

**The taxonomy, phylogeny and ecology of
Botryosphaeriaceous fungi occurring on various woody
hosts**

This dissertation is presented in partial fulfillment of the requirements of the degree

PHILOSOPHIAE DOCTOR

in the Faculty of Natural and Agricultural Sciences, Department of Microbiology and
Plant Pathology, University of Pretoria

by

Bernard Slippers

August 2003

Promotor: Prof Michael J Wingfield
Co-Promoters: Prof Teresa A Coutinho
Prof Pedro W Crous
Prof Brenda D Wingfield



Declaration

I, the undersigned, hereby declare that the thesis submitted herewith for the degree *Philosophiae Doctor* to the University of Pretoria contains my own independent work.

This work has hitherto not been submitted for any degree at any other University.

Bernard Slippers

Bernard Slippers

August 2003



I dedicate this thesis to my wife, Jana.

You were with me through every bit of this experience, all the way to the end. You are truly my other half. I cannot wait for the rest of what lies ahead for us.

TABLE OF CONTENTS

Acknowledgements	1
Preface	3
Research Chapters	
Chapter 1	6
Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as <i>Botryosphaeria dothidea</i>	
Chapter 2	44
Phylogenetic and morphological re-evaluation of the <i>Botryosphaeria</i> anamorphs causing diseases of <i>Mangiferae indica</i> in Australia	
Chapter 3	68
Multiple gene sequences delimit <i>Botryosphaeria australis</i> sp. nov. as a sister species to <i>B. lutea</i>	
Chapter 4	99
Phylogenetic comparison of <i>Botryosphaeria</i> isolates from pome and stone fruit trees in South Africa and other regions of the world	
Chapter 5	122
Sympatric speciation and distribution of <i>Botryosphaeria</i> spp. on native and introduced <i>Eucalyptus</i> trees	
Chapter 6	158
Preliminary studies on <i>Botryosphaeria</i> species from <i>Wollemia nobilis</i> and related southern hemisphere conifers in Australasia and South Africa	
Chapter 7	176
A contribution to the taxonomy of <i>Botryosphaeria obtusa</i> , <i>B. stevensii</i> and <i>B. quercuum</i> based on molecular and morphological data	
Chapter 8	195
Development of SSR and RFLP markers for <i>Botryosphaeria</i> spp. with <i>Fusicoccum</i> anamorphs	



Chapter 9	203
Differentiation and phylogeography of cryptic species in the <i>B. parva</i> - <i>B. ribis</i> complex	
Chapter 10	236
Evolving species concepts in <i>Botryosphaeria</i> over the past 140 years	
Summary	270
Opsomming	275

ACKNOWLEDGEMENTS

I am sometimes overwhelmed by the wonder of the biological world. This love for biology is deeply rooted and has been there since I was a child. I ended up studying fungi during the part of my life that this thesis represents, and have gained immense pleasure and satisfaction from it. Perhaps any part of biology would do, but fungi will no doubt always have a special place in my heart. I also truly believe the wonder of life was created, with a purpose, and I see myself as part of that creation. Therefore, looking back on this study, I am deeply grateful to the Creator for the purpose and love, the opportunity, the inspiration and guidance, and making me part of it all. I cannot wait to continue on this road and learn more.

The thesis was not produced on my effort alone, but was shaped by the social structure that I am part of. This includes family, friends, colleagues and other people that I have had contact with. Sometimes it is difficult to put boundaries around these categories of people – like some species studied during this project. Some friends are family, some colleagues are friends, etc. But no matter where you think you belong in the classification of relationships in my life, I need to and want to thank you.

There are a few people I need to specifically identify when saying thank you. My wife, Jana, has been the one I leaned on and taken from most during this time. Working patiently with me through this phase of our lives; caring and encouraging. It is so wonderful to have you closest to me as we discover life. Now, with Yvonne's arrival, also as a family. My parents and family, as well as Jana's parents and family have been intimately involved and interested in us and in this project. We are thankful for this circle of love and prayer around us that promises to also be there in future. Similarly, our friends from the neighborhood, church, the past, work and other areas have blessed our lives. Both family and friends have shared the good and the bad moments, prayed, loved, helped carry the load and made this journey so much more worth while. May we have grace to give our lives and love in return to the measure you have.

I realize that I am privileged to have done this study at the Forestry and Agricultural Biotechnology Institute on the campus of the University of Pretoria. Few other places in my field can boast with better equipment or more opportunities and stimulation. The most valuable parts of this institute are, however, not to be found in its hardware or infrastructure, but in its people. More than anything else, however, it

was a privilege to work with a very special team of promoters. I have gained so much from you in terms of knowledge, guidance and support. I can hardly imagine more passionate and enthusiastic biologists than you. In FABI I have also met an amazing diversity of people from South Africa and the rest of the world – a survey last year showed that 30 languages were spoken at that stage in FABI. I have learned from them all and now have friends all over the world. Many of you were also intricately involved in this thesis by teaching me techniques, discussing ideas, editing manuscripts and much more. Thank you. I hope I can be a worthy friend in return.

