Cape Peninsula
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Introduction pathways of phytopathogenic fungi and their potential role in limiting plant invasions: The case of *Banksia* spp. (Proteaceae) in the Cape Floristic Region

By

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Thesis submitted in fulfilment/partial fulfilment of the requirements for the degree

Master of Conservation Science

In the Faculty of Applied Sciences at the Cape Peninsula University of Technology

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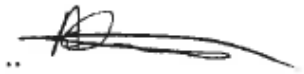
September 2018

Declaration

I Axola Qongqo, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Chapter 2 and 3 are written aimed for submission to different journals and have more than one author hence the use of "we". Me the student is the first author in all chapters and had the main responsibility for designing the study, field work, data collection, data analysis and manuscript writing while the supervisors helped with conceptualising ideas, planning and commenting on manuscript drafts.

The thesis contains a single bibliography to minimise duplication of referencing across chapters.



12/09/2018

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Abstract

Introduction pathways of fungal pathogens in South Africa are far less quantified in the literature than those for plants, animals and human infectious diseases. Phytopathogens continue to be introduced to South Africa via several pathways at an unprecedented rate. A number of these species pose a significant threat to South African ecosystems and biodiversity. Despite this, fungal pathogens could also be beneficial when they are used as bio-control agents to control alien invasive plant species. Nevertheless, recent studies revealed pathogens are most likely to be studied after they have caused a detrimental impact on the environment. Invasive fungal pathogens, such as *Phytophthora cinnamomi* (Oomycota) do not only pose a threat to native species of the family Proteaceae but could also potentially be bio-control agents for emerging alien plant invaders. In this thesis, firstly, I review current knowledge of phytopathogenic fungi introduction pathways in South Africa; secondly, I aim to understand the importance of fungi in limiting plant invasions using *Banksia* as a case study in the Cape Floristic Region. In chapter two I investigate introduction pathways and dispersal vectors that facilitate the spread of fungal pathogens. I compiled comprehensive list of fungal pathogens in South Africa, and evaluated the dispersal vectors and introduction pathways for each species. I found fifty five casual species, three naturalised species, six invasive species and thirty six pathogens for which invasion status was not classified due to insufficient data. Agriculture is responsible for the introduction of most fungal pathogens in South Africa. Wind was identified to be the prominent dispersal vector facilitating the spread of pathogens. I conclude that knowing introduction pathways of pathogens and their dispersal vectors will assist in developing quarantine protocols that could improve bio-security. Lastly, I provide recommendations for the national invasive microbe species list. In chapter three the study investigates the variability in mortality rate of *Banksia* species in the Cape Floristic. Species abundance was calculated across known *Banksia* populations in the Cape Floristic Region to determine survival and mortality rates. Soil and leave samples were taken from *Banksia* plants to evaluate potential microbial pests that were present. Also, acetone leaf extracts of twelve *Banksi*a species were screened for antimicrobial activity against *P. cinnamomi* (Oomycota). Lastly, a post-border risk assessment was conducted for 14 *Banksia* species –present in South Africa – using the Australian Weed Risk Assessment protocol, to evaluate potentially invasive species. The results indicated that survival and mortality rate varied across species; I found the two invasive species, *B. integrifolia* and *B. ericifolia* to have the highest survival rate. *Phytophthora cinnamomi* was the most prominent isolated fungal pathogen sampled from *Banksia* species roots. The detection of antifungal activities in the minimum inhibitory concentration (MIC) bioassay provided evidence that some

Banksia species (*B. ericifolia, B. integrifolia, B. hookeriana* and *B. formosa)* have antimicrobial chemical constituents that could possibly inhibit infection and colonisation by *P. cinnamomi*. The weed risk assessments conducted on *Banksia* species showed five species pose a high risk of invasion while seven species required further evaluation. I conclude that *P. cinnamomi* could potentially regulate invasive *Banksia* species such as *B. speciosa* with minimal antimicrobial activity against the pathogen. I recommend an in-situ and ex-situ inoculation trials of *Banksia* species against *P. cinnamomi* to be conducted to evaluate pathogenicity, under different watering regimes since the pathogens proliferation is favoured by soils that are high in moisture. I present the main conclusions from this thesis in chapter four and provide recommendations for management and invasive species legislation.

Acknowledgements

I would like to thank my supervisors for their ideas, comments and friendly guidance Prof Sjirk Geerts and Prof Felix Nchu.

I am grateful to Phumlani Roto for field trip assistance and Prof Charles Laubscher for granting him permission to accompany me on my trips.

I thank Mrs Deborah Erasmus and Denise Daniels for their excellent administrative assistance.

Special thanks to all my friends who have always supported me along the way.

I would like to thank my mother Miss Z. Qongqo for supporting me throughout my studies.

We would like to thank *Banksia* farmers, Andre Brink, Wessels Swart, Bertus Van Zyla and Jaco Oosthuizen for allowing us to conduct research on their farms.

This work was supported by the South African National Department of Environment Affairs through its funding of the South African National Biodiversity Institute Invasive Species Programme and, as such, we are thankful for such a huge contribution to our study.

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1.1 Chapter one: General introduction

Human-mediated dispersal of non-native species began with the first human migration and the beginning of agriculture, when humans accidentally or deliberately transferred species beyond their native range (Hodkinson & Thompson, 1997; Pyšek & Richardson, 2008). During the dawn of global trade (Post-Columbian era) the number of intentionally introduced species to several continents increased and this was partially attributed to transcontinental exchange of organisms (Crosby, 1988). Ornamentation is one of the main human motivations for introducing non-native plant species, this is the case for some Proteaceae species (Richardson et al., 1990; Weber, 2004; Geerts et al., 2013). Quite often the potential effects the introduced species might have in the new habitat is not considered (Reichard & Hamilton, 1997; McNeely et al., 2001). Consequently, prevention of intentional introductions has increased at intra and inter-continental scale (Mack, 2003). However, more species still manage to get through bio-security; therefore, information on species introduction pathways and factors which may limit invasion success of a species is essential.

1.2 Biological invasions

Research on biological invasions has received substantial attention in the past few decades (Kitching et al., 2011; Downey & Richardson, 2016) and yet the fundamental question of why some introduced species become more abundant and wide spread than others, only remains partially answered (Kolar & Lodge, 2001; Keane & Crawley, 2002; van Kleunen et al., 2010). A phenomena that could possibly explain this is that when a species is introduced in an exotic area, its abundance and distribution is likely to increase depending on the absence or decline of natural enemies (enemy release hypothesis) (Mack et al., 2001; Catford et al., 2009). Nonetheless, only a few introduced established species will spread and become problematic (Williamson, 1996; Kolar & Lodge, 2001; Richardson et al., 2006). Natural enemies of plants come from a wide variety of taxonomic groups (vertebrates, invertebrate herbivores, fungal pathogens, bacterial and viral diseases) (Keane & Crawley, 2002; Klein, 2011). Plants are immobile and cannot physically escape their natural enemies like animals do; therefore, they synthesise a wide range of phenolic compounds as defence mechanisms against pathogen attack (Bell, 1980). These phenolic compounds can be antimicrobial agents against fungi or bacterial viruses, they can also be anti-nutritional or have unpalatable properties (Lattanzio et al., 2006).

In order to manage biological invasions efficiently it is essential to understand the mechanisms which favour biological invasions and their impact on biodiversity (Keane & Crawley, 2002). Predicting impact of alien invasive species is challenging (Leung et al., 2012) and the prioritisation and management of alien invasive species has been highlighted in the Strategic Plan for Biodiversity 2011-2020 (CBD COP 10). The Convention on Biodiversity promotes a mandate that all relevant parties need to prevent the introduction of species and control or eradicate invasive alien species (Glowka et al., 1994). Quite often eradication of an invader covering large areas is not feasible (Myers et al.,

2000). Therefore preventing introduction of a high risk species needs to be prioritised as this is the most effective control method (Leung et al., 2012; Novoa et al., 2015). Introduced species need regular reassessment as most invaders undergo a lag phase followed by a rapid expansion (Crooks & Soule 1999; Murren et al. 2014) such as the invasive *Banksia ericifolia* which became invasive after a few fire events in the Cape Floristic Region (Geerts et al., 2013). Frameworks have been developed to assess the transition stages of a species from being an introduced species to becoming invasive (Fig.1.1) (Blackburn et al., 2011).

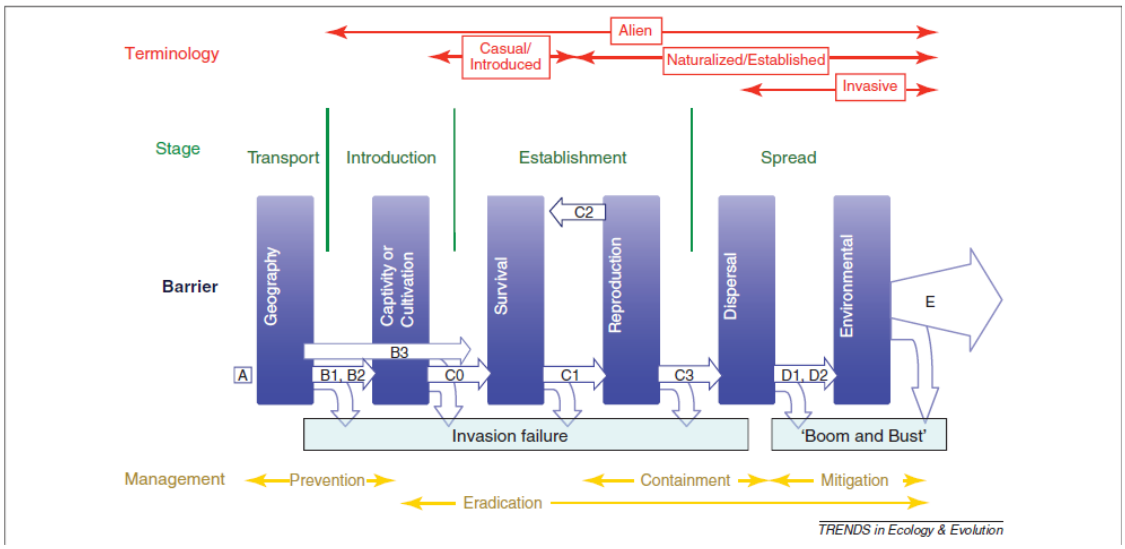

Figure 1.1: Schematic representation of barriers which alien species must overcome in order to progress across to different invasion stages adopted from Blackburn et al. (2011)

1.3 Impact of biological invasions

Alien invasive taxa are non-native species with the ability to change the character, conditions or nature of an ecosystem (Richardson et al., 2006). An introduced species primarily has to be able to reproduce and establish a self-sustaining population with no human intervention in order to become invasive (Kolar & Lodge, 2001; Murren et al., 2014). Some alien invasive plants contribute to native species loss and extinction (Sax et al., 2002); they erode natural capital, compromise the delivery of ecosystem goods and services and threaten economic productivity (Richardson & van Wilgen, 2004). There are various factors that influence the degree of invasion in an ecosystem; these include fires, historical habitat modification, propagule pressure and habitat suitability (Honig et al., 1992; Sundaram et al., 2015). In South Africa the worst plant invaders are *Melia azedarach, Pinus pinaster, Pinus patula, Acacia mearnsii,* and *Lantana camara;* each of these species has invaded about 2 million ha of South Africa and Lesotho (Richardson et al. 1990; Le Maitre et al. 2000).

About 10 million ha (8.28%) of South Africa and Lesotho landscapes are affected by IAPs, of which 1.7 million ha (1.39 %) are completely covered with alien vegetation (Le Maitre et al., 2000). South Africa spends an estimated R6.5 billion every year on IAPs, which will rise significantly if IAPs are unmanaged (de Lange & van Wilgen, 2010). Increased globalisation has increased the spread of IAPs (Kolar & Lodge, 2001; Meyerson & Mooney, 2007), but also new plant pests and diseases such as Ramorum disease caused by the *Phytophthora ramorum* pathogen (Mills et al., 2011).

Interestingly, many problematic invasive alien species are not per se invasive in their native range. For example, the emerald ash borer (*Agrilus planipennis*), after its introduction into the US, destroyed many ash tree (*Fraxinus spp.*) populations (Flower et al., 2013) but is not an important pest in its native range (Flower et al., 2014). Another example is our indigenous *Chrysanthemoides monilifera* which was introduced to Australia over a 100 years ago (Gallagher et al., 2010) and today is a major invader in coastal sand dunes in southern Australia. *Chrysanthemoides monilifera* leaves decompose faster than those of the common Australian coastal shrubs *B. integrifolia* and *A. longifolia* (Lindsay & French, 2004). This could ultimately speed up nutrient cycling which will favour *C. monilifera* growth at the expense of the Australian native vegetation (Lindsay & French, 2004). In South Africa Holmes & Cowling (1997) observed that invasion by *Acacia saligna* resulted in 70% reduction of native plant species richness on stands where *Acacia* thickets have established after two or more fire cycles. The adverse impact *of A. saligna* in the Cape Floristic Region resulted in control measures to be implemented. However mechanical and chemical control are costly, therefore the only feasible and viable method of control is biological control (Morris, 1991; Wood & Morris, 2007).

1.4 Research background

Preliminary surveys of *Banksia spp*. in South Africa revealed that *Banksia* species are parasitized by unidentified pathogens and it appears there is variability in the susceptibility of the different *Banksia* species to these pathogens. Reports from Australia have linked the phytopathogen, *Phytophthora cinnamomi* to be responsible for dieback of some *Banksia* species (Bathgate et al., 1996; Davis et al., 2014) Ornamental crops that are grown outside their native range might act as potential host and be invaded by endemic microbial pathogens of the new location (Kroon et al., 2012). This knowledge could potentially be used to manage alien invasive plants (Reinhart et al., 2010). This can act both ways; where we need an understanding of local fungi for potential management of invasive species, but also understand the alien fungi which can negatively influence native plant species. The rate of infection and severity is influenced by the evolutionary interaction between the pathogen and host, which might be commensalistic, mutualistic or parasitic (Gilman et al., 2010). In the late 1980s, Richardson et al. (1990) conducted a post-border risk assessment and predicted that four *Banksia* species (*B. burdettii, B.coccinea, B. hookeriana, B. prionotes*) are high risk species and are likely to become invasive in the Cape Floristic Region (CFR). Prior to this study, Von Broembsen (1984) found

these four species to be parasitized by the phytopathogen *P. cinnamomi* in the south Western Cape. These findings correlate with several *Banksia* studies from their native range in Australia (Shearer & Dillon, 1996; Davis et al., 2014).

The genus *Banksia* presents an ideal study group to understand the importance of fungi in limiting plant invasions. Firstly, because we have a good understanding of *Banksia* invasions in South Africa (Moodley et al., 2013; Richardson et al., 1990; Moodley et al., 2014; Geerts et al., 2013; Moodley et al., 2016). Secondly, within this genus there are non-invasive, naturalised and invasive species in South Africa; lastly, susceptibility to fungi differs between *Banksia* species. Fungi introduction pathways have not been well-documented in literature; however, the ornamental plant trade is a major pathway for introducing exotic fungi (Desprez-Loustau et al., 2007). We know little about the fungal species of countries trading with South Africa and more species will continue to be introduced in the country (van Rensburg et al., 2011) through several pathways (Wilson et al., 2009) and the best way to prevent this is to focus on pathways of introductions (Campbell, 2001; Novoa et al., 2015).

1.5 Research problem

The earliest records of *Banksia* introductions in the Western Cape date back to the 1970s when they were initially propagated for floriculture (Geerts et al., 2013). Currently, there are fourteen *Banksia* species grown in the Western Cape for floriculture and horticulture (Moodley et al., 2014). Within the genus *Banksia*, there are 172 species, (Moodley et al., 2013) of which more species with desirable horticultural and floricultural traits could be introduced to South Africa in the near future. From the fourteen introduced species, *Banksia formosa* and *Banksia speciosa* have naturalised, but *B. speciosa* has been recognised as an emerging invader in the Agulhas National Park (pers. obs.); *Banksia ericifolia* and *Banksia integrifolia* are invasive in the Cape Floristic Region (CFR) (Moodley et al., 2013; Geerts et al., 2013). Invasion success of *Banksia* species can largely be attributed to propagule pressure, natural dispersal, suitable climate, fire (Richardson et al., 1990; Geerts et al., 2013; Moodley et al., 2013; Moodley et al., 2014;), but also resistance to phytopathogens (pers. obs.).

The most cost-efficient management for invaders is to direct efforts at prevention (Novoa et al., 2015). Therefore, in order to reduce the risk posed by phytopathogens to the agricultural, horticultural and floricultural industries we need to understand their pathways of introduction to South Africa. In this thesis, I collate data on fungal pathogens in these industries and identify species introduction pathways and their dispersal vectors.

In this thesis, I also intend to identify factors that may modulate differential invasion status of *Banksia* species. Preliminary investigations suggest that *Banksia* species are parasitized by *P. cinnamomi*, *Phythium* spp. and nematodes; however, some species seem not be affected by these pathogens. This suggests that susceptibility to the infective agents varies amongst *Banksia* species. If this assertion is

true, then it is important to determine whether there is any association between *Banksia* species susceptibility to the infective agents and if this relates to species invasion status.

1.6 Thesis outline

Chapter one is a general literature review on biological invasions and includes justification of the study. Chapter two reviews current knowledge of fungi in South African (with the main focus on horticultural, floricultural and agricultural crops) and identifies potential introduction pathways of pathogens. Chapter three assesses the antifungal activity of *Banksia* species and relate these to the current invasion status. Chapter four is a general synthesis for the thesis and recommendations.

Chapter 2 and 3 are written aimed for submission to different journals and have more than one author hence the use of "we". I the student am first author in all chapters and had the main responsibility for designing the study, field work, data collection, data analysis and manuscript writing while the supervisors helped with conceptualising ideas, planning and commenting on manuscript drafts.

1.7 Research aims

The aim of this thesis is to:

- Review current knowledge of alien phytopathogenic fungi in South Africa and identify prominent introduction pathways of fungal pathogens.
- Understand the importance of fungi in limiting plant invasions by using the genus *Banksia* in the Cape Floristic Region as a case study.
- Provide recommendations for listing fungal pathogens and species of the genus *Banksia* for management.

2 Chapter 2: Invasion pathways of phytopathogenic fungi in South Africa

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Keywords: Pathways, phytopathogens, invasive species, NEM: BA, dispersal vectors

Abstract

Globalisation has resulted in a vast increase of phytopathogens introductions to novel environments through anthropogenic activities. Consequently, the dissemination and spread of phytopathogens through various pathways occurs far more rapidly than via natural processes. Introduction pathways of terrestrial plants, invertebrates, and human infectious disease are well quantified in South Africa. However, information on introduction pathways and dispersal vectors for plant pathogens and their invasion status has received almost no attention. We compiled a list of comprehensively studied phytopathogenic fungi in South Africa and identified prominent introduction pathways of these phytopathogens. Using the categorisation from the unified framework, species were categorised according to their invasion status. Our findings suggest agriculture to be the most prominent introduction pathway of pathogens and wind to be an important dispersal vector in facilitating their spread. Eighteen pathogens are undergoing expansion and three species are recognised as ''fully invasive'' (*Fusarium oxysporum, Phytophthora cinnamomi, Fusarium circinatum*) and we recommend that these species should be prioritised for management. This study advances our understanding of fungal invasions in South Africa and provides an avenue to inform management of pathways that need prioritisation.

2.1 Introduction

Invasion by alien phytopathogens is of great concern in conservation sciences since these species are prominent drivers of biodiversity losses (Vitousek et al., 1996; Desprez-Loustau, 2009). Well studied fungal pathogens are mostly those with large scale impacts, most of these are tree pathogens from the northern hemisphere (Wingfield et al., 2017). These include chestnut blight, caused by *Cryphonectria*

parastica; white pine blister rust (Milgroom & Lipari, 1995) but also the southern hemisphere such as protea root rot caused by *P. cinnamomi* in Australia and South Africa (Von Broembsen & Brits, 1985; Linde et al., 1997).

In South Africa, understanding fungi invasion dynamics is restricted because there is limited data on fungi. The baseline information on fungi biodiversity is limited, making it difficult to classify origins of newly discovered fungi and whether the fungi is alien or indigenous to South Africa (Wood, 2017). However, fungi data generated from global studies suggests that South Africa has 171 500 indigenous fungal species (Crous et al., 2006). Only a few of these species are well documented in the literature and this can be attributed to their detrimental impact on agriculture, rather than their impact in invasion ecology (Desprez-Loustau, 2009).

The spread and dispersal of alien species occur more rapidly than we would anticipate in nature (Hulme et al., 2008; Wilson et al., 2009). Human-mediated dispersal is the main reason of distributing species globally (Mack, 2003; Blackburn & Ewen, 2017). Dispersal pathways differ remarkably with time at different spatial scales and with the human motivation for introducing a species (Poschlod & Bonn, 1998; Mack et al., 2001; Pyšek & Hulme, 2005; Pauchard & Shea, 2006; Kowarik & von der Lippe, 2008). Current human-mediated dispersal requires special consideration as the unintentional transportation of biological material continues at unparalleled spatial and temporal scale (Golan & Pringle, 2017). Fungal pathogens present complex networks of vectors that facilitate their spread and movement globally, within cities and in natural environments (Desprez-Loustau, 2009; Dickie et al., 20 17). When these pathways of an alien invasive species are known, effective control measures can be implemented to mitigate future introductions and impacts (Caldwell et al., 2007).

Dispersal vectors are one of the most important traits when evaluating the distribution and invasion success of a pathogen. Hulme et al. (2008), categorises pathways of introduction from the greatest to the least amount of human intervention as follows: (1) Release: the intentional introduction of an organism to a new environment (e.g. bio control agents); (2) Escape: intentional introduction of a species, that escapes unintentionally into the novel environment (e.g. pest); (3) Contaminant: the unintentional introduction a species with a commodity (e.g. plants infected with pathogens); (4) Stowaway: the unintentional introduction attached to or within a transport vector (e.g. insect and fungi symbiotic relationship); (5) Corridor: the unintentional introduction or organism via human built corridors that link previously unconnected regions (e.g. lessepsian migrants); (6) Unaided: the unintentional introduction of organisms through natural dispersal into novel environments.

Similar to other invasive species management, the main three objectives for fungi should also be prevention, eradication and control (Hulme, 2006; Hulbert et al., 2017). Early detection is the key to eradication success (Desprez-Loustau, 2009), but in many cases eradication of an established species

is not feasible and scientists recommend that government prioritises methods to prevent the introduction of alien species (Campbell, 2001; Novoa et al., 2015). In South Africa, under the National Environmental Management: Biodiversity Act (Act No. 10 of 2004) (NEM: BA A&IS regulations) as of 29 July 2016, 7 fungal species are listed as invasive fungi and all of these species listed, are categorised as 1b species (1b species are major invaders which require government assistance to be removed/contained). The list published under NEM: BA for invasive fungi does not reflect invasive fungi in South African natural ecosystems, but rather reflects that there is no researcher(s) employed to undertake studies on alien invasive fungi in natural ecosystems (Wood, 2017). Recent studies have described and categorised introduction pathways (Hulme et al., 2008; Wilson et al., 2009; Pyšek et al., 2011; Padayachee et al., 2017), but most of these studies focus on the spread of animals and plant species. In invasion ecology, far less attention has been given to the spread of microorganisms (virus, fungi, bacteria) (Desprez-Loustau et al., 2007; Dickie et al., 2017).

The aim of this chapter is to review information of alien phytopathogens in South Africa and, a) identify the most prominent introduction pathways, b) identify dispersal vectors and determine species invasion status and c) to provide recommendations to inform management on fungal pathogens that need to be prioritised and monitored.

2.2 Methods

2.2.1. Selection of pathogens

There are no South African databases with specific information on fungal species or pathogens found in South Africa. Newly discovered fungal pathogens have little or no information to make inferences on species pathways or invasion status (Wingfield et al., 2017; Wood, 2017), therefore these were excluded in this study. Pathogens included in this study were selected from published literature, government and institutional publications that indicated these as species of concern. One of the key sources of information which served a as a baseline for this study is the book “Phytopathogenic Fungi From South Africa” by Crous et al. (2000). Fungal species from this book were screened for the attributes measured in this study namely: region origin, species invasion status, date of introduction (Table 2; Supplementary material 1); however, most pathogens were neither problematic nor invasive, or posed any significant threat to South African ecosystems. Therefore we also screened abstracts of peer reviewed papers to identify problematic species or species that have been well documented in literature with an adverse impact on the environment. We noticed that some species did not have peer reviewed publications but, rather, there is evidence in grey literature of a species causing an adverse impact in agriculture or on the environment. Here we define “grey literature’’ as unpublished data or data that has been published in a non-commercial form such as government reports, statutes, conference proceedings, thesis etc. The following databases were consulted to collate information of the pathogen attributes included in this study: Web of Science, Google Scholar, Science Direct, CBS-

KNAW, and Centre for Agriculture and Bioscience International. The following search terms – or parts thereof – were used: phytopathogenic fungi, South Africa, diseases type, dispersal vectors, and region of origin, invasion status and pathways (Table 2.1). There was no systematic approach adhered to when data was collated since for some species most of the information could be found in two or three sources but for some species multiple sources were used. This resulted in 108 plant pathogens being included in this study.

2.2.2. Pathway and dispersal vectors data collection

Species introduction pathways were identified by employing the framework developed by Hulme et al. (2008). Six principal pathways of introduction are identified in this framework (release, escape contaminant, stowaways, corridor and unaided). Species dispersal vectors information and pathway data were dealt with separately, based on data gathered prominence was given to six-dispersal vectors: wind-borne, insect-borne, rain, soil-borne, seed-borne and tuber-borne. Another variable which was evaluated is whether phytopathogens were undergoing expansion or not. No criterion was developed to quantify this variable; the decision regarding a species was largely based on evidence found in the literature. The following information was taken into consideration when a decision was made regarding a species: the number of new records of the species in the country or in a province over time. The categorisation framework by Blackburn et al. (2011) was used as a tool to classify species invasions status.

The region of origin for the pathogens was considered; however, 85% of pathogens region of origin could not be identified; therefore, no further analyses was conducted. We only found three species to be native to South Africa, two from the Fertile Crescent, one United States of America, two Mexico, one Europe, one South America, (three) Asia (see Supplementary material 1). From these phytopathogens' origins, it can be noted that not for all pathogens was the origin identified to the same level; for some pathogens the country was identified while for others the continent. It is important to note that origin referred to here is not the region which the pathogen was introduced from into South Africa but rather its potential native range.

2.2.3. Invasion status categorisation

Species invasion status was categorised using the framework of Blackburn et al. (2011) (see Appendix 2.1).

2.2.4. Analysis

We rated species distribution on a scale from 1- 9, where one has the smallest distribution meaning the species occurs only in one province and nine has the largest distribution where the species is found in all South African provinces. Species dispersal vector was scaled from 1-6, one having the least dispersal vectors and six having all dispersal vectors identified in this study. A generalised linear

model was developed to evaluate the relationship between species distribution and dispersal vector. This was done to test the hypothesis whether a species with more dispersal vectors is likely to be more widely distributed than a species with fewer dispersal vectors. Prior to conducting statistical analysis, inconsistent records were removed from the dataset (see supplementary material 4). All records lacking species dispersal vector data or distribution data were excluded from the analysis; this resulted in a statistical analysis to being conducted on 47 of the 108 species included in this study.

Statistical analyses were conducted in R version 3.4.3 (R Core Team 2015).

2.3 Results

2.3.1. Pathway and dispersal vectors

Agriculture is the most common introduction pathway for pathogens with forty-three species, followed by horticulture (twelve species) biological control (eight species) forestry (four species) and viticulture (three species) (Fig. 2.1). For thirty-seven percent (forty-one species) the introduction pathways were unknown. In this study, we identified six dispersal vectors of phytopathogens (insect-borne, wind-borne, rain, soil-borne, seed-borne and tuber-borne) (Table 2.1). Wind-borne was the most prominent dispersal vector (twenty-seven pathogens). Three other dispersal pathways are noted to be important dispersal vectors, seed-borne (eleven species), rain dispersed (fifteen species) and soil-borne (twelve species). Six species were dispersed by insects while two species were tuber-borne (supplementary material 1).

2.3.2. Invasive species categorisation

On the regression model we observed a significant relationship between species distribution and dispersal vectors ($z= 3.9$, $df = 45$, $p < 0.01$) (see appendix 2.2 and supplementary material 4). Eighteen species were identified to be undergoing range expansion (Table 2.1), thirty-nine species not so, and for forty-four species this was unknown. According to our classification criteria, we identified fifty-five casual pathogens, three naturalised species, six invasive species and thirty-six pathogens for which invasion status was not classified due to insufficient data. Based on the evidences gathered here, we identified three ''fully invasive'' [(category E) see Appendix 2.1 (Blackburn et al., 2011)] pathogens (*F. oxysporum*, *P. cinnamomi*, *F. circinatum*) that should be prioritised for management.

2.3.3. Distribution

KwaZulu-Natal (KZN) had the most fungal pathogens (98) recorded, the vast majority of species occurring in this province are from the phylum Ascomycota (36.9%) followed by Basidiomycota (28.8%), but for the majority of species recorded, phylum was unknown (40.9%) (Fig.2.2), the least number of pathogens were encountered in Limpopo (8) and Gauteng (11).

2.4 Discussion

We found agriculture to be the most prominent introduction pathway, and wind has proven to be an important dispersal vector in the spread of pathogens. Six species are identified to be invasive, and three species were recognised to fall under category E under Blackburn et al. (2011) (see Appendix 2.1). The identification of introduction pathways and the classification of species invasion status are essential for an effective response to invasions (Faulkner et al., 2016). This study focused on identifying the most prominent introduction pathways of phytopathogenic fungi and dispersal vectors, which facilitates their spread in the novel environment.

Most pathogens were introduced through agriculture; this is largely attributed to anthropogenic activities; thus these pathogens are most likely contaminants of agricultural products. Due to the nature of the agricultural and horticultural industries, more pathogens are likely to be introduced as plant material contaminants of these industries. This is due to lack of pathogen awareness on contaminated plant material in agriculture, horticulture and wholesale nurseries (suppliers and consumers) (Hulbert et al., 2017). Phytosanitary policies in South Africa of all plant material imports states that all consignments imported to the country need to be evaluated for any pathogens or pests which may be present on them. Should there be pests encountered they should be treated with pesticides and then confirmed to be uncontaminated before import (Saccaggi & Pieterse, 2013). Despite this, consignments imported to South African quite often tend to go through bio-security tests with undetected pest from contaminated plant material (Saccaggi & Pieterse, 2013). Evidence in literature suggests the introduction of alien species in South Africa continues to increase at an unprecedented rate through various pathways (Novoa et al., 2015; Faulkner et al., 2016; Hulbert et al., 2017; Padayachee et al., 2017; Wingfield et al., 2017).

We identified plant pathogens found species introduced as bio-control agents (Klein, 2011). The introduction of a species as a bio-control agent is a pathway as per classification by Hulme et al. (2008) —is recognised as the ”release pathway”, and it is also regarded as the intentional introduction of a species into the novel environment. Two pathogens *Acremonium zonatum* and *Cercospora piaropi* were found occurring naturally and were not released as bio-control agents by the Agricultural Research Council (ARC), but rather could have potentially have been co-introduced with their host species (invasive *Eichhornia crassipes*) to South Africa (Klein, 2011; Ray & Hill, 2012). To date, there is no evidence of these pathogens spreading to other native or alien plant species, but rather have proven to be effective bio-control agents of water hyacinth (Morris, 1991; Morris et al., 1999; Ray & Hill, 2012; Ray & Hill, 2016). To the best of our knowledge there are no bio-control agents released by the ARC which have escaped from their target species or have spread to other alien species or native species (Ray & Hill, 2016; Morris, 1991; Wood & Morris, 2007).

Dispersal vectors play an important role in the distribution and spread of pathogens. Of the six dispersal vectors identified in this study, wind was the most prominent dispersal vector. Here we identified eighteen species that are under expansion and fifty-five casual species and three naturalised species. The number of invasive fungal pathogens is likely to increase as more of these naturalised and casual species spread; therefore, these species should be carefully monitored.

The vast majority of pathogens were encountered from the Ascomycota and Basidiomycota phyla. These findings are rather not surprising since the vast majority 98% of fungi described belong to these two dominant phyla Ascomycota and Basidiomycota (Stajich et al., 2009). These two dominant phyla contains a variety of fungal pathogen saprophytic, endophytic entomopathogenic and phytopathogenic fungal species (Kirk et al., 2008). From our findings we found more plant pathogens in KZN; however, the reasons for encountering more plant pathogens in KZN are not clear. It might be that more collections were made in this province, or this could partially be attributed to the warm and humid climate in the province which favours the rapid development of pathogens (Muedi et al., 2015). KZN boasts of a thriving trade industry, as it is the home of the country's largest port. Therefore, it is expected there would be more pathogens encountered in a region that has the busiest port in South Africa (Guastella, 1994). We suggest a bio-security efficacy study be conducted in this region to determine if the current bio-security measures are adequate to detect pathogens on plant materials.

A review of the list published under National Environmental Management: Biodiversity Act (NEM: BA) in 2016 is partially consistent with our findings because we observed three species *P. cinnamomi*, *Fusarium critinum* and *F. oxyspoum* that are on the list (category 1b) and are invasive. However, the other four species on the list are not included in our plant pathogen list (see supplementary Table 1); this may be because the three species listed (*Phyophthora. kernoviae*, *P. pinifolia*, *Mycosphaerella cryptica*) have no records in South Africa (Wood, 2017). However, it is interesting to note that these species are also listed on www.invasivespecies.org. Another pathogen listed is a recently discovered species, *Kirramyces destructans*, which was first reported as occurring only in one locality in Zululand (Greyling et al., 2016). Since this is a recently discovered species, we argue that there is insufficient information to quantify the invasion status of this species in South Africa. Based on the evidence gathered from this study, we recommend that three more species should be listed as category 1b species under NEM: BA. These three species are *Alternaria solani*, *Aspergillus niger* and *Botryosphaeria dothidea*, which are recognised as category D2 under Blackburn et al. (2011) (see Table 2.1).

2.5 Conclusion

Wood (2017) argues that the list published under NEM:BA does not reflect important phytopathogens in South African natural ecosystems and our findings in this review are consistent with Wood (2017). We, therefore, advise that the national invasive species list of microbes be revised and a governing protocol to list species should be developed in order to avoid misconceptions in future. The present study advances our understanding of fungal invasions not just in South Africa but globally by identifying prominent vectors that facilitate the spread and dispersal of phytopathogens. Understanding pathways and dispersal vectors of pathogens will help in developing quarantine protocols for handling infected host species, and in doing so more species can be detected by bio-security.

2.6 List of Figures

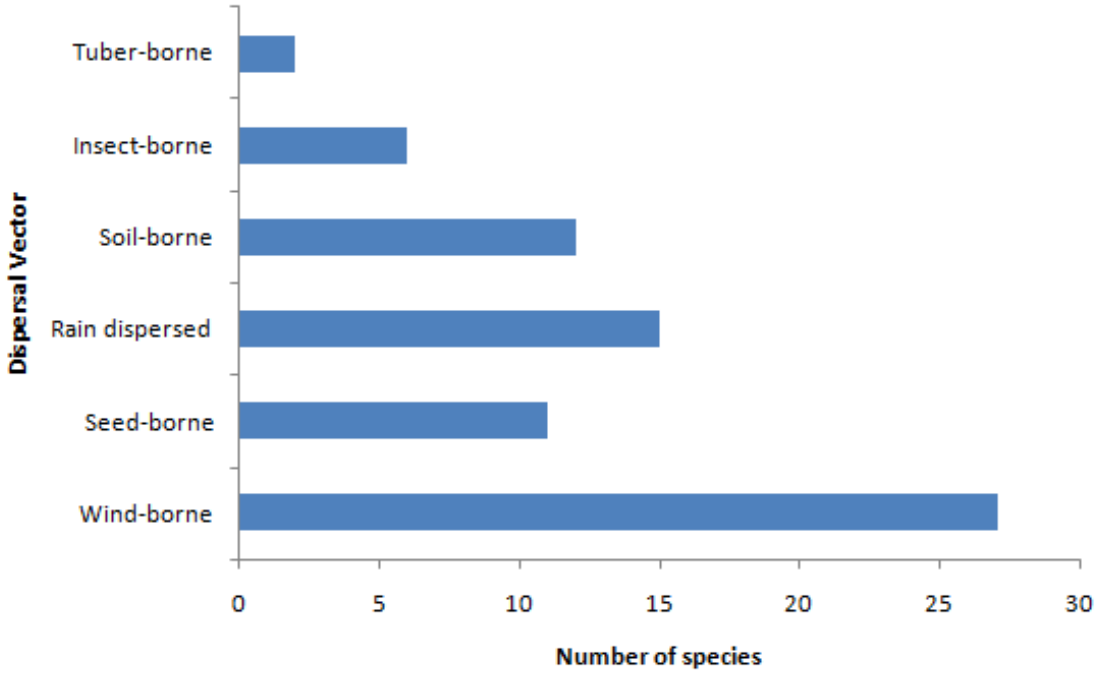

Figure 2.1: Dispersal vectors facilitating the introduction and spread of phytopathogens in South Africa

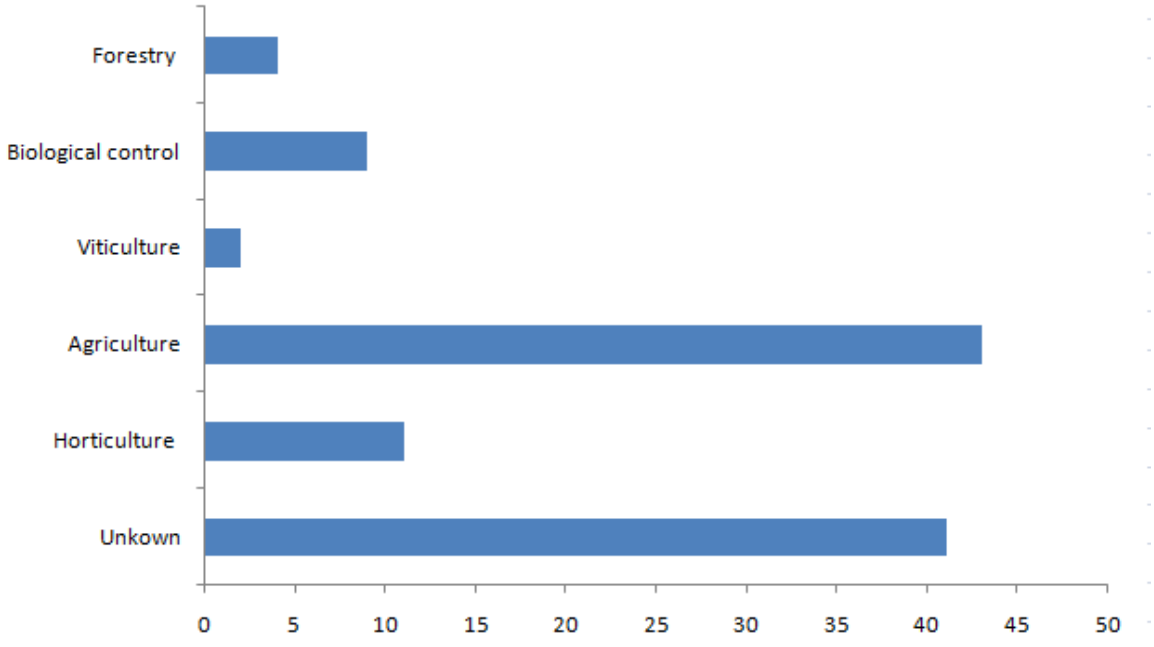

Figure 2.2: Different pathways facilitating introduction and establishment of plant pathogens in South Africa

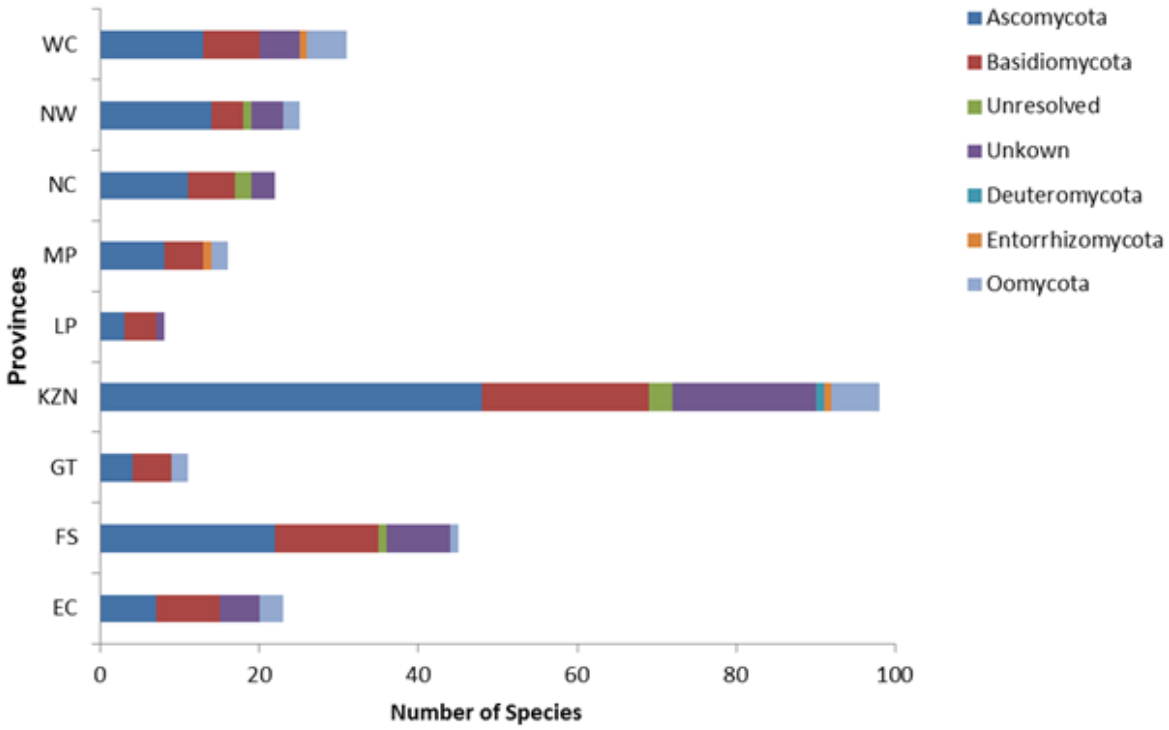

Figure 2.3: Phytopathogens distribution ordered by phyla in South African provinces

2.7 Tables

Table 2.1: Species undergoing rapid population growth in South Africa, their invasion status as defined by Blackburn et al. (2011) and dispersal vectors (sub-set data: see supplementary material 1 for full data)

Pathogen	Dispersal Vectors	Invasion status	Origin	Invasive	Date of first publication/record in SA	Source(s)
Alternaria alternata	W	C3	Unknown	Casual	1990	Woudenberg et al. (2015); Basim et al. (2017)
Alternaria solani	I, W,R	D2	Unknown	Casual	1900	van der Waals et al. (2004); Santiago et al. (2015)
Aspergillus niger	W,S	D2	Unknown	Casual	1921	Crous et al. (2000); Borin et al. (2017)
Botryosphaeria dothidea	W,S,R	D2	California	Casual	1989	Smith et al. (1994); Slippers et al. (2004); Pillay et al. (2013); Marsberg et al. (2017)
Cercospora zeina	W	B3	Africa	Casual	1988	Ward et al. (1999); Pedro W Crous et al., (2006); Neves et al. 2015; Barnes et al. (2016)
Colletotrichum gloesporioides	R	B2	Caribbean Coast of Mexico	Casual	1989	Crous et al. (2000); Sanders et al. (2000); Sanders & Korsten, 2003; Rampersad et al. (2013)
Erysiphe orontii Cast. var. orontii/Golovinomyces orontii/ G.	W	C3	Northern Hemisphere	Naturalised	1977	Gorter (1993); Crous et al. (2000); Seinosuke, (2003); Haupt, (2007); Lebeda & Mieslerová, (2011); Pei et al.

cichoracearum						(2012)
Fusarium circinatum/Gibberella circinata	SB, W	E	Mexico	Invasive	1994	Coutinho et al. (2007): Wingfield et al. (2008)
Fusarium oxysporum	SB, I	E	No data	Invasive	1953	Koenig et al. (1997); Gracia-Garza et al. (1998); Gracia-Garza et al. (1999); Karangwa et al. (2016)
Peronospora destructor	W	B3	No data	Casual	1953	Crous et al. (2000); Wright et al. (2002); Kennedy & Wakeham, (2008)
Phytophthora cinnamomi	R, S	E	Papua New Guinea	Invasive	1931	Brasier et al. (1993); Brasier, (1996) Beaulieu et al. (2017)
Puccinia graminis	W, R	B2	No data	Casual	1925	Le Roux,(1987);Terefe et al. (2010); Visser et al., (2011)
Puccinia hordei	No data	B1	No data	Casual	1953	van Niekerk et al. (2001); Farber & Mundt, (2017)
Puccinia striiformis	W, R	B2	Himalayan	Casual	1996	Boshoff et al., (2002); Visser et al. (2011; Ali et al. (2014)
Pythium aphanidermatum	S	B2	Unknown	Casual	1941	Crous et al. (2000); McLeod et al. (2009); Binagwa et al. (2016)
Rhizoctonia solani	SB, TB	B1	No data	Casual	1918	Truter & Wehner,(2004); Strausbaugh et al. (2011); Muzhinji et al. (2016)
Sclerotinia sclerotioum	W	B2	No data	Casual	1979	Crous et al. (2000); McLaren & Craven, (2008); Qandah & del Río Mendoza, (2011)

Sclerotium rolfsii	SB	B1	No data	Casual	1926	Moore, (1926); Shim et al. (1998); Flores-Moctezuma et al. (2006)

W wind-borne, I insect-borne, R rain, S soil-borne, SB seed-borne, TB tuber-borne,

Appendices

Appendix 2.1 Plant pathogen categorisation and related definitions as defined by Blackburn et al. (2011)

Definition	Category
Not transported beyond limits of the native range	A1
Individuals transported beyond limits of the native range, and in captivity or quarantine (i.e. individuals provided with conditions suitable for them, but explicit measures of containment are in place)	B1
Individuals transported beyond limits of the native range, and in cultivation (i.e. individuals provided with conditions suitable for them but explicit measures to prevent dispersal are limited at best)	B2
Individuals transported beyond limits of the native range, and directly released into the novel environment	B3
Individuals released into the wild (i.e. outside of captivity or cultivation) in a location where introduced, but incapable of surviving for a significant period	C0
Individuals surviving in the wild (i.e. outside of captivity or cultivation) in a location where introduced, no reproduction	C1
Individuals surviving in the wild in a location where introduced, reproduction occurring, but population not self-sustaining	C2
Individuals surviving in the wild in a location where introduced, reproduction occurring, and population self-sustaining	C3
Self-sustaining population in the wild, with individuals surviving a significant distance from the original point of introduction	D1
Self-sustaining population in the wild, with individuals surviving and reproducing a significant distance from the original point of introduction	D2
Fully invasive species, with individuals dispersing, surviving and reproducing at multiple sites across a greater or lesser spectrum of habitats and extent of occurrence	E

Appendix 2.2: The table represents the species dispersal vectors and species distribution (see methods for categorising species distribution and dispersal vector)

Species	Dispersal vector	Distribution
Alternaria alternata	1	2
Alternaria porri	2	4
Alternaria solani	4	5
Aspergillus niger	2	2

Athelia rolfsii	1	1
Blumeria graminis	2	2
Botryosphaeria dothidea	3	1
Botryosphaeria obtusa/ Diplodia seriata	1	1
Botrytis cinerea	1	1
Cercospora penzigii	1	1
Cercospora piaropi	1	1
Cercospora zeina	1	3
Colletotrichum circinans	2	2
Colletotrichum gloesporioides	1	3
Colletotrichum spp.	1	3
Didymella pinodes	2	1
Erysiphe orontii Cast. var. orontii/Golovinomyces orontii/ G. cichoracearum	1	1
Fusarium circinatum/Gibberella circinata	1	2
Fusarium graminearum	1	3
Fusarium oxysporum	2	3
Fusarium solani	1	3
Gaeumannomyces graminis/ Rhaphidophora graminis	3	1
Glomerella cingulata	1	4
Guignarda citricarpa	2	6
Leptosphaeria nodorum/ Septoria nodorum/Phaeosphaeria nodorum	3	2
Oidium mangiferae	1	2
Ophiostoma ulmi	1	1
Peronospora destructor	1	5
Phyllosticta odinae	2	1
Phytophthora cinnamomi	2	2
Phytophthora porri	2	1
Pleospora alli/ Stemphylium vesicarium	1	3
Puccinia allii	2	1
Puccinia isoglossae	1	1
Puccinia phyllocladiae	2	1
Puccinia striiformis	1	7
Pyrenochaeta terrestris	1	5

Pythium aphanidermatum	2	2
Rhizoctonia solani	1	4
Rhynchosporium secalis	1	1
Sclerotinia sclerotioum	1	3
Septoria lannae	2	1
Stagonospora curtisii/ Peyronellaea curtisii/Phoma narcissi	1	1
Uromyces badius	1	1
Uromyces hypoestis	1	3
Ustilago scitaminea	1	1
Verticillium dahliae race 2	1	4

3 Chapter 3: The potential role of phytopathogenic fungi in limiting plant invasions: the case of Australian *Banksia* (Proteaceae) in the Cape Floristic Region

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Keywords: Invasion, Horticulture, phytopathogen, minimum inhibitory concentration (MIC), National Environmental Biodiversity Act (NEM: BA), Invasive Alien Plants (IAPs), weed risk assessments (WRA).

Abstract

Fungus invasions have until recently received little attention in invasion biology. This is largely attributed to little or non-existent information about these inconspicuous organisms. The exceptions are a few well-known cases of the devastating impact of phytopathogenic fungi on native ecosystems and agriculture. In this chapter, we investigated whether the invasiveness of the Australian genus *Banksia* in South Africa was limited by the phytopathogens, *Phytophthora cinnamomi.* Furthermore the antifungal activity against *P. cinnamomi* was determined for these alien *Banksia* species. The risk posed by each species in the region was assessed using the Australian Weed Risk Assessment protocol (A-WRA*).* The presence of *P. cinnamomi* in *Banksia* root and soil samples was evaluated using morphological and molecular techniques. Isolates were cultured onto selective media and polymerise chain reactions (PCR) and internal transcribing spacers (ITS) were used to identify the fungi. Acetone leaf extracts of twelve *Banksia spp.* were screened for antifungal activity against *P. cinnamomi*, using the minimum inhibitory concentration (MIC) assay. A total of 3840 *Banksia* individuals from seven localities were surveyed, 1063 individuals were recorded as dead and 2772 were alive. *P. cinnamomi* was consistently isolated from *Banksia* species root and soil samples. Out of the twelve *Banksia* species that were screened for antifungal activity against *P. cinnamomi*, four introduced species *B. burdettii, B. coccinea, B. hookeriana* and *B. prionotes* exhibited high antifungal activity. Risk assessments indicated that five of the fourteen species pose a high risk of invasion, eight species required further evaluation and one species, namely, *B. coccinea,* has a low risk for invasion. The invasive *B. integrifolia* and *B. ericifolia* had high antifungal activity against the *P. cinnamomi* strain

(696/12). Consequently, on the basis of these results, these *Banksia* species should be prioritised for management. Furthermore, we recommend the listing of *B. ericifolia* and *B. integrifolia* as category 1b and the introduced *B. spinulosa* and *B. quercifolia, B. hookeriana, and B. formosa* as category 2 species under the National Environmental Biodiversity Management Act (NEM: BA) AIS regulations.

3.1 Introduction

Fungi are essential organisms for the proper functioning of most ecosystems, as food sources, mutualists, decomposers or pathogens (Desprez-Loustau et al., 2007; Gladieux et al., 2015). Despite this, the ecological impact of alien fungi in natural ecosystems has not been well quantified, mainly because fungi are so inconsipicous and only attract attention when plants are dying on a large scale (Desprez-Loustau et al., 2007; Gladieux et al., 2015). Other than a few well-known cases of phytopathogenic fungi causing devastating economic and ecological impact, such as Dutch elm disease epidemics on *Ulmus* spp. caused by *Ophiostoma ulmi* (Loo, 2009; Scott et al., 2009; Davis et al., 2014) and the decline of *Banksia* woodlands caused by *Phytothphthora cinnamomi* in Australia, alien invasive fungi are mostly studied in an agricultural, rather than an invasion context (Desprez-Loustau et al., 2007).

One of the most prominent questions in invasion biology is why some alien species are more widespread than others (Catford et al., 2009). There are various factors that arise when answering this question, such as the species life history (trait-mediated effect), biogeographic range and climate (Richardson et al., 1990;Werner & Peacor, 2003; Geerts et al., 2013; Moodley et al., 2013), the presence of natural enemies and immunity to pathogens (Hatcher et al., 2006; Dunn et al., 2012). One of the main hypotheses explaining the rapid establishment of an alien plant species in a new geographic range, is the enemy release hypothesis (Keane & Crawley, 2002). This hypothesis is based on the assumption that the spread of a species in their native range is regulated by the presence of natural enemies. However, a species may not escape entirely from natural enemies in the recipient community. In some instances, the species could be co-introduced with its natural enemies or a cosmopolitan species is already established in the introduced range (Keane & Crawley, 2002; Dunn & Hatcher, 2015). This can prevent invasion or slow down the spread of an invasion.

Phytophthora cinnamomi is a global invasive pathogen and has been listed amongst the top 100 most invasive species in the world (Lowe et al., 2000; Hatcher et al., 2006; Hansen, 2008). In Australia, this species is associated with the mortality of *Eucalyptus marginata* and the decline of Proteaceae species in the Jarrah Forest, including species of the genus *Banksia* (Shea et al., 1982; Hardham, 2005; McCredie et al., 1985). *Phytophthora cinnamomi* is the most damaging invasive phytopathogenic fungi in South Africa (Wood, 2017) and is widely distributed throughout South Africa (Linde et al., 1997). *Phytophthora cinnamomi* has been reported to cause die-back of commercially cultivated native and alien Proteaceae species in the Cape Floristic Region (CFR) (Von Broembsen, 1985). *P. cinnamomi* is alien to South Africa (Linde et al., 1997) and was most likely to have been co-introduced with contaminated potatoes from Papua New Guinea in the 1800s (Galindo & Zentmyer, 1964; Zentmyer, 1988; Linde et al., 1997; Linde et al., 1999).

The genus *Banksia* belongs to the family Proteaceae and has the highest number of introduced species from this plant family in South Africa (Moodley et al., 2014). Species of this genus were introduced to South Africa for floriculture in the 1970s. The potential risk posed by *Banksia* species was assessed by Richardson et al. (1990) and, quite recently, *Banksia ericifolia* by Geerts et al. (2013). As knowledge advances, continuous evaluation of the risk posed by alien species is crucial for species prioritisation and management. Weed Risk Assessments (WRA) present an ideal tool for supporting the exclusion of potentially invasive species before their introduction in the novel environment (pre-border assessment) (Pheloung et al., 1999), as well as for assessing the potential impact of an established species in the novel environment (post-border assessment) along the invasion continuum (Blackburn et al., 2011; Moodley et al., 2017).

The objectives of this study are, therefore, to (a) evaluate the presence and impact of *P. cinnamomi* on *Banksia* species in South Africa; (b) screen the antifungal activity of introduced, naturalised and invasive *Banksia* species; (c) conduct post-border weed risk assessments; and (d) make inferences on *Banksia* species invasion potential, based on antifungal activity to *P. cinnamomi*, in South Africa.

3.2 Material and Methods

3.2.1. Study area and study species

The study was conducted at seven *Banksia* species localities in the Cape Floristic Region of South Africa (Fig 3.1). The Cape Floristic Region is categorised by a sub-Mediterranean climate with cold winters and warm, dry summers. The region is approximately 90,000 km^2 and contains over 9,000 plant species (Collins & Rebelo, 1987; Cowling & Richardson, 1997; Moran & Hoffmann, 2012). The vast majority of plant species in the region are fire prone and thrive on nutrient poor soils (Cowling & Richardson, 1997; Moran & Hoffmann, 2012). *Banksia* localities were obtained from Geerts et al. (2013), Moodley et al. (2014), Moodley et al. (2016) and consultations with local experts and conservationists. During surveys we added localities, which had not been previously recorded for *B. coccinea*, *B. baxteri*, *B. hookeriana*, *B. prionotes*, *B. serrata*, *B. formosa* and *B. intergrifolia*.

3.2.2. *Banksia* mortality surveys

Out of the fourteen *Banksia* species occurring in the CFR, the survival percentage of eleven species was determined. The other three species were not found during the field surveys and some had been cleared in some areas in the region. At each locality, the number of transects established were determined by the number of species occurring. A minimum of two transects were established per locality for a species and thereafter these transects were averaged. Transects were 50 m long and 5 m wide. Each point of origin was arbitrarily chosen and marked with a handheld GPS unit (Garmin GPSMAP 64st).

3.2.3. *Phytophthora cinnamomi* collection and isolation

At seven *Banksia* localities in the CFR, twenty three, approximately 1500 g of soil samples from the top 10 cm at the bases of *Banksia* individuals showing necrosis were collected and sealed in a plastic bag. Isolation from soil and diseased plant samples was performed within 48 h of collection. Bait solution was prepared by mixing soil sample (20 g) with 100 ml of sterile deionised water (dH_2O); the bait solution was mixed using a vortex mixer for three minutes. Sterile *Citrus sp*. leaf sections (0.5 m^2) were introduced into the bait solution for three days. After three days, *Citrus sp*. leaf sections were plated onto selective NARP (Natamycin. Ampicillin, Rifamyicin, Pentachloronitronzene) agar (Jeffers & Martin, 1986) and incubated in the dark for five days at 25 ºC. Root samples 1cm^2 were dissected, rinsed for 5 minutes and surface sterilised with 70% ethanol and left to air dry. Sterile root samples were plated on NARP agar and incubated in the dark for 5 days at 25 ºC. In order to obtain pure cultures, 1 cm diameter of solid agar containing actively growing fungus was transferred onto clean Petri-dish with NARP new agar.

3.2.4. Identification of *Phytophthora cinnamomi*

Actively growing 1 cm diameter mycelia on NARP agar was transferred to 10% V8 broths. Mycelium of isolates grown in V8 broth was harvested and rinsed with dH_2O, excess water was removed with a filter paper and the mycelia was placed in two millilitre microfuge tubes, and lyophilised with VirTris Advantage BenchTop Tray Lyophilizer (SP Scientific, UK) overnight. The dried mycelium was then transferred into sterile microfuge tubes with two three-millimetre metal beads. This was followed by extraction of the total genomic deoxyribose nucleic acid DNA and amplification of target genes. A polymerase chain reaction (PCR) was carried out using a modification of the protocol described by Winton and Hansen (2001). The Internal Transcribed Spacer regions of the rDNA (ITS1 and ITS2) were amplified using the primers ITS6 (Cooke & Duncan, 1997) and ITS4 (White et al., 1990) and the cytochrome oxidase subunit I (coxI) was amplified using the primers FM84 and FM83 (Martin et al., 2003) with optimum temperatures of 55 ºC and 50 ºC, respectively. Amplified DNA was purified with a high pure PCR product purification kit (Roche, RSA) and sequenced with the same primers. Cloning was conducted using pGEM-T® Easy vectors (Promega, USA). The manufacturer's instructions were followed when the sequences of the isolates could not be read due to DNA polymorphism. Sequences of the isolates were uploaded and aligned in Geneious v. R6. The most appropriate substitution model was determined using jModelTest (Posada, 2008). The TIM3+G (ITS) and GTR+I+R (coxI) models were selected and used in the Bayesian analysis (Ronquist & Huelsenbeck, 2003).

3.2.5. Zoospore preparation

Phyopthora cinnamomi isolate (696/12 12g) was selected for the minimum inhibitory concentration bioassay and this was partially attributed to its consistent proliferation and spread when it was cultured on NARP agar. A diameter (1 cm) of *P. cinnamomi* was cut from the margins of the NARP medium using a sterile cork borer and transferred into a selective medium; 1000 mL Nutrient Broth Merck (Pty. Ltd., South Africa) containing antibiotics (25 mg/mL Pimaricin, 100 mg/mL Ampicillin, 5 mg/mL Rifamycin, 100 mg/ mL Pentachloronitrobenzene) (Jeffers & Martin, 1986), and then incubated for 60 minutes at 23 ºC. A haemocytometer was used to count zoospores. The final spore concentration for the MIC was maintained at 1×10^5 cells/mL (Nchu et al., 2010).

3.2.6. Acetone leaf extracts

Fresh leaf materials were collected from twelve *Banksia* spp. which was oven-dried at 30 ºC for five days. The dried leaves were ground into fine powder using a Jankel and Kunkel Model A10 mill. Ground leaf material (5 g) was extracted with 100 ml of acetone in a glass beaker using a vortex mixer for 15 min and then filtered through Whatman No.1 filter paper. The plant extracts were left to air dry in a fume cabinet overnight at room temperature (22 ± 2 ºC).

3.2.7. Minimum Inhibitory Concentration (MIC) bioassay

The MIC assay, previously described by Eloff (1998) and Nchu et al. (2010), was adopted with modifications. Each *Banksia* species had six replicates. The bioassay was conducted using 96-well microplates; 100 µl of dH_20 was added to each well, followed by a serial successive dilution of acetone plant extract to obtain an initial concentration of 6 mg/ml. A concentration of 100 µl of *P. cinnamomi* (1×10^5 cells/mL) was added to each well, and finally 40 µl of (0.2 mg/mL of p-iodonitrotetrazolium (Sigma) dissolved in dH_20, was also added to each well. The bio-reagent p-iodonitrotetrazolium salt acted as an electron acceptor and displayed a red colour due to biological active organisms (Eloff, 1998). Microplates were sealed with plastic and incubated at 25 ºC in the dark. Minimum inhibitory concentration (MIC) values were recorded periodically every six hours for 24 hours. The same protocol was used for the negative control by substituting acetone plant extract with acetone and for the positive control, 480 µg/mL Amphotericin b dissolved in acetone. Antifungal activity was rated in these four categories: (i) ($1 \leq 3$ mg/mL) relatively high antifungal activity; (ii) ($3.1 \leq 3.5$ mg/mL) relatively optimum antifungal activity; (iii) ($3.6 \leq 5.9$ mg/mL) relatively low antifungal activity; and (iv) (≥ 6 mg/mL) no activity.

3.2.8. Weed risk assessments

We employed the Australian Weed Risk Assessment (A-WRA), developed by Pheloung et al. (1999), to evaluate the potential risk posed by *Banksia* species in the CFR. The A-WRA is an assessment of a species based on its biology, biogeography, history and ecology. The A-WRA is a useful system to predict potentially invasive plants. The assessment consists of 49 questions and each question is awarded points of between -3 and 5. The final answer of the assessment results is based on the possible three outcomes regarding the species; if a species has score (< 1) it can be introduced, if a species has a score (> 6) the species cannot be introduced; and lastly if a species outcome score is (1 - 6) the species requires further evaluation. Species data available in literature may be insufficient to answer all 49 questions. In this case, a minimum of ten questions is required for the assessment (Moodley et al., 2017).

3.2.9. Soil nutrient analysis

One kilogram of each of the soil sample was sent to a commercial laboratory (Bemlab Pty Ltd, in Somerset West, South Africa) for a complete soil chemical analysis. The following protocols were implemented for soil analysis: the soil was air dried, sieved through a 2 mm sieve for determination of stone fraction (weight/weight basis) and analysed for pH (1.0 M Kill), P (Bray II) and total extractable cations, namely K, Ca, Mg and Na (extracted at pH = 7 with 0.2 M ammonium acetate) and organic carbon by means of the Walkley-Black method (The Non-affiliated Soil Analyses Work Committee, 1990). Micro-nutrients (Zn, Mn, Cu & Fe) were extracted with Di-ammonium EDTA (0.02 M) and boron (B) using a 1:2 hot water ratio (The Non-affiliated Soil Analyses Work Committee, 1990).

Sulphur (S) was extracted with concentrated phosphoric acid (at pH = 4) according to an adapted method as described in Pansu and Gautheyrou (2006). The extracted solutions was analysed with a Varian ICP-OES optical emission spectrometer.

Salinity was determined by measuring the resistance of saturated paste in an electrode cup according to the method described by the Non-affiliated Soil Analyses Work Committee (1990). Total P in the soil was determined by a method adapted from that described by Sommers and Nelson (1972). The P was extracted from soil through acid digestion using a 1:1 mixture of 1 N nitric acid and hydrochloric acid at 80ºCfor 30 minutes.

The P concentration in the extract was then determined with a Varian ICP-OES optical emission spectrometer. Both total C and N content of soil were determined through total combustion using a LecoTruspec® CN N analyser. Ammonium and nitrate are extracted from soil with 1N KCl. Ammonium-N (NH_4^+-N) is determined colorimetrically on a SEAL AutoAnalyzer 3 after reaction with a sodium salicylate, sodium nitroprusside and sodium hypochlorite solution that was buffered at a pH of 12.8 to 13.0. Nitrate-N (NO_3^--N) concentration in the extract was also determined colorimetrically on a SEAL AutoAnalyzer 3 through the reduction of NO_3^- to NO_2^-, using a copper-cadmium reduction column, where after the nitrate reacts with sulphanilamide under acidic conditions, using N-1-naphthylethylenediamine dihydrochloride.

3.2.10. Statical analysis

A one-way ANOVA was employed to analyse differences in antimicrobial activity among *Banksia* species and the means were compared with a post-hoc Tukey HSD test. Polynomial regression was used to compare species survival and WRA score relationship ($y \sim x + x^2$) this model was the best fit compared to the linear regression. Statistical analysis was conducted by using the software program R statistics ver.3.4.3 (R Core Team, 2015).

3.3 Results

3.3.1. *Banksia* mortality surveys

A total of 3840 *Banksia* individuals were surveyed in this study; 1068 individuals were recorded as dead and 2772 individuals were alive. The survival rate varied between species (Fig. 3.2) the two invasive species *B. integrifolia* (98%) and *B. ericifolia* (95%), had the highest survival rate (Appendix 3.2). The survival rate of introduced species varied from high in *B. spinulosa* (85%) and *B. hookeriana* (89%) to *B. serrata* (48%) as the lowest. Dying *Banksia baxteri* (72%) and *Banksia coccinea* (51%) individuals showed clear symptoms of necrosis and were rotten at the base of the stem (Fig. 3.3a and 3.3b).The two naturalised species, *B. speciosa* (53%) and *B. formosa* (61%) (Fig. 3.3c), had an intermediate survival rate.

3.3.2. Minimum inhibitory concentration (MIC) bioassay

The minimum inhibitory concentration activity was significantly different among the species (one way ANOVA, F=18.2, df 13, $P < 0.001$). While *B. speciosa* showed low antifungal activity against *P. cinnamomi,* four species (B. *integrifolia*, *B. ericifolia*, *B. formosa* and *B. hookeriana*) exhibited high antifungal activity against *P. cinnamomi* (1<3mg/ml), after 18 hours (Fig 3.4 and supplementary material 3). Among these four, were the two invasive species *B. integrifolia* and *B. ericifolia,* and two introduced species, *B. hookeriana* and *B. formosa*. Five species, the naturalised *B. coccinea* and introduced species *B. baxteri*, *B. quercifolia*, B. *prionotes* and *B. spinulosa* after 18 hours, showed optimum antifungal activity against the pathogen. *B. speciosa* after 18 hours exhibited no antifungal activity. The positive and negative control showed no antifungal activity against *P. cinnamomi* after 18 hours.

3.3.3. Weed risk assessments

The risk assessment outcome of five species is a reject (6<), meaning that these species pose a high risk of invasion. Eight *Banksia* species require further evaluation (1-6). This is largely attributed to insufficient data to assess the risk posed by each species (appendix 3.1). There was a significant relationship observed ($r^2 = 0.6122$; $P < 0.05$) between the weed risk assessment scores and species survival rate in the Cape Floristic Region (Fig. 3.5).

3.3.4. Soil nutrient analyses

The average soil texture (7% clay, 8% silt and 86% sand) on *Banksia* localities was regarded as loamy sand. Generally, the soils in all the sites were acidic with an average pH of 4.6; soil nutrients seemed to be optimal for *Banksi*a species. No significant difference in soil nutrients were observed between the various sites or were correlated to *Banksia* mortality ($P > 0.05$ for all correlations) (see supplementary material 2).

3.4 Discussion

Our study reveals that for the two invasive species, *B. ericifolia* and *B. integrifolia,* antifungal activity was high against the tested isolate of *P. cinnamomi*. Arguably, these two *Banksia* species contain chemical constituents which could potentially suppress infection by *P. cinnamomi.* The antifungal activity of the naturalised *B. speciosa* to *P. cinnamomi* was relatively low, this could possibly explain the low survival rate observed in the field. The weed risk assessments depicted five species, namely: *B. speciosa, B. ericifolia, B. integrifolia, B. quercifolia,* and *B. spinulosa,* to pose a high risk of invasion. *B. ericifolia* and *B. integrifolia* are known to be invasive species and *B. speciosa* was previously reported as an emerging invader (Geerts et al., 2013; Moodley et al., 2013; Moodley et al., 2014). *Banksia speciosa* was previously reported by Moodley et al. (2013) to have all the morphological traits to invade in the CFR, however from our findings we have established that this species invasion will be limited by the prevalence *P. cinnamomi* in the CFR. These species have low antifungal activity against *P. cinnamomi* and has relatively low survival rate on *P. cinnamomi* infested sites in the region.

Phytophthora cinnamomi is quite prevalent in the CFR (Von Broembsen, 1984; Von Broembsen, 1985; Linde et al., 1999). Similarly it was isolated from 90% of *Banksia* localities we surveyed in our study. Only at two localities with *B. integrifolia* – one on coastal dunes in Pringle Bay (Fig 3.4d) and one on a farm – did we not isolate *P. cinnamomi*. However, it is worth noting that these populations consisted of only a few individuals, which were not more than eight years old.

The screening of *Banksia* species acetone leaf extracts provided evidence that there are *Banksia* species which have the ability to inhibit infection and colonisation by *P. cinnamomi*. Thus, this is one of the underlying factors which may possibly explain the difference in species mortality rate in the region. Antifungal activity findings from this study are consistent with the seedling inoculation trials conducted by McCredie et al. (1985) and Tynan et al. (1998) with the exception of *B. hookeriana,* where the species was reported to susceptible against *P. cinnamomi*. However in this study the species was found to have high antifungal activity against the pathogen and in the field we observed high survival rate on *P. cinnamomi* infested *Banksia* localities. Soil nutrients analysis revealed that soil conditions were optimum to favour *Banksia* species since Proteaceae species prefer low nutrients soils (Collins & Rebelo, 1987; Cowling & Lamont, 1987; Cowling & Richardson, 1997; Denton et al., 2007). Therefore, we can conclude that these species were not under soil nutrient stress.

The ability of some *Banksia* species to suppress *P. cinnamomi* infection is one trait for *Banksia* species to successfully invade in the CFR. However there are other important traits that are required in order for a species to establish successfully or invade in a region such as climate suitability, propagule

pressure, pollinators and fire. Fire was observed by Geerts et al. (2013) as an important factor which facilitated invasion success of *B. ericifolia* after a lag phase in the CFR. The importance of *Banksia* pollinators was studied by Moodley et al. (2016), and because most *Banksia* species reproduced via autonomous self-pollination this does not affect the naturalisation or invasion status of *Banksia* species.

Species of the genus *Banksia* are of economic importance to the horticultural and floricultural industries, although some species pose a significant threat to be invasive. However, we recommend that not all species which pose a high risk of invasion be prohibited for cultivation, but rather should be monitored and kept in captivity. In the event of new *Banksia* species being introduced for floriculture, pre-border risk assessments should be conducted, to evaluate if species pose a high risk of invasion. Species that are highly susceptible to *Phytophthora cinnamomi* could potentially be safely introduced into the country and still be profitable to grow. The plant pathogens can be treated in plantations and the flowers used for floriculture. In an event where these species escape plantations into native vegetation, invasion will be curbed by *P. cinnamomi* infection.

We acknowledge that, even though we have not conducted a plant species inoculation experiment, the bioassay conducted in this study gave us a good indication as to which species are able to suppress infection and colonisation by *P. cinnamomi*. Conducting species minimum inhibitory concentration bioassay can be used as a reliable tool to predict possible resistance of a plant species to a pathogen.

The significant relationship observed between weed risk assessment scores and species survival rate, indicates that the survival rate of species, which pose high risk of invasion, is relatively high, therefore supporting evidence that weed risk assessments can be used as a reliable tool to predict the risk posed by a species even though it cannot accurately predict species invasion success (Moodley et al., 2014).

There are no *Banksia* species listed in South Africa's National Environmental Management: Biodiversity Act (10/2004) AIS regulations. However *Banksia ericifolia* was placed on the suspect list in 2013 by SANBI (Wilson et al., 2013). Nevertheless, given the accumulative evidence gathered from previous studies, [Geerts et al., (2013), Moodley et al., (2013) and, Moodley et al., (2014)], as well as this study, we recommend that *B. ericifolia* and *B. integrifolia* be listed under NEM: BA; as category 1b species. *B. spinulosa, B. quercifolia, B. hookeriana, and B. formosa* these species have shown that they have antimicrobial activity against *P. cinnamomi* and also possess other morphological invasive traits, and therefore we suggest these species be listed as category 2 species under NEM: BA.

3.5 Conclusion

In this study we show that selected *Banksia* species are resistant to *P. cinnamomi* and pose a high risk of invasion in the Cape Floristic Region. Consequently, as a first step to reducing the risk posed by these species, this study suggests that selected *Banksia* species (*B. ericifolia, B. integrifolia, B. hookeriana, and B. formosa*) be prioritised for management. Furthermore, under drought conditions, *P. cinnamomi* susceptible *Banksia* such as *B. speciosa* might be less affected, since *P. cinnamomi* reproduction is favoured by moist soils. For future research, we, therefore, recommend in-situ and ex-situ *P. cinnamomi* inoculation trials on *Banksia* species under different watering regimes.

List of figures

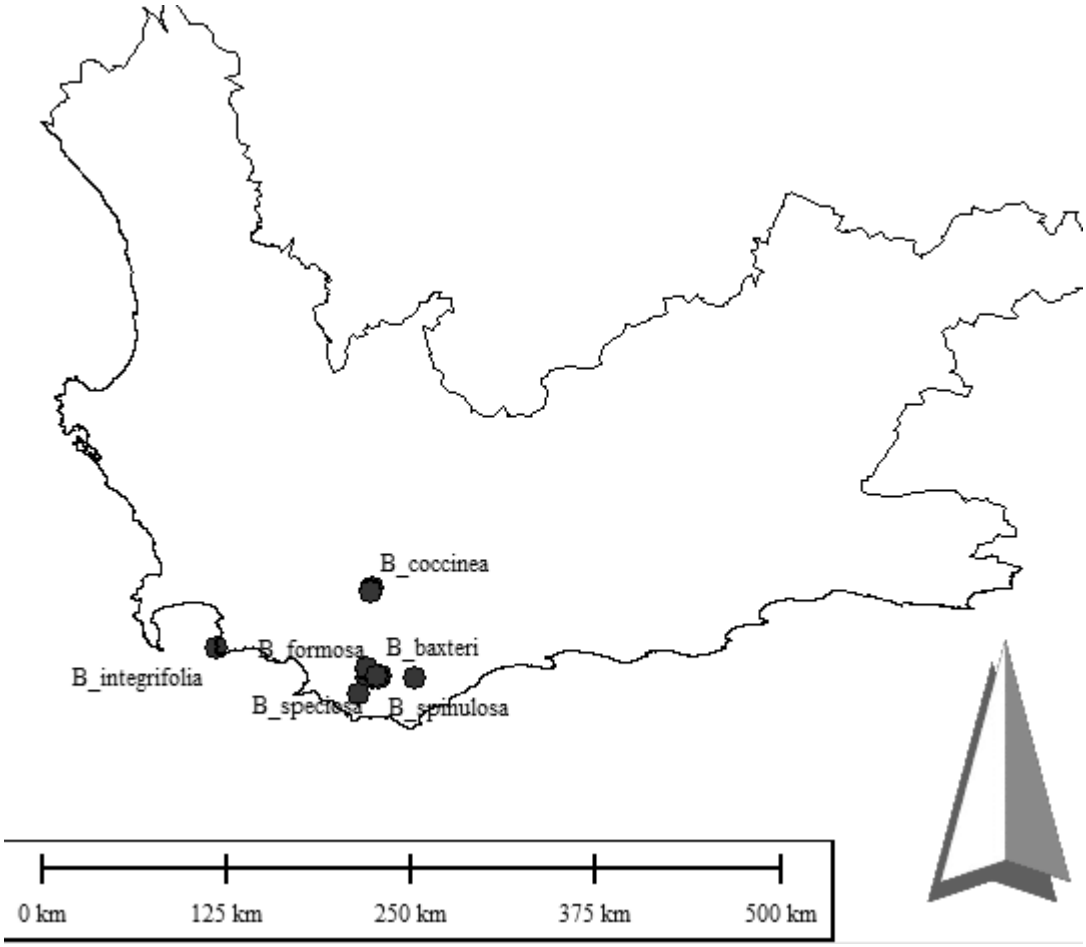

Figure 3.1: *Banksia* species (Proteaceae) localities of this study in the Cape Floristic Region (Western Cape), (see supplementary material 1)

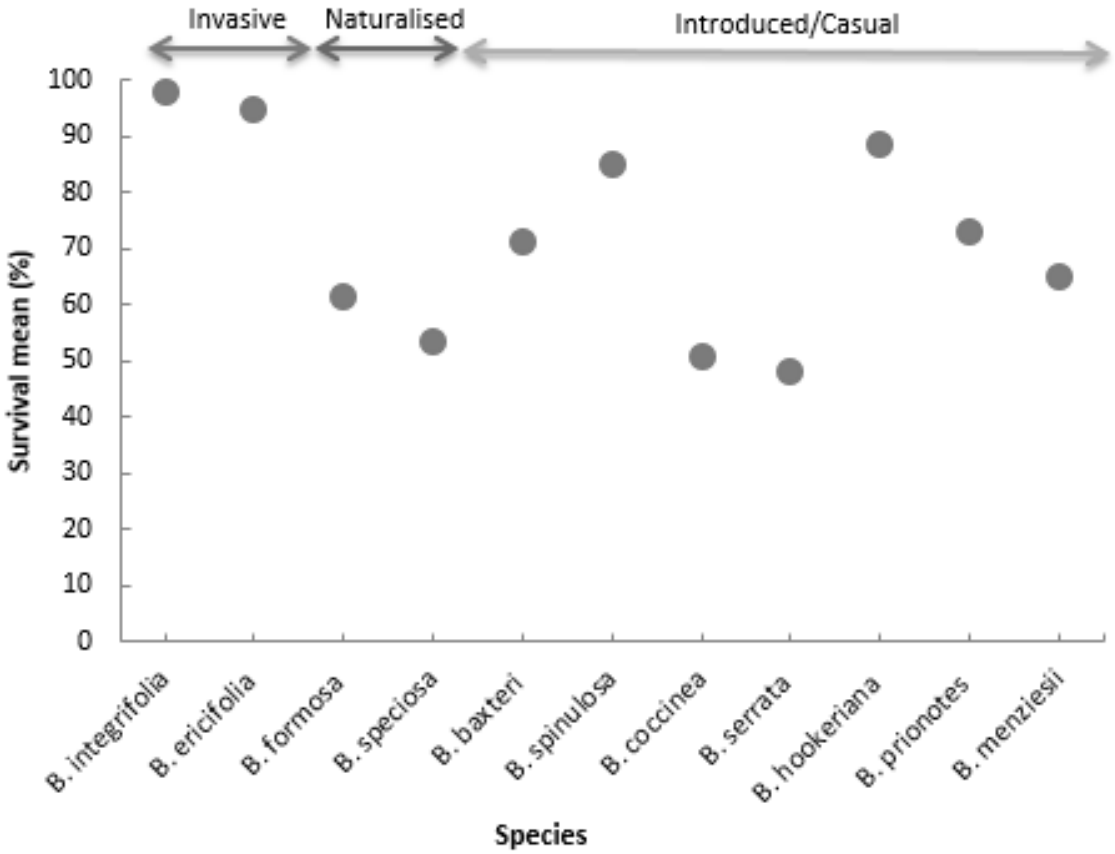

Figure 3.2: Survival mean (%) of the 11 *Banksia* species at *P. cinnamomi* infested localities in the Cape Floristic Region. Grey circles depict mean. Species invasion status indicated at the top of the graph

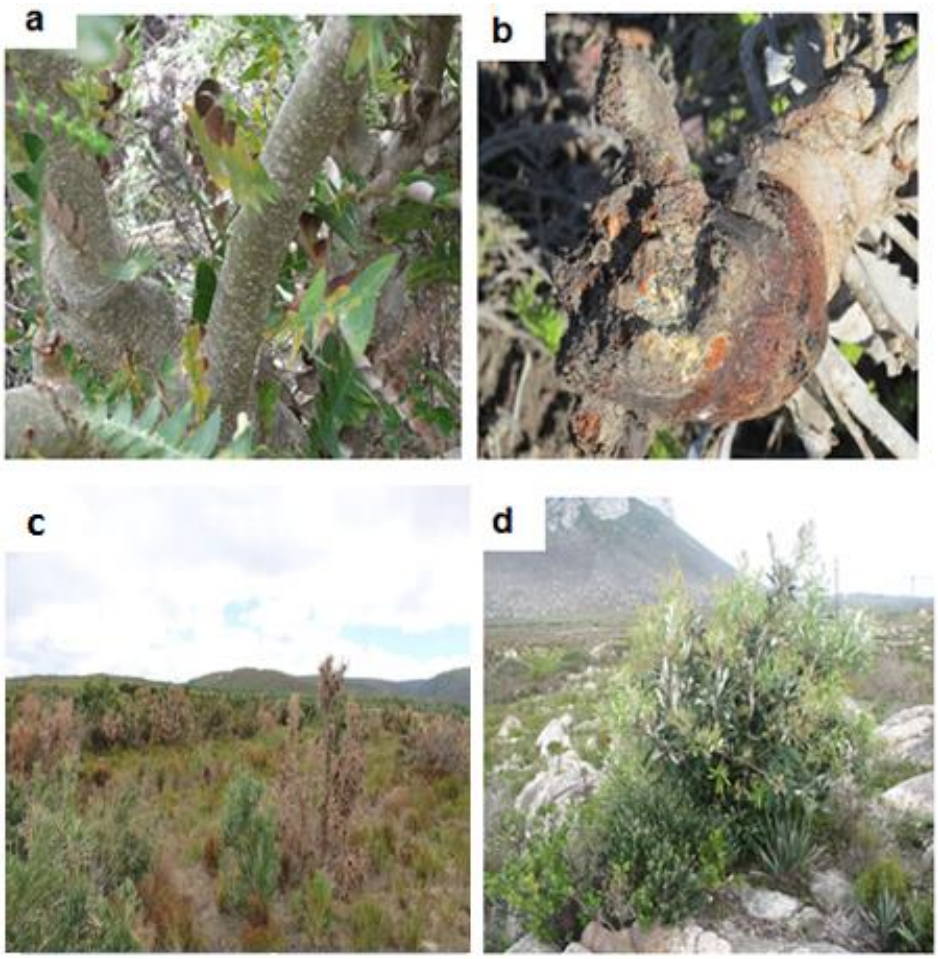

Figure 3.3: a) *P. cinnamomi* infested *B. baxteri* showing leaf lesions; b) *P. cinnamomi* infested *B. coccinea* base, rotten showing ‘’canker’’; c*). P. cinnamomi* infected *B. formosa* plants dying; d).*B. integrifolia* growing on coastal dunes in Pringle Bay.

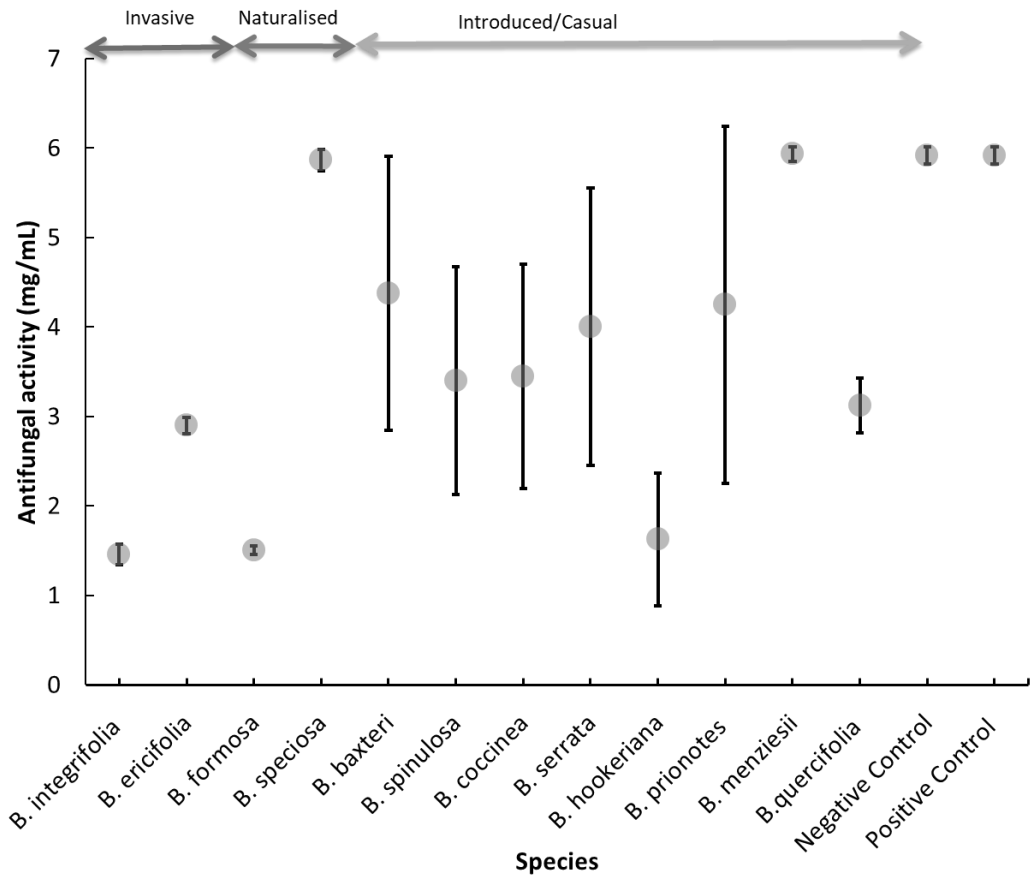

Figure 3.4: Antifungal activity of *Banksia* species acetone leaf extracts against *Phytophthora cinnamomi* (positive and negative control had no antifungal activity after 18 hours against *P. cinnamomi).* Species invasion status indicated at the top of the graph

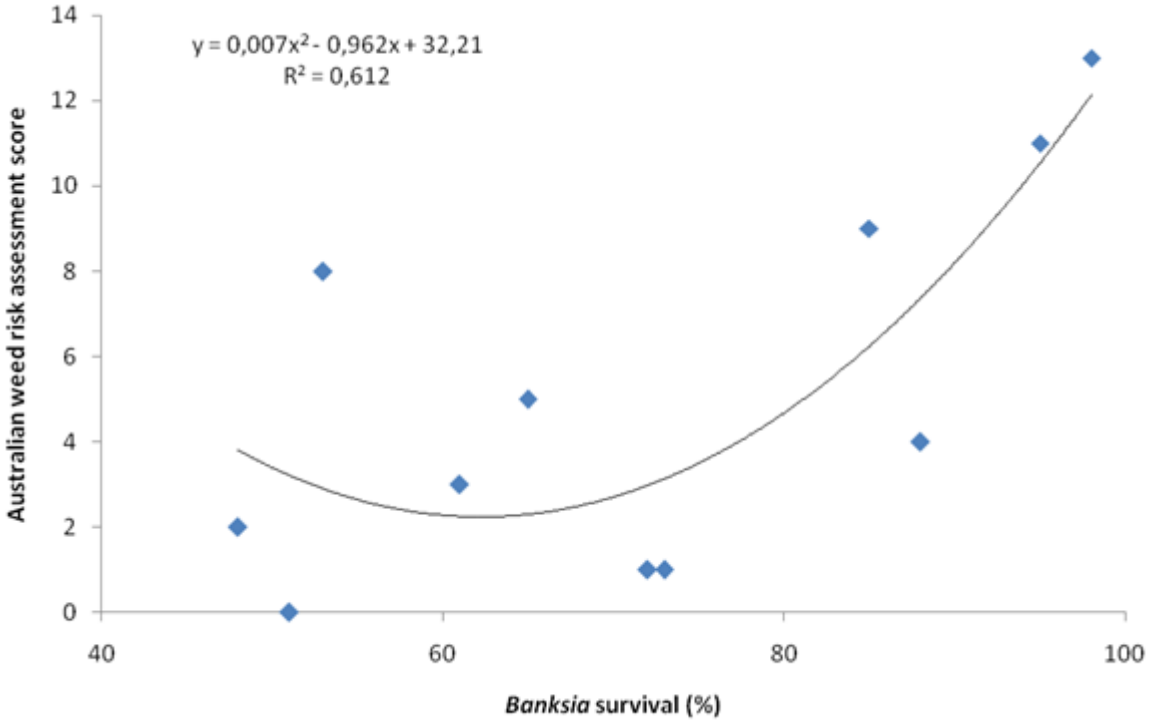

Figure 3.5: Weed risk assessment score (Biogeography, Undesirable traits, Biology, Ecology) polynomial regression to *Banksia* (Proteaceae) survival in the Cape Floristic Region.

Appendices

Appendix 3.1: *Banksia* species survival mean, weed risk assessment outcome, and antifungal activity of *Banksia* species acetone leaf extracts

Species	**Survival mean (%)**	**A-WRA score**	**Outcome**	**Antifungal activity (mg/mL)**
B. integrifolia	98,00	13	Reject	1.45
B. ericifolia	95,00	11	Reject	2.9
B. coccinea	51,00	2	Further evaluation	3.45
B. speciosa	53,00	8	Reject	5.87
B. baxteri	72,00	1	Further evaluation	4.375
B. spinulosa	85,00	9	Reject	3.4
B. formosa	61,00	3	Further evaluation	1.51
B. serrata	48,00	2	Further evaluation	4
B. hookeriana	88,00	4	Further evaluation	1.63
B. prionotes	73,00	1	Further evaluation	4,25
B. menziesii	65,00	5	Further evaluation	5.93
B. quercifolia		7	Reject	3.45
B. burdetti		2	Further evaluation	×
B. sphaerocarpa		8	Reject	×

×, no antifungal activity was conducted on these species, because populations have been removed or not found.

Appendix 3.2: *Banksia* species and *P. cinnamomi* survey data in the Cape Floristic Region included in this study

	GPS			***P. cinnamomi* presence**						
Site	**Latitude**	**Longitude**	**Species**	**soil**	**roots**	**Dead**	**Alive**	**Survival**	**pH (KCl)**	**Soil Type**
Blomkloof	S34,520694	E 19.794278	*B. baxteri*	Yes	Yes	40	64	62%	4.1	LmSa
Blomkloof	S34,520694	E 19.794278	*B. speciosa*	Yes	Yes	89	11	11%	5.1	LmSa
Blomkloof	S34,527639	E 19.810472	*B .spinulosa*	Yes	Yes	1	99	99%	4.6	LmSa
Blomkloof	S34,527639	E 19.810722	*B. formosa*	Yes	Yes	28	72	72%	4.5	LmSa
Blomkloof	S34,523444	E 19.820861	*B. serrata*	Yes	Yes	73	27	27%	4.8	Sa
Blomkloof	S34,533	E 19.773333	*B.integrifolia*	Yes	Yes	0	100	100%	5.2	LmSa
Blomkloof	S34,518861	E 19.796167	*B. coccinea*	Yes	Yes	9	91	91%	4.8	LmSa
Blomkloof	S34,519028	E 19.796194	*B.hookeriana*	Yes	Yes	21	79	79%	4.7	LmSa
Blomkloof	S34,532972	E 19.773306	*B. ericifolia*	Yes	Yes	3	97	97%	4.8	LmSa
Blomkloof	S34,518694	E 19.796	*B. prionotes*	Yes	Yes	24	76	76%	4.0	LmSa
Blomkloof	S34,519222	E 19.796444	*B. manziesii*	Yes	Yes	0	100	100%	4.6	LmSa
	S34,530947	E 19.732125	*B.integrifolia*	Yes	Yes	0	60	100%	5,3	Sa
Viljoens Hof	S34,532661	E 20.030428	*B. ericifolia*	Yes	Yes	2	25	93%	4,5	LmSa
Eenvoud	S34,476794	19.740042	*B. coccinea*	Yes	Yes	26,00	4	13,33%	4,9	Sa
Akkersdrif	S34,3536	E18.819535	*B.integrifolia*	No	No	0	6	100%	3,8	Sa
Eenvoud	S34,479055	E19.738841	*B. baxteri*	Yes	Yes	16,00	24,00	67%	4,5	Sa
Eenvoud	S34,480273	E19.739462	*B.hookeriana*	Yes	Yes	2	92	98%	5	Sa
Eenvoud	S34,480329	E19.739627	*B.prionotes*	Yes	Yes	0	8	100%	4,1	Sa
Eenvoud	S34,476654	E19.739962	*B. serrata*	Yes	No	23	51	69%	4,7	Sa
Eenvoud	S34,475383	E19.741710	*B. formosa*	Yes	Yes	42	68	62%	4,7	Sa

McGregor	S33,998312	E19.762645	*B. speciosa*	Yes	Yes	51	49	49%	4,5	LmSa
McGregor	S33,994841	E19.758957	*B. formosa*	Yes	Yes	22	22	50%	4,7	LmSa
McGregor	S34,001124	E19.764166	*B. coccinea*	Yes	Yes	55	50	48%	4,3	LmSa
Napier	S34,630045	E 19.690283	*B. baxteri*			100	75	43%		
Napier	S34,630012	E 19.69024	*B. menziesii*			39	17	30%		
Napier	S34,630067	E 19.690023	*B. coccinea*			15	127	89%		
Napier	S34,630699	E 19.692081	*B. prionotes*			100	75	43%		
Napier	S34,630716	E 19.691784	*B. prionotes*			96	224	70%		
Napier	S34,520305	E 19.794963	*B. coccinea*			107	147	58%		
Napier	S34,520736	E 19.794137	*B. speciosa*			0	417	100%		
Napier	S34,520375	E 19.794999	*B. coccinea*			28	73	72%		
Napier	S34,52774	E 19.806551	*B. spinulosa*			56	139	71%		
Napier	S34,520375	E 19.794999	*B. coccinea*			0	203	100%		

Abbreviations: LmSa: Loam Sandy soil Sa: Sandy soil

4 Chapter 4: Conclusion and Recommendations

This thesis comprises of two studies set out to gain a better understanding of fungi introduction pathways in South African and understanding their potential role in limiting invasion success of invasive plants; using *Banksia* as case study in the Cape Floristic Region.

Plant trade is the most common introduction pathway of undesirable organisms into novel environments (Saccaggi & Pieterse, 2013; Padayachee et al., 2017). However, there is no researcher in South Africa employed to evaluate fungal invasions in our natural ecosystem (Wood, 2017) and most research conducted on fungi invasions is mainly after they have had a detrimental impact in agricultural or horticultural industries (Desprez-Loustau et al., 2007; Dickie et al., 2017). There is not enough information on fungi invasions and introduction pathways in South Africa. Therefore assessing introduction pathways of fungi is necessary to determine the most prominent introduction pathways. My findings suggests that agriculture is the most prominent introduction pathways for most pathogens, and that wind is the most important dispersal vector that facilitates the spread of pathogens. I also identified three pathogens (*F. oxysporum*, *P. cinnamomi*, *F. circinatum*) that are highly invasive; hence, I recommend that these species need to be prioritised for management. Lastly, in chapter two I identify eighteen pathogens that are under expansion in South Africa. This knowledge could potentially assist bio-security to detect more contaminated plant material at our ports of entry. There is a high probability of pathogens being introduced with their host species (Saccaggi & Pieterse, 2013). Understanding dispersal vectors of pathogens will also assist in developing control regimes for fungal pathogens to prevent their spread and dissemination of spores.

Here I show that the national invasive species list of microbes published under NEM: BA as of July 2016 has three species (*Phytophthora kernoviae*, *Phytophthora pinifolia*, *Tetratosphaeria cryptica*) with no records in South Africa. These findings are consistent with that of (Wood, 2017)• , and therefore, advise that the list be revised (chapter two). The listed species selection was most likely based on invasion success elsewhere in the world and expect opinion. We do not dispute that these species will not be invasive in South Africa in the near future but rather recommend that they be removed due to the lack of information on their impact in South African ecosystem. It is quite important to note that not all invasive species reported elsewhere will be invasive in other regions.

I also show that a species' ability to suppress infection and colonisation by a pathogen or pests may be an important trait to determine invasion success (chapter 3). As I have observed in *Banksia speciosa*, that a species may have all the morphological traits for invasion but its invasion success can be limited by being susceptible to pathogens like *Phytophthora cinnamomi*. *Phytophthora cinnamomi* is an invasive plant pathogen in South Africa and has been listed as a category 1b species under NEM:BA (Linde et al., 1999; Moodley et al., 2013; Wood, 2017). Even though this species is invasive it can also curb invasion success of potentially invasive plants of the family Proteaceae.

Future studies should consider conducting an inoculation experiment of *Banksia* with the pathogen *P. cinnamomi* under different watering regimes, primarily because *P. cinnamomi* reproduction and proliferation is favoured by soils that are high in moisture. Evaluating *Banksia* species pathogenicity under different watering will help us to understand the role in which water or moisture plays during infection of *Banksia* species by *P. cinnamomi*. Secondly, by understanding this relationship it would be beneficial in the development of a control or bio-control programme should it be required to control invasive *Banksia* species in future.

A study should be conducted to evaluate the efficacy of our bio-security protocol and the probability of a pathogen being associated with its host species at the port of entry; I suggest that KZN be the region where this study should be primarily conducted. Mainly because this is where we found most pathogens in this study and secondly this region has the busiest port in South Africa.

4.1 Recommendations for management

- I recommend that the three species (*Phytophthora kernoviae*, *Phytophthora pinifolia*, *Tetratosphaeria cryptica*) be removed from the national invasive species lists of microbes.
- I recommend that the species *Alternaria solani*, *Aspergillus niger* and *Botryosphaeria dothidea* be listed as category 1b on the national invasive species list of microbes.
- I also recommend that a governing protocol be developed for listing microbial species under NEM: BA and that species listing should not solely be based on expert opinion.
- I recommend the listing of *Banksia* ericifolia and *Banksia integrifolia* under NEM: BA as category 1b species and *Banksia quercifolia* and *Banksia spinulosa* as category 2 species.

Supplementary material 1: Summary data collected for the thesis, the table below is data collected for chapter 2**.**

Pathogen	Dispersal Vectors	Invasion status	Origin	Invasive	Date of first publication/record in SA	Source(s)
Alternaria alternata	*W*	C3	Unknown	Casual	1990	Woudenberg et al. (2015); Basim et al. (2017)
Alternaria solani	I, W,R	D2	Unknown	Casual	1900	van der Waals et al. (2004); Santiago et al. (2015)
Aspergillus niger	W,S	D2	Unknown	Casual	1921	Crous et al. (2000); Borin et al. (2017)
Botryosphaeria dothidea	W,S,R	D2	California	Casual	1989	Smith et al. (1994); Slippers et al. (2004); Pillay et al. (2013); Marsberg et al. (2017)
Cercospora zeina	W	B3	Africa	Casual	1988	Ward et al. (1999); Pedro W Crous et al., (2006); Neves et al. 2015; Barnes et al. (2016)
Colletotrichum gloesporioides	R	B2	Caribbean Coast of Mexico	Casual	1989	Crous et al. (2000); Sanders et al. (2000); Sanders & Korsten, 2003; Rampersad et al. (2013)
Erysiphe orontii Cast. var. orontii/Golovinomyces orontii/ G. cichoracearum	W	C3	Northern Hemisphere	Naturalised	1977	Gorter (1993); Crous et al. (2000); Seinosuke, (2003); Haupt, (2007); Lebeda & Mieslerová, (2011); Pei et al. (2012)
Fusarium circinatum/Gibberella circinata	SB, W	E	Mexico	Invasive	1994	Coutinho et al. (2007): Wingfield et al. (2008)

Fusarium oxysporum	SB, I	E	No data	Invasive	1953	Koenig et al. (1997); Gracia-Garza et al. (1998); Gracia-Garza et al. (1999); Karangwa et al. (2016)
Peronospora destructor	W	B3	No data	Casual	1953	Crous et al. (2000); Wright et al. (2002); Kennedy & Wakeham, (2008)
Phytophthora cinnamomi	R, S	E	Papua New Guinea	Invasive	1931	Brasier et al. (1993); Brasier, (1996) Beaulieu et al. (2017)
Puccinia graminis	W, R	B2	No data	Casual	1925	Le Roux,(1987);Terefe et al. (2010); Visser et al., (2011)
Puccinia hordei	No data	B1	No data	Casual	1953	van Niekerk et al. (2001); Farber & Mundt, (2017)
Puccinia striiformis	W, R	B2	Himalayan	Casual	1996	Boshoff et al., (2002); Visser et al. (2011; Ali et al. (2014)
Pythium aphanidermatum	S	B2	Unknown	Casual	1941	Crous et al. (2000); McLeod et al. (2009); Binagwa et al. (2016)
Rhizoctonia solani	SB, TB	B1	No data	Casual	1918	Truter & Wehner,(2004); Strausbaugh et al. (2011); Muzhinji et al. (2016)
Sclerotinia sclerotioum	W	B2	No data	Casual	1979	Crous et al. (2000); McLaren & Craven, (2008); Qandah & del Río Mendoza, (2011)
Sclerotium rolfsii	SB	B1	No data	Casual	1926	Moore, (1926); Shim et al. (1998); Flores-Moctezuma et al. (2006)

W wind-borne, I insect-borne, R rain, S soil-borne, SB seed-borne, TB tuber-borne,

The symbols under the column dispersal vector depict: W wind-borne, I insect-borne, R rain, S soil-borne, SB seed-borne, TB tuber-borne,

Supplementary material 2: The table below shows summary data obtained from the soil nutrient analyses from *Banksia* localities in the Cape Floristic Region used in chapter 3.

Species	pH (KCl)	Resist. (Ohm)	H+	Stone (Vol %)	P (mg/kg)	K	Ex. cations (cmol(+)/kg)				Cu	Zn	Mn	B	Fe mg/kg	C	N	NO3-N	NH4-N	Clay	Silt	Sand
Species			(cmol/kg)		Bray II	mg/kg	Na	K	Ca	Mg	mg/kg					%	%	mg/kg	mg/kg	%	%	%
B. baxteri	4.1	2350	1.21	1	3	33	0.24	0.08	1.64	0.66	0.1	0.3	0.5	0.13	90	1.50	0.056	0.74	6.84	5	14	81
B. speciosa	5.1	3890	0.75	1	2	52	0.15	0.13	1.54	0.65	0.1	0.2	0.5	0.19	61	1.46	0.059	0.48	6.22	7	14	79
B. spinulosa	4.6	4080	0.70	2	1	38	0.12	0.10	0.76	0.41	0.1	0.2	0.4	0.06	42	0.94	0.054	0.64	5.03	7	12	81
B. formosa	4.5	4360	1.08	1	1	44	0.09	0.11	0.63	0.31	0.1	0.2	0.2	0.05	61	1.44	0.064	0.65	5.86	9	12	79
B. serrata	4.8	6010	0.67	1	7	25	0.07	0.07	0.90	0.32	0.1	0.2	0.7	0.10	88	0.84	0.058	0.46	5.70	5	6	89
B. integrifolia	5.2	1080	0.71	5	3	56	0.68	0.14	1.95	1.22	0.1	0.2	2.4	0.57	72	2.12	0.067	0.48	7.23	7	6	87
B. coccinea	4.8	1760	0.78	1	1	35	0.22	0.09	1.36	0.54	0.1	0.2	0.3	0.13	89	1.08	0.057	0.64	4.84	7	12	81
B. hookeriana	4.7	1670	0.83	1	2	41	0.16	0.11	1.64	0.63	0.1	0.2	0.4	0.05	56	1.37	0.062	0.81	6.22	7	18	75
B. ericifolia	4.8	1890	0.66	1	1	41	0.19	0.11	1.02	0.60	0.1	0.2	0.7	0.03	72	1.39	0.064	0.46	6.94	9	8	83
B. prionotes	4.0	7530	0.90	1	2	19	0.06	0.05	0.76	0.35	0.1	0.2	0.2	0.03	84	1.01	0.050	0.48	8.89	5	10	85
B. manziesii	4.6	7900	0.77	1	3	50	0.07	0.13	1.46	0.55	0.1	0.2	0.2	0.04	56	1.10	0.053	2.24	12.48	9	18	73
B. integrifolia	5.3	1190	0.58	6	3	31	0.4	0.08	2.31	1.17	0	0.2	0.4	0.87	57	1.75	0.067	2.57	7.08	5	6	89

B. ericifolia	4.5	710	0.7	50	3	28	0.42	0.07	1.44	0.86	0	0.2	0.2	0.22	179	1.59	0.057	1.46	4.85	9	4	87
B. integrifolia	3.8	1420	0.7	1	6	18	0.17	0.05	0.91	0.58	0	0.3	0.6	0.18	31	1.12	0.062	1.2	9.04	5	2	93
B. coccinea	4.9	5310	0.28	1	3	15	0.09	0.04	0.66	0.4	0	0.1	0.1	0.16	55	0.72	0.071	1.49	6.86	5	4	91
B. baxteri	4,5	2320	0.66	1	3	18	0.13	0.05	0.89	0.6	0	0.3	0.3	0.24	39	1.35	0.068	4.59	7.68	7	4	89
B. hookeriana	5	3840	0.34	1	3	26	0.1	0.07	1.62	0.51	0	0.3	0.1	0.17	29	1.02	0.07	1.77	5.52	5	4	91
B. prionotes	4.1	11570	0.24	1	1	14	0.06	0.04	0.39	0.34	0	0.2	0.1	0.1	17	0.62	0.063	1.39	5.37	5	4	91
B. serrata	4.7	5620	0.25	1	2	15	0.08	0.04	0.46	0.38	0	0.1	0	0.12	25	0.57	0.053	1.69	5.06	5	4	91
B. speciosa	4.5	2240	0.61	47	3	27	0.18	0.07	2.45	1.02	0.1	0.3	11.7	0.2	82	1.71	0.077	2.93	8.04	7	6	87
B. formosa	4.7	1270	0.64	58	3	45	0.28	0.11	2.6	1.3	0.1	0.2	5.8	0.32	138	1.56	0.089	2.05	9.61	9	6	85
B. coccinea	4.3	2920	0.83	70	1	24	0.15	0.06	2.67	0.92	0.1	0.3	6.6	0.17	91	1.43	0.082	1.89	7.27	7	6	87

Supplementary material 3: The table below shows averages of antifungal (mg/mL) data collected from the Banksia species antifungal bioassay after 18 Hours used in chapter 3

B. formosa	*B. speciosa*	*B. integrifolia*	*B. ericifolia*	*B. baxteri*	*B. spinulosa*	*B. coccinea*	*B. serrata*	*B. hookeriana*	*B. prionotes*	*B. menziesii*	*B. quercifolia*	Negative Control	Positive Control
1.5083333	5.8666667	1.453146667	2.9	4.375	3.4	3.45	4	1.625	4.25	5.9333333	3.125	5.9166667	5.9166667

Supplementary material 4: Summary statistics on the relationship between species distribution and dispersal vectors

```
> r1<-glm (Distribution~dispersal.vectors, family=poisson, data=data)
> Summary (r1)
Call:
glm (formula = Distribution ~ dispersal.vectors, family = poisson,
data = data)
Deviance Residuals:
Min       1Q   Median       3Q      Max
-0.9938  -0.9772  -0.1958   0.4085   2.4481
Coefficients:
Estimate Std. Error z value Pr (>|z|)
(Intercept)       0.87777    0.22321   3.933 8.41e-05 ***
dispersal.vectors -0.02465    0.13649  -0.181    0.857
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Dispersion parameter for poisson family taken to be 1)
Null deviance: 42.285  on 46  degrees of freedom
Residual deviance: 42.252  on 45  degrees of freedom
AIC: 167.67
Number of Fisher Scoring iterations: 5
> coef (r1)
(Intercept) dispersal.vectors
0.8777675       -0.0246545
> exp (-0.0246545)
[1] 0.9756469
>
```

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