediate leaves of *E. grandis* x *E. urophylla* also corresponds to reports from Sumatra, Indonesia, where *T. destructans* was found affecting younger leaves, buds and even immature shoots in plantations (Wingfield *et al.* 1996).

Spore source was found to be an important factor in both the germination and infection studies conducted. After observing very low spore germination for spores obtained from cultures in this study, it was decided to test spore germination of fresh spores obtained directly from field infected leaves. It was found that germination of *T. destructans* conidia obtained directly from leaves was much greater than those from culture. The optimum temperature for germination of conidia from leaves was 20 ˚C, while it varied between isolates for spores from culture. Similarly, although both spore sources caused infection of plants as well as the production of fruiting bodies the production of fruiting bodies resulting from spores obtained from naturally infected leaves was more abundant that that of spores obtained from cultures (data not presented). This is an important consideration for future

studies of this pathogen and shows that laboratory conditions cannot necessarily be extrapolated to natural conditions on the plant under field conditions for *T. destructans*.

Images taken by the scanning electron microscope revealed that *T. destructans* spores were alive after landing on the leaf surface. Once they landed on the leaf surface, they formed germ tubes which extended and grew towards the stomatal openings. Similarly, spores of *T. cryptica* survived for up to four days on the leaf surfaces with very little loss of infectivity (Park 1988a). The reason why some experiments did not work in this study is unknown, although speculations can be made. The first experiment which made use of spores obtained from cultures of two isolates did not cause infection. The second experiment which made use of spores obtained from cultures of a different isolate however, caused infection; although the spore suspension did not contain Tween20. In this case, it could be assumed that the isolate had an impact on the infection by *T. destructans*. This can further be supported by the positive results from experiment six which also used spores from the same isolate as in experiment two. Three out of the six experiments were done using spores obtained from naturally infected leaves from the same areas. However, only one experiment was successful despite using similar protocols. More research is therefore needed to determine which adjustments to the parameters for infection are needed to obtain consistent infection rates.

CONCLUSIONS

Teratosphaeria destructans is an economically important pathogen causing leaf and shoot disease of *Eucalyptus* species. Its recent discovery in South Africa has raised some serious concerns about the long-term viability of *Eucalyptus* plantations in the sub-tropical regions of the country. A better understanding of the pathogen's biology is thus needed to support the development of control strategies. In this study, optimum environmental conditions were determined for culture growth, conidial germination and infection of *T. destructans* and we could show that these parameters closely match the climatic conditions of *Eucalyptus* plantations in the sub-tropical regions of South Africa. Furthermore, conidial germination rates up to 88% were observed from spores obtained from naturally infected leaves showing a high viability and infectivity of inoculum in the field, which could give rise to epidemic disease outbreaks. *Teratosphaeria destructans* was able to infect young and intermediate leaves of *E. grandis* x *E. urophylla* at temperatures between 20 and 25 ˚C and leaf wetness periods of two - eight days. However, fruiting bodies developed on a greater number of plants

at 25 ˚C in comparison to 20 ˚C, which shows high levels of inoculum are more likely at higher temperatures. This is concerning, as global climate change scenarios predict an increase in temperature of between 2 to 5 ˚C for many *Eucalyptus* growing regions of South Africa (Toucher and Schulze, 2008, Booth, 2013). Due to this imminent threat, more research is required to support the management of Destructans leaf blight by developing control strategies and resistant varieties.

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Table 1. Origin of *Eucalyptus* material for obtaining *Teratosphaeria destructans* cultures.

Isolate number	Host	Collection date	Field/ nursery	Location
2413	*E. grandis* x *E. urophylla*	March 2016	Nursery	Cramond (KZN)
2496	*E. grandis* x *E. urophylla*	March 2016	Field	Pietermaritzburg, KZN
24163	*E. grandis* x *E. urophylla*	April 2016	Field	Mtubatuba, KZN
24193	*E. grandis*	April 2016	Field	Mtubatuba, KZN
24421	*E. grandis*	February 2017	Field	Whiteriver, Mpumalanga (MPU)
24445	*E. grandis*	February 2017	Field	Mkhondo, MPU

Table 2. Origin of leaf material containing cirri of *T. destructans* that were used to harvest spores for germination and infection studies.

Leaf sample/ Isolate	Experiment	Host	Location
2496	one, three	*E. grandis* x *E. urophylla*	Pietermaritzburg, (KZN)
24163	one, three	*E. grandis* x *E. urophylla*	Mtubatuba, KZN
24445	one, three	*E. grandis*	Mkhondo, Mpumalanga (MPU)
2413	three	*E. grandis* x *E. urophylla*	Wartburg, KZN
24193	three	*E. grandis*	Mtubatuba, KZN
24421	three	*E. grandis*	Whiteriver, MPU
Leaf 1	two	*E. grandis* x *E. urophylla*	Mtubatuba, KZN
Leaf 2	two	*E. grandis* x *E. urophylla*	Mtubatuba, KZN
Leaf 3	two	*E. grandis* x *E. urophylla*	Mtubatuba, KZN
Leaf 4	two	*E. grandis* x *E. urophylla*	Mtubatuba, KZN
Leaf 5	two	*E. grandis* x *E. urophylla*	Mtubatuba, KZN
Leaf 1	two, three	*E. grandis* x *E. urophylla*	Wartburg, KZN
Leaf 2	two, three	*E. grandis* x *E. urophylla*	Wartburg, KZN
Leaf 3	two, three	*E. grandis* x *E. urophylla*	Wartburg, KZN
Leaf 4	two, three	*E. grandis* x *E. urophylla*	Wartburg, KZN
Leaf 5	two, three	*E. grandis* x *E. urophylla*	Wartburg, KZN

Table 3 Summary of artificial inoculation experiments conducted.

	Start date	Isolates/Leaf samples	Temperatures tested (°C)	Leaf wetness period tested	No. plants per isolate	No. plants per temperature	No. plants per leaf wetness period	Termination date
			Spores obtained from cultures					
Experiment 1	11 May 2017	2433, Wartburg 2488, Pietermaritzburg	20, 25 and 28	12 hours, 1, 4, 6 and 8 days	90	60	6	6 July 2017
Experiment 2	5 February 2018	2496, Wartburg	20 and 25	6 and 12 hours, 1, 2 and 8 days	50	25	5	2 April February 2018
			Spores obtained directly from leaf material					
Experiment 3	7 July 2018	Leaves 1-5, Wartburg Leaves 1-5, Mtubatuba	20 and 25	7 days	10	10	1	1 September 2018
Experiment 4	1 October 2018	Leaves 1-2, Wartburg Leaves 1-2, Mtubatuba	20	0, 2,4 and 7 days	6	24	8	26 November 2018
Experiment 5	18 March 2019	Leaf 1, Mtubatuba	20 and 25	1, 3, 5, 7 and 10 days	100	50	10	13 May 2019
Experiment 6	5 March 2018	2496, Pietermaritzburg	20 and 25	24 hours, 4 and 7 days	4	2	2 leaves	30 April 2018

Table 4. Three-way Analysis of Variance Table for the first culture growth experiment (complete model)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
poly(Temperature, 3)	3	43.854	14.6180	184.9081	**<0.001**
FIsolate	5	29.540	5.9079	74.7309	**<0.001**
light.dark	1	0.045	0.0451	0.5700	0.451
poly(Temperature, 3):FIsolate	15	6.731	0.4487	5.6761	**<0.001**
poly(Temperature, 3):light.dark	3	1.515	0.5050	6.3877	**<0.001**

FIsolate:light.dark	5	2.085	0.4169	5.2740	**<0.001**
Residuals	267	21.108	0.0791		

Table 5. Three-way Analysis of Variance table for the second culture growth experiment (complete model)

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
poly(Temperature, 3)	3	67.020	22.3400	644.1047	**<0.001**
FIsolate	5	2.243	0.4486	12.9329	**<0.001**
light.dark	1	0.003	0.0026	0.0752	0.784
poly(Temperature, 3):FIsolate	15	5.378	0.3585	10.3376	**<0.001**
poly(Temperature, 3):light.dark	3	1.291	0.4303	12.4053	**<0.001**
FIsolate:light.dark	5	1.547	0.3093	8.9190	**<0.001**
poly(Temperature, 3):FIsolate:light.dark	15	2.992	0.1995	5.7514	**<0.001**
Residuals	252	8.740	0.0347		

Table 6. Three-way Analysis of Variance table for the first culture spore germination experiment: day one (complete model)

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
poly(Temperature, 1)	1	0.19146	0.191458	25.1735	**<0.001**
FIsolate	2	0.00493	0.002466	0.3242	0.725
light.dark	1	0.01743	0.017431	2.2918	0.137
poly(Temperature, 1):FIsolate	2	0.01976	0.009881	1.2992	0.282
poly(Temperature, 1):light.dark	1	0.05879	0.058787	7.7295	**<0.05**
FIsolate:light.dark	2	0.00612	0.003061	0.4025	**<0.001**
poly(Temperature, 1):FIsolate:light.dark	2	0.05617	0.028084	3.6925	**<0.05**
Residuals	48	0.36507	0.007606		

Table 7 Three-way Analysis of Variance table for the first culture spore germination experiment: day two (complete model)

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
poly(Temperature, 1)	1	0.10961	0.109609	5.6845	**<0.05**
FIsolate	2	0.31333	0.156664	8.1248	**<0.001**
light.dark	1	0.00270	0.002703	0.1402	0.710

poly(Temperature, 1):FIsolate	2	0.02668	0.013339	0.6918	0.506
poly(Temperature, 1):light.dark	1	0.07835	0.078350	4.0633	**<0.05**
FIsolate:light.dark	2	0.01641	0.008204	0.4255	0.656
poly(Temperature, 1):FIsolate:light.dark	2	0.33861	0.169304	8.7803	**<0.001**
Residuals	48	0.92554	0.019282		

Table 8 Three-way Analysis of Variance table for the first culture spore germination experiment: day three (complete model)

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
poly(Temperature, 1)	1	0.00182	0.001820	0.1222	0.728
FIsolate	2	0.23856	0.119282	8.0069	**<0.001**
light.dark	1	0.00088	0.000883	0.0592	0.809
poly(Temperature, 1):FIsolate	2	0.01944	0.009720	0.6524	0.525
poly(Temperature, 1):light.dark	1	0.13672	0.136722	9.1775	**<0.05**
FIsolate:light.dark	2	0.02898	0.014488	0.9725	0.385
poly(Temperature, 1):FIsolate:light.dark	2	0.14056	0.070281	4.7177	**<0.05**
Residuals	48	0.71508	0.014897		

Table 9 Two-way Analysis of Variance table for the third culture spore germination experiment (complete model)

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
poly(Temperature, 3)	3	1.9124	0.63745	17.7056	**<0.001**
FIsolate.leaf	5	0.7947	0.15893	4.4144	**<0.001**
poly(Temperature, 3):FIsolate.leaf	15	1.5513	0.10342	2.8725	**<0.001**
Residuals	126	4.5364	0.03600		

Table 10 Occurrence of symptoms and signs of *T. destructans* infection in the second artificial inoculation experiment eight weeks after inoculation with isolate 2496

Temperature	Leaf wetness period	Percentage of plants with *T. destructans* symptoms: leaf blight	Percentage of plants with *T. destructans* symptoms: fruiting bodies
20 ˚C	6 hours	0	0
20 ˚C	12 hours	0	0
20 ˚C	24 hours	0	0
20 ˚C	48 hours	40%, n=5	0
20 ˚C	8 days	100%, n=5	40%, n=5
25 ˚C	6 hours	0	0
25 ˚C	12 hours	0	0
25 ˚C	24 hours	0	0
25 ˚C	48 hours	60%, n=5	0
25 ˚C	8 days	100%, n=5	80%, n=5

Table 11 Occurrence of symptoms and signs of *T. destructans* infection in the third artificial inoculation experiment eight weeks after inoculation, at a leaf wetness period of seven days

Temperature	Leaf Spore source	Percentage of plants with *T. destructans* symptoms: leaf blight	Percentage of plants with *T. destructans* symptoms: fruiting bodies
20 ˚C	Wartburg Leaf 1	60%, n=5	40%, n=5
20 ˚C	Mtubatuba Leaf 1	40%, n=5	20%, n=5
25 ˚C	Wartburg Leaf 1	100%, n=5	60%, n=5
25 ˚C	Mtubatuba Leaf 1	80%, n=5	60%, n=5

Table 12 Occurrence of symptoms and signs of *T. destructans* infection in the sixth artificial inoculation experiment, eight weeks after inoculation with isolate 2496

Temperature	**Plant source**	**Leaf wetness period**	***T. destructans* conidia under SEM**	**Percentage of plants with *T. destructans* symptoms: leaf blight**	**Percentage of plants with *T. destructans* symptoms: fruiting bodies**
20 ˚C	Plant 1, 2 leaves	7 days	Present	100%, n=2	50%, n=2
20 ˚C	Plant 2, 2 leaves	7 days	Present		
25 ˚C	Plant 3, 2 leaves	7 days	Present	100%, n=2	100%, n=2
25 ˚C	Plant 4, 2 leaves	7 days	Present		

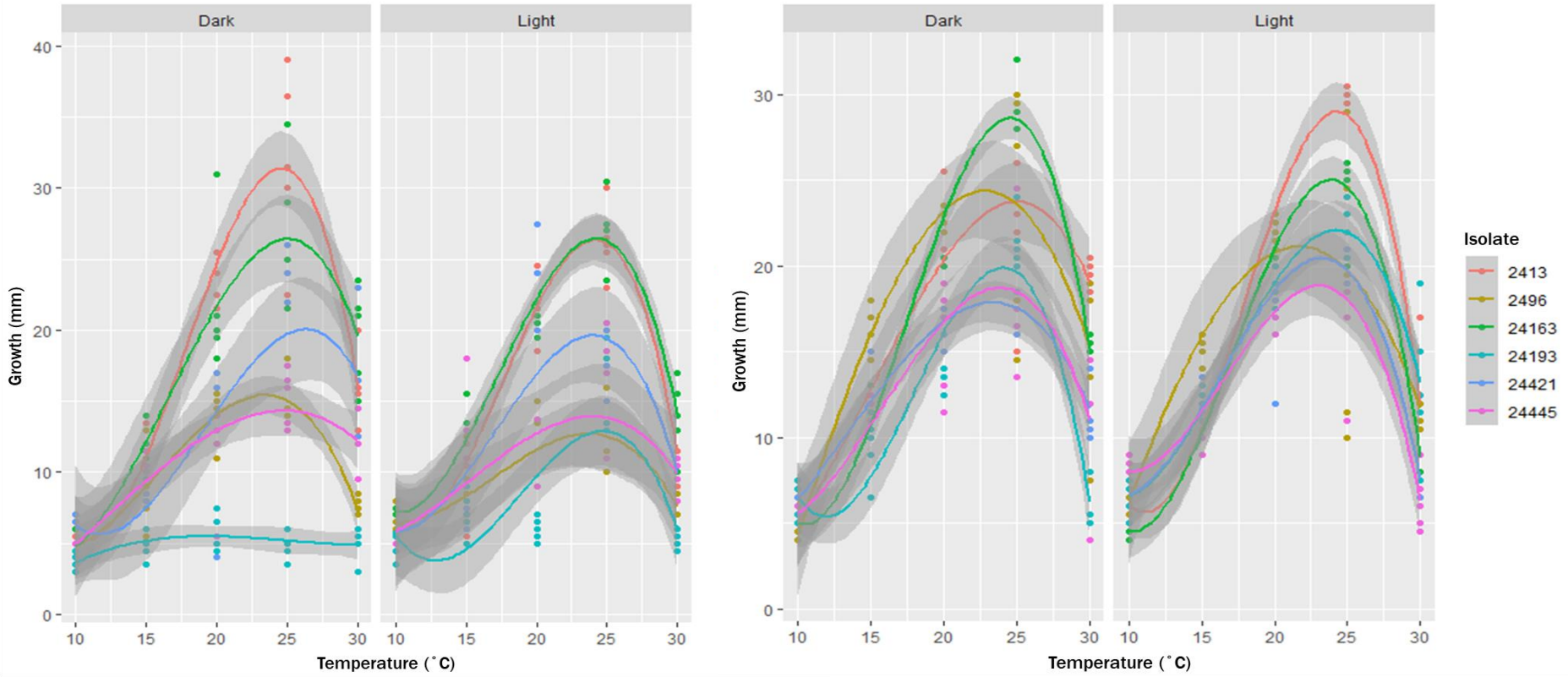

Fig. 1 The effect of temperature, isolate and light on the culture growth of *T. destructans* in experiment one (a) and experiment two (b) at temperatures 10 – 30 ˚C with or without light condition

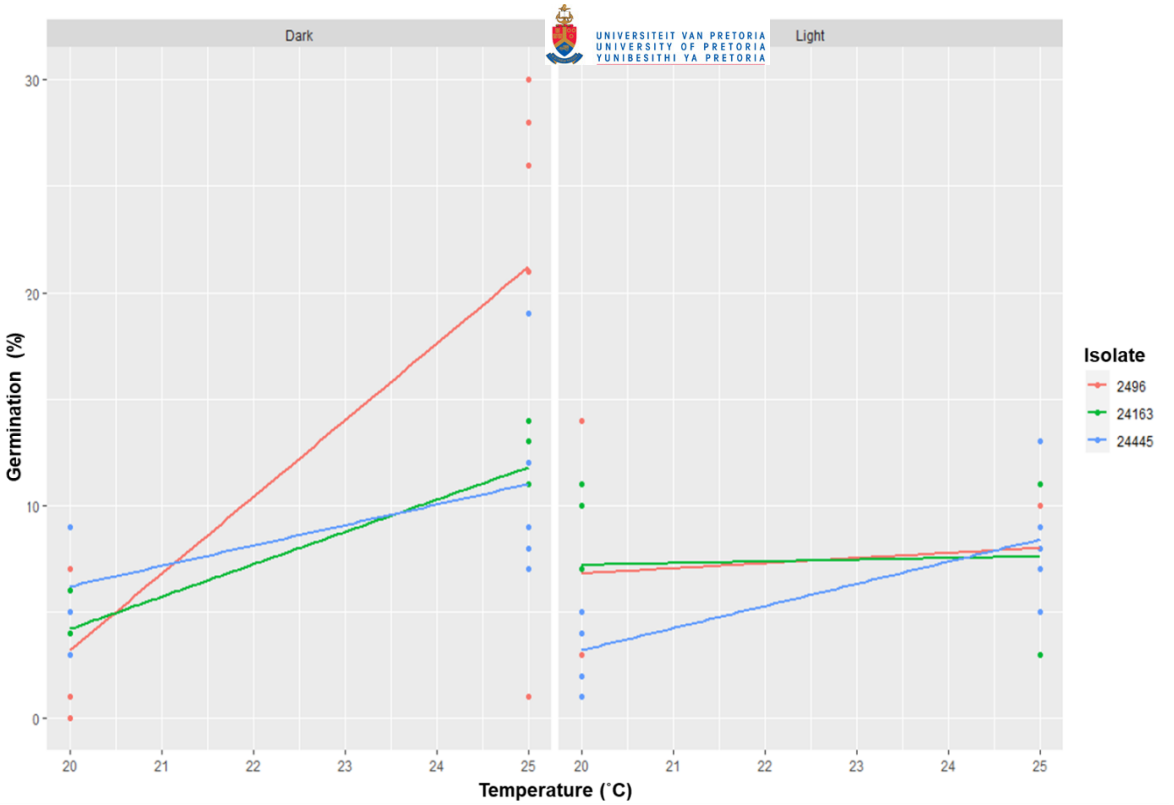

Fig. 2 The effect of temperature, isolate (spores obtained from cultures) and light on the germination of *T. destructans* spores from experiment one, day one.

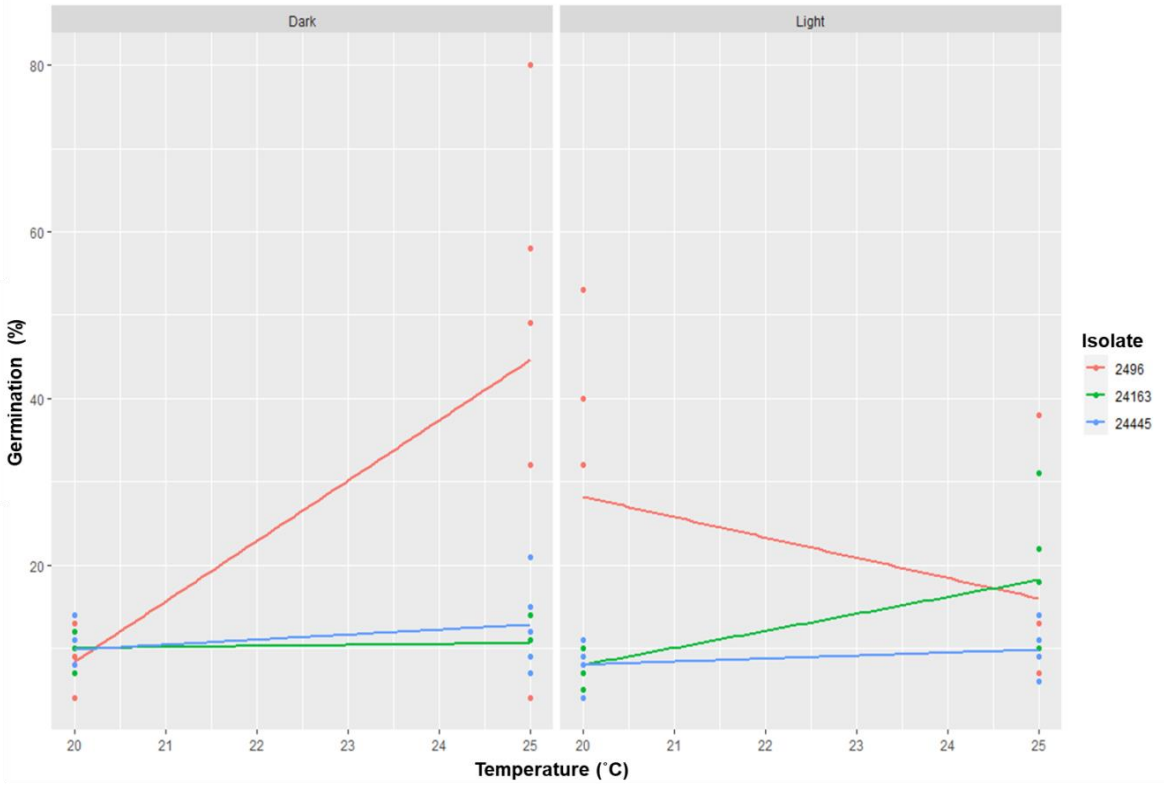

Fig. 3 The effect of temperature, isolate (spores obtained from cultures) and light on the germination of *T. destructans* spores from experiment one, day two.

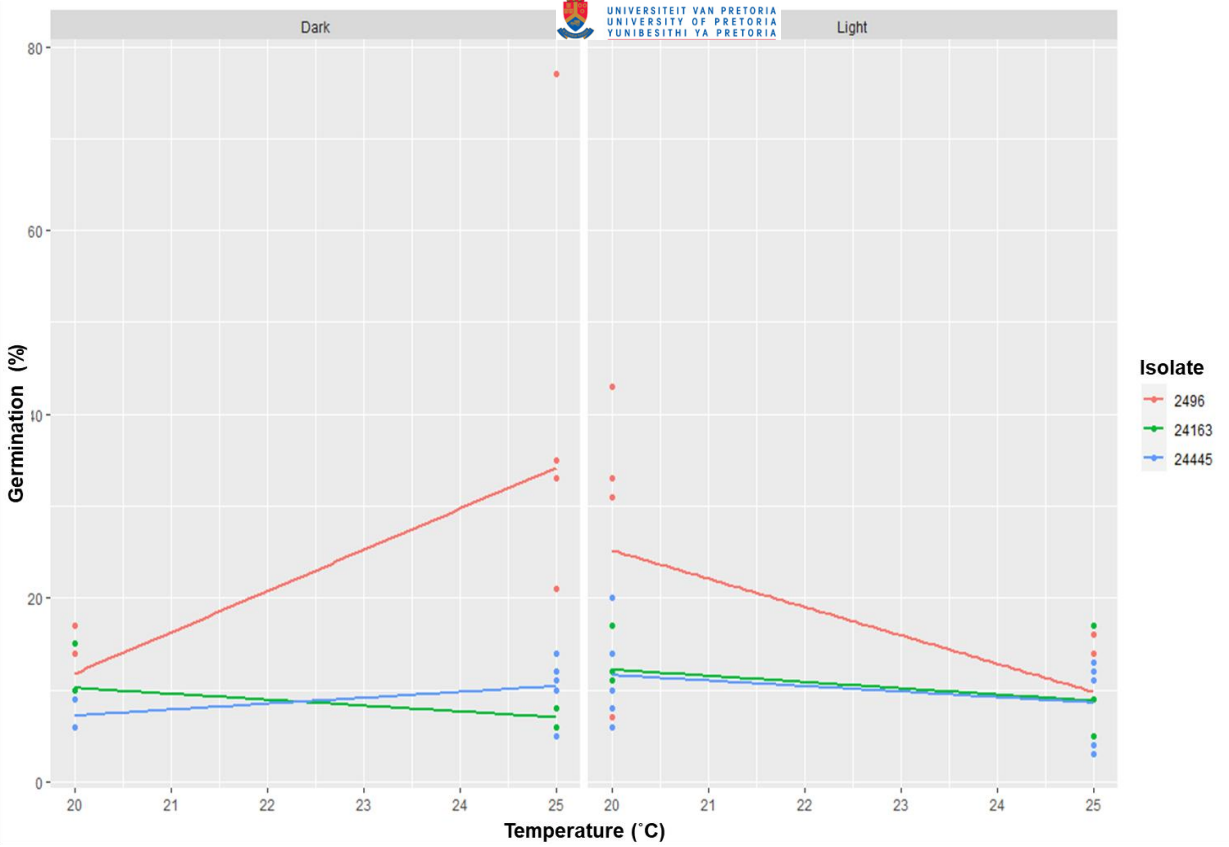

Fig. 4 The effect of temperature, isolate (spores obtained from cultures) and light on the germination of *T. destructans* spores from experiment one, day three.

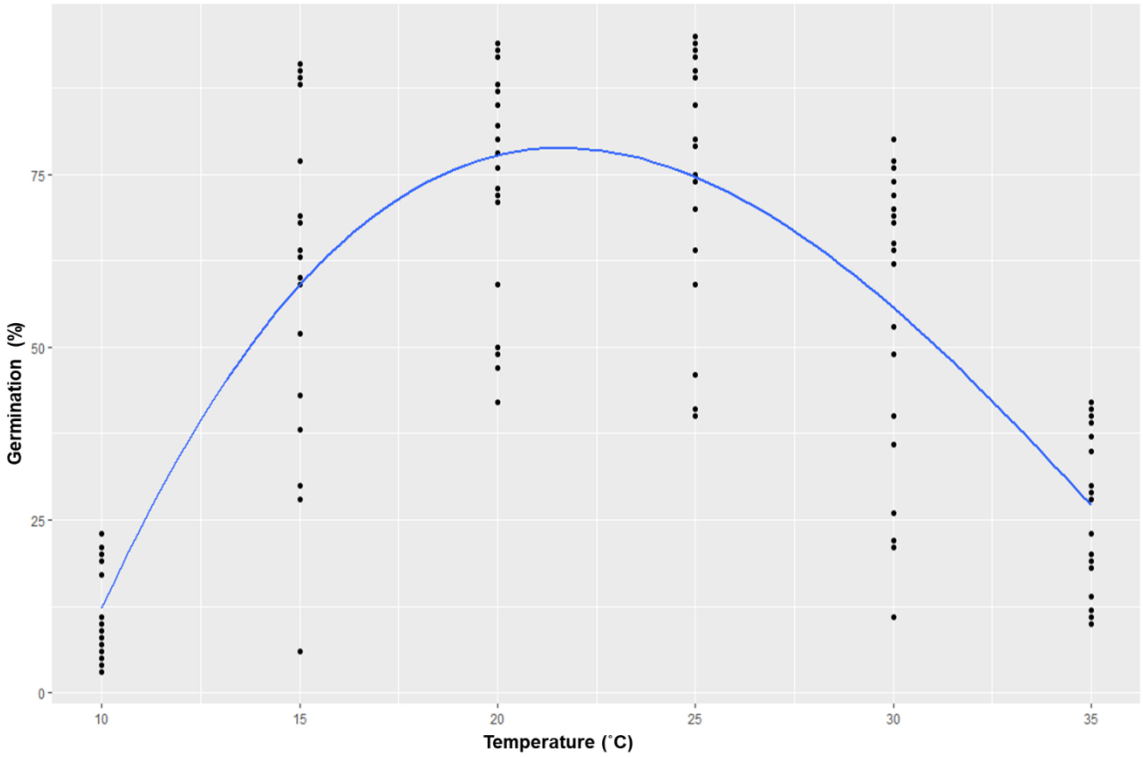

Fig. 5 The effect of temperature on the germination of *T. destructans* spores obtained from leaf material from experiment two, day one. (ANOVA, d.f = 3, F = 76.287, P < 0.001).

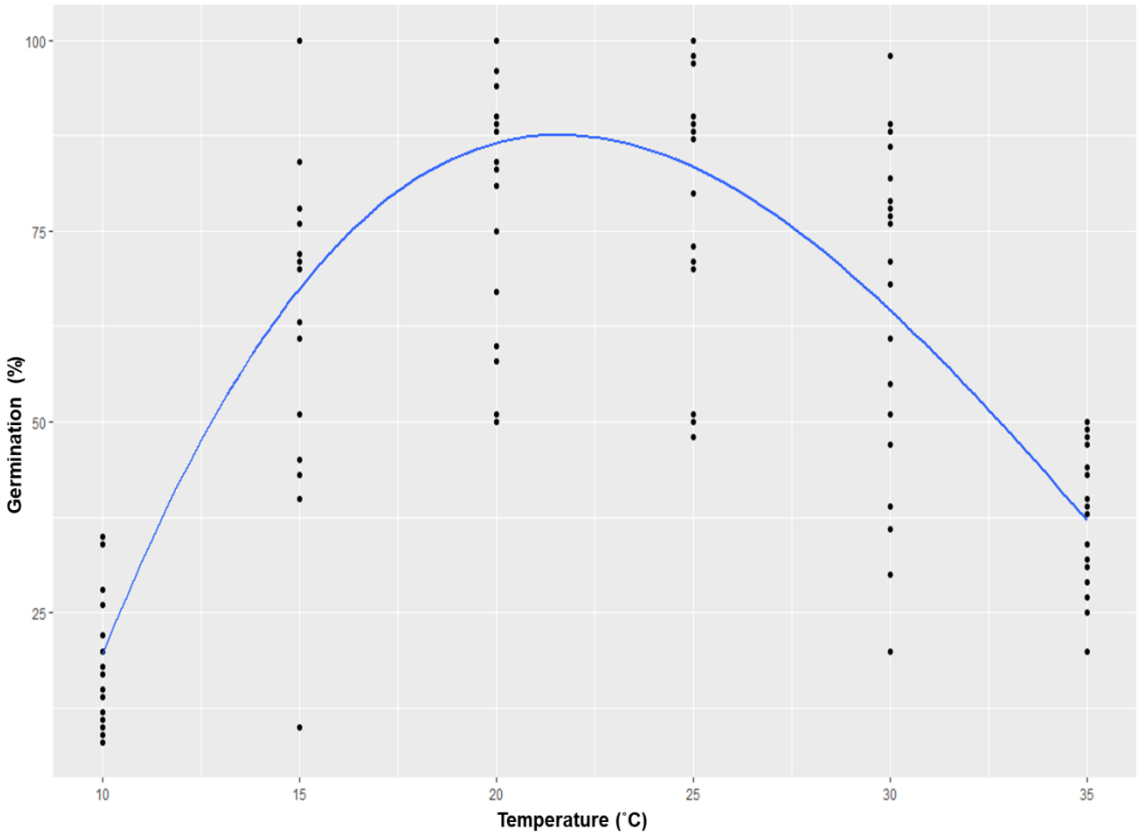

Fig. 6 The effect of temperature on the germination of *T. destructans* spores obtained from leaf material from experiment two, day two. (ANOVA, d.f = 3, F = 57.556, P < 0.001).

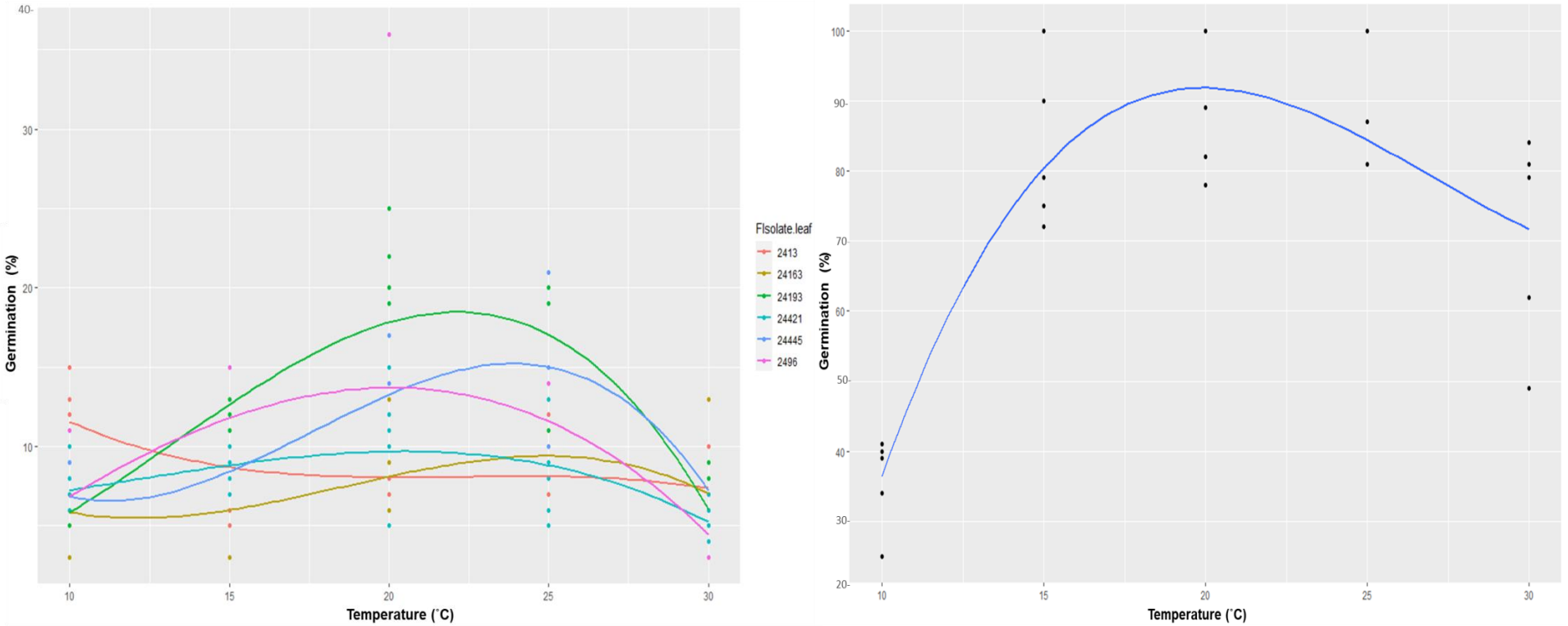

Fig. 7 The effect of temperature on the germination of (a) spores obtained from cultures and (b) spores obtained from leaf material (ANOVA, d.f = 3, F = 14.097, P < 0.001)from experiment three, day two

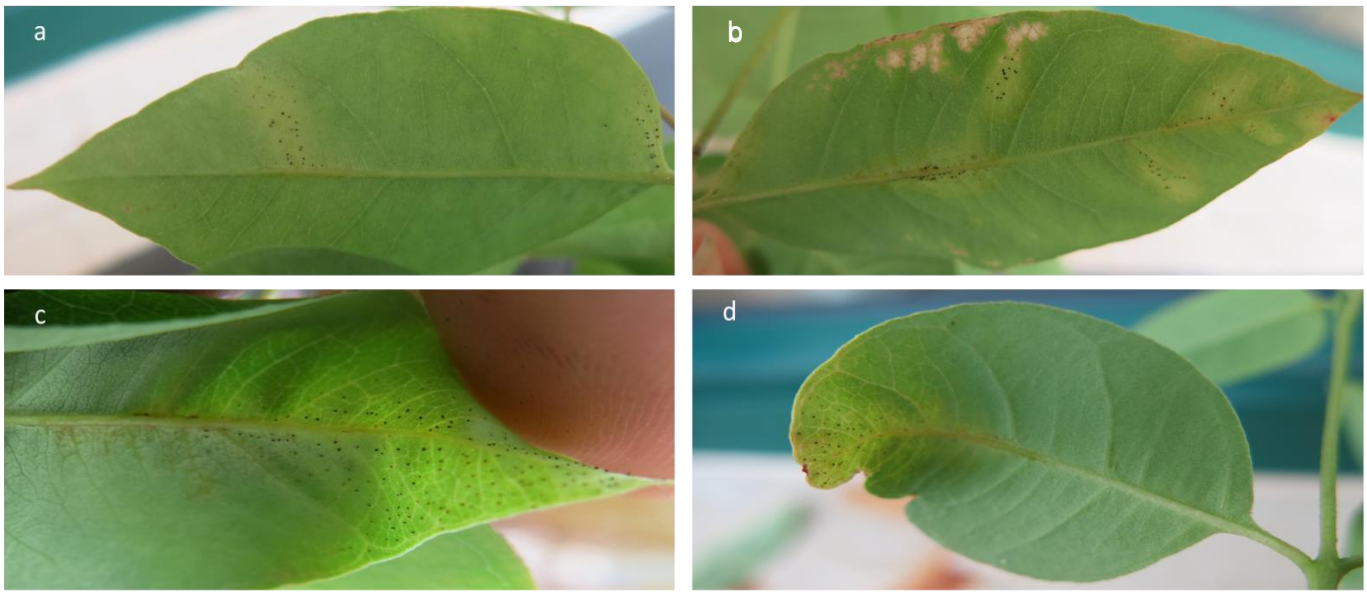

Fig. 8 Symptoms and signs of *T. destructans* infection eight weeks after artificial inoculation. (A) experiment two at 20 ˚C , (B) experiment three at 20 ˚C, (c) experiment three at 25 ˚C, (d) experiment six at 20 ˚C.

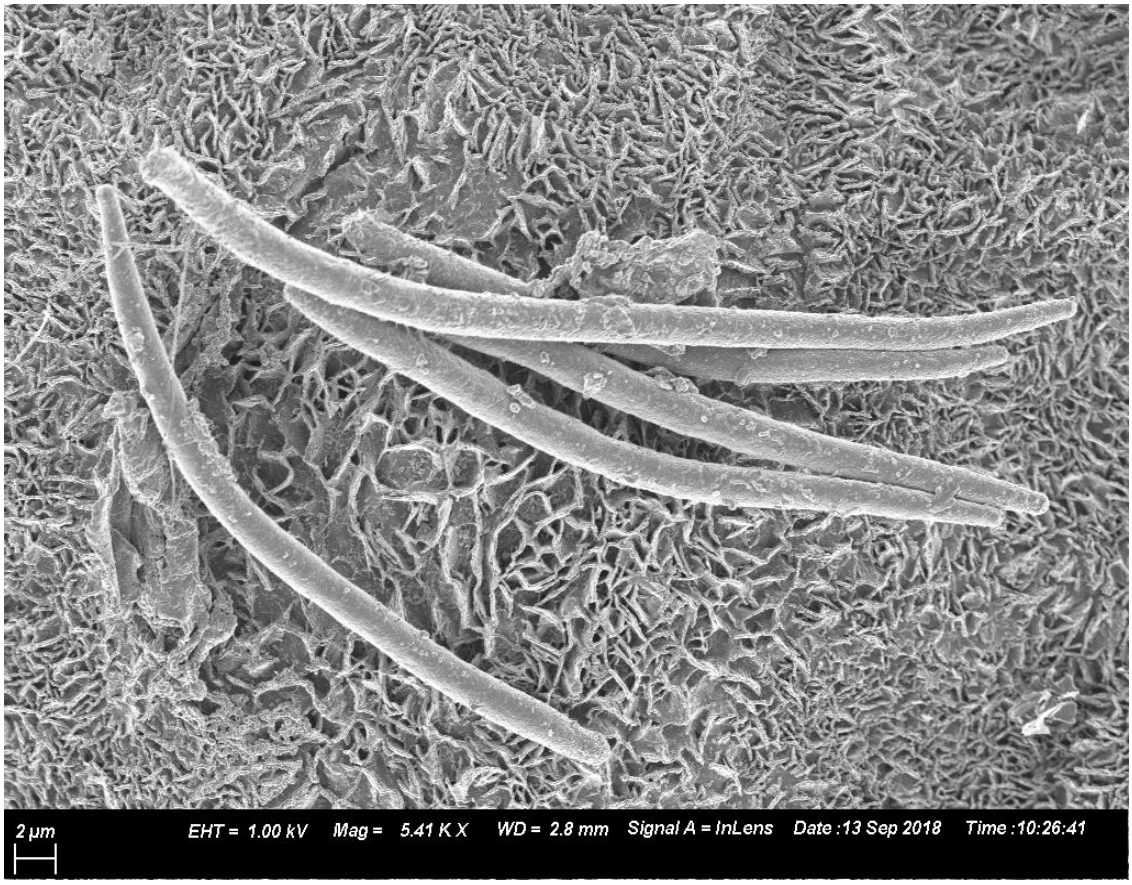

Fig. 9 Conidia of *T. destructans* on the abaxial leaf surface post inoculation at a leaf wetness period of one day at temperature 25 ˚C

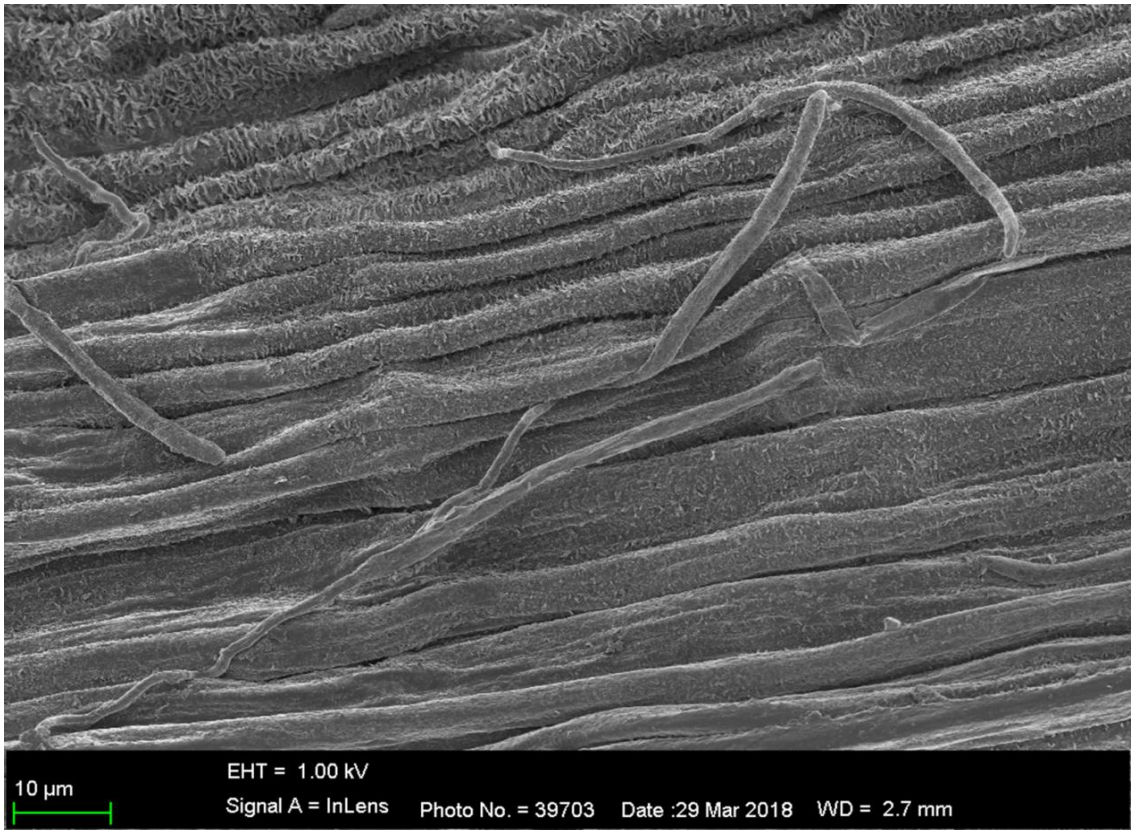

Fig. 10 Developing germ tube of *T. destructans* after a leaf wetness period of four days at temperature 25 ˚C

Fig. 11 Extending germtube of *T. destructans* around the stomatal area after a leaf wetness period of seven days at temperature 25 ˚C

Fig. 12 Sporulation of *T. destructans* after a leaf wetness period of seven days at temperature 25 ˚C

SUMMARY

Commercial plantations comprising of *Eucalyptus* species and their hybrids make up approximately 44 % of the South African forestry industry. The majority of *Eucalyptus* plantations in the country are planted in the KwaZulu-Natal and Mpumalanga provinces, with smaller areas in the Limpopo, Eastern and Western Cape Provinces. The constant increase of pests and pathogens from native and exotic environments threatens the productivity, health status and ultimately the economic importance of *Eucalyptus* species. Globally, species of *Calonectria*, *Coniella*, *Quambalaria*, *Teratosphaeria*, the rust fungus *Austropuccinia psidii* and the bacterial species, *Pantoea*, are considered to be important *Eucalyptus* leaf pathogens. The diseases caused by these leaf pathogens include leaf and shoot blight, leaf spot and leaf blotch, which may cause shoot death, tip die-back, defoliation and even plant death.

Studies conducted for this dissertation updated and increased knowledge pertaining to the foliar pathogens of *Eucalyptus* genotypes in South Africa. Firstly a survey of commercial *Eucalyptus* plantations was conducted in the KwaZulu-Natal, Mpumalanga and Limpopo Provinces to identify possible important leaf diseases of these trees. Based on symptoms, spore and culture morphology as well as DNA sequence data, *Calonectria pauciramosa*, *Quambalaria eucalypti* and *Teratosphaeria destructans* were identified from diseased leaves. Importantly, this study expanded the geographic range of *T. destructans* in the country and also provides the first report of Calonectria leaf spot in a South African plantation. This disease was previously only known from nurseries in the country.

Teratosphaeria destructans is one of the most important leaf pathogen of *Eucalyptus* in South Africa. The results obtained from chapter two confirm that the presence of *T. destructans* in South Africa has resulted in an expanded geographic range and even more destructive impact. This prompted the need to further study the epidemiology of this pathogen. The results obtained from chapter two confirm that the presence of *T. destructans* in South Africa has resulted in an expanded geographic range and even more destructive impact. This prompted the need to further study the epidemiology of this pathogen. Prior to the research conducted for this dissertation nothing was known about optimal conditions for spore germination, growth and infection of this pathogen. The growth of *T. destructans* cultures took place at an optimum temperature of 25 ˚C irrespective of light conditions. Conidial germination obtained from cultures took place at optimum temperatures of 20 and 25 ˚C, while those obtained from fresh leaves took place mostly at 20 ˚C, with or without

light. Greater conidial germination was observed from spores that were obtained from naturally infected leaves (88 %) as opposed to that of spores obtained from cultures (12 %). *Teratosphaeria destructans* was able to infect young and intermediate leaves of *E. grandis* x *E. urophylla* at temperatures of 20 and 25 ˚C and leaf wetness periods of two to eight days with symptoms developing approximately four weeks after inoculation. The production of fruiting bodies was observed at 20 and 25 ˚C and at leaf wetness periods of seven (spores obtained from fresh leaves) and eight (spores obtained from cultures) days four - eight weeks after inoculation. This information can be used for the development of artificial inoculation protocols for use in breeding programs to select more disease resistant planting material for commercial deployment.