The access that we enjoyed to cultures from across the world reflects the good collaboration between FABI and other scientists. I wish to thank a number of these colleagues who have been involved in specific chapters by providing access to cultures, diseased material, added ideas or gave technical assistance. These are Dr. Sandra Denman (Chapter 1), Dr. Greg I. Johnson (Chapter 2), Gerda Fourie (Chapters 3, 5), Dr. Adriaan W. Smit (Chapter 4), Dr. Angus Carnegie (Chapter 5), Dr. Brett Summerell and Dr. Mike Ivory (Chapter 6), Dr. Thomas A. Gordon (Chapter 7) and Dr. Treena Burgess (Chapters 8, 9). The Latin translation of the species descriptions in Chapters 3 and 5 were done by Dr. Hugh Glen.

Many of the techniques used in this study require specific equipment and have substantial running costs. Many institutions contributed financially and by providing infrastructure. In this regard I wish to thank the University of Pretoria, the Forestry and Agricultural Biotechnology Institute, the members of the Tree Pathology Co-operative Program, THRIP, the Mellon Foundation and the National Research Foundation.

The people who played the most direct part in this work:

Alan. Altus. Adriaan. Andrea. Ana Maria. Bongani. Brenda. Carlos. Chris. Claudia.
Danie G. Danie N. Draginja. Eddie. Elizabeth. Ellen-Anne. Elsje. Elzaan. Emma.
Esta. Ester. Gavin. George. Gerda. Gerrit. Gideon. Gina. Hannes. Helen. Herman.
Irene. Jacquie. Jana. Johan. Johannes. Jolanda. Juanita. Koos. Liesl. Lilanie. Liliane.
Lise. Lizette. Ma Marietjie. Ma Yvonne. Marlé. Marieka. Mariette. Marinda. Marius
N. Marius S. Martin. Melinda. Michele. Mike. Nico. Nonnie. Ntsane. Oliver. Orlando.
Oupa. Pa Freddie. Pa Marius. Pedro. René. Riaan. Riana. Rikus. Ronald. Rodrigo.
Ronel. Rose. Sandra. Schalk. Sonja B. Sonja dB. Stephan. Stefan. Susan-Karin.
Teresa. Teuns. Thys. Treena. Wilhelm. Willem. Winnie. Yvonne.

And those ones I forgot.

PREFACE

Identification of *Botryosphaeria* spp. is difficult. The sexual state (teleomorph) is not commonly seen and there is considerable overlap in morphological characters across the species. Anamorphs are more common and often have more distinctive morphological features. However, closely related anamorph taxa also have overlapping morphological characters. Traditional identification using these data has, therefore, caused considerable confusion in the taxonomy of these fungi. In more recent years, comparisons of DNA sequence data have contributed significantly to *Botryosphaeria* taxonomy. Such studies, which are mostly based on single gene phylogenies or dominant molecular markers, have, however, not resolved some long-standing taxonomic controversies. It is evident from an overview of the current literature that this commonly encountered and economically important genus of fungi remains in taxonomic disarray. The main aims of this study were, therefore, to address questions relating to identification of species, as well as relationships between species and their geographic distribution.

The first question addressed in this thesis concerns the identity of the type species of the genus *Botryosphaeria*, namely *B. dothidea*. Since its description in 1863, this taxon has been applied to a wide diversity of species in the genus. The result is that there is considerable confusion over the use of this name, especially concerning its relationship to *B. ribis*. Many researchers use these taxa as referring to separate species, while others view them as conspecific. Further confusion arises from the morphological and ITS rDNA sequence similarity between isolates identified as *B. ribis* and *B. parva*. In the first chapter we consider the morphology of type specimens and original descriptions of these fungi. An attempt is also made to clarify confusion regarding these names by selecting neotypes, lectotypes and epitypes to represent these names. From ex-type and other morphologically characterized isolates, multiple gene sequence genealogies are produced in order to distinguish especially *B. ribis* and *B. parva*, which are difficult to distinguish using all other data.

Botryosphaeria species occur on a wide diversity of plants, world-wide. Some species are also important pathogens. Hosts include many economically important agricultural and forestry crops. A growing concern is that pathogenic *Botryosphaeria* spp. from introduced hosts can move onto native hosts, and *visa versa*. In Chapters 2 to 6 *Botryosphaeria* species and species complexes are identified from a number of

SUMMARY

The identification of species is controversial in many groups of organisms. This is also true for species in the genus *Botryosphaeria*. One of the reasons is that phenotypic and ecological characters that have been traditionally used to identify species boundaries, tend to be interpreted subjectively. Modern techniques based on DNA sequence analyses can be used more objectively to make comparisons between species. The drawback of DNA based techniques is that the biology of an organism is not reflected by DNA, but in the expression of the information contained in the DNA and the eventual interaction with the physical and biological environment. Therefore, the combination of DNA and phenotypic data can be used very effectively to identify species boundaries and subsequently to characterize the populations that make up a species. This approach has thus been followed in developing chapters of this thesis focused on the taxonomy and identification of *Botryosphaeria* species.

Chapter 1. *Botryosphaeria dothidea* epitypification, *B. parva* and *B. ribis*

Botryosphaeria dothidea is the type and thus the anchor species for the genus. Controversy has persisted regarding the correct identity of this taxon, especially concerning closely related species such as *B. berengeriana*, *B. ribis* and *B. parva*. In order to clarify this situation, epitype material for *B. dothidea* was collected in 2001 from the same sites (Italy and Switzerland) and hosts as the original collections in 1863. Material for *B. ribis* was collected on our behalf in New York from where this species was first described in 1911. Material from the original collections was also obtained from herbaria from Rome, New York and New Zealand. Using these collections and combining morphology and multiple DNA genealogies, we were able to identify and describe each of the named species. *Botryosphaeria dothidea* and *B. berengeriana* were, however, synonymized. *Botryosphaeria ribis* and *B. parva* are shown to be distinct species, albeit very closely related and cryptic when studying individual gene phylogenies and morphology.

Chapter 2. *Botryosphaeria* on *Mangifera indica*

Botryosphaeria spp. cause die-back of mango trees, as well as severe pre- and post-harvest diseases of mango fruit. For a number of years a plethora of species in the anamorph genera *Dothiorella*, *Natrassia* and *Fusicoccum* have been applied to the

fungi isolated from this tree. Using DNA sequence data from two gene regions, we could identify four species involved in causing diseases of mango. These species are also easily distinguished using anamorph morphological characters. The four species group with other species of *Botryosphaeria*, but no specific teleomorph names could be linked to two of these taxa. They were identified as *B. parva*, *B. dothidea*, *F. mangiferum* (comb. nov.) and an unknown *Fusicoccum* sp.

Chapter 3. *Botryosphaeria australis* sp. nov. and *B. lutea*

There are a number of *Acacia* spp. that are native to Australia. Some of these species are fast growing and are important commercial and informal forestry crops around the world. *Botryosphaeria* spp. are known to affect these trees where they have been introduced. Little is, however, known about potential pathogens of these trees in Australia. In this study, a group of isolates were identified that closely resembled *B. lutea* in morphology and ITS rDNA sequence. Concordance between phylogenies of the ITS rDNA and two other gene regions, however, confirmed the phylogenetic separation of *B. lutea* and the isolates from Australian *Acacia*. This previously unknown species is described here as *B. australis* sp. nov. (anamorph *F. australe*).

Chapter 4. *Botryosphaeria* spp. infecting pome and stone fruit trees

A number of *Botryosphaeria* spp. affect pome and stone fruit trees world-wide. Confusion over names of these species complicates comparisons of results of different studies and the eventual control of the diseases. Sequence data and morphology were thus used in this study to compare South African collections with data from other parts of the world. *Botryosphaeria obtusa* was identified as the main species that affects these hosts in South Africa. The newly described *B. australis* is also identified from these hosts for the first time. Based on data from GenBank, *B. dothidea*, *B. stevensii* and fungi in the *B. ribis* – *B. parva* complex, were also identified from fruit trees in other parts of the world. A rapid PCR RFLP technique was developed to effectively identify all these *Botryosphaeria* species from fruit trees.

Chapter 5. *Botryosphaeria* spp. from *Eucalyptus*

Eucalyptus trees are amongst the most important sources of fiber, especially for the production of paper, in South Africa and in other countries in the tropics and southern hemisphere. *Botryosphaeria* spp. are one of the most significant pathogens of

Eucalyptus trees in areas where they are planted as exotics. In this chapter, isolates from native *Eucalyptus* trees in Australia and exotic trees in South Africa, Hawaii and Uruguay were compared based on morphology and molecular data. A PCR RFLP technique was also developed to identify the fungi from this host. *Botryosphaeria dothidea*, which has commonly been reported from *Eucalyptus*, was shown to be rare on this host. This fungus and *B. ribis* have been mistaken for *B. parva*, which is commonly found on exotic *Eucalyptus*. A new and cryptic species closely related to *B. eucalyptorum* was also identified by studying concordance between genealogies of three gene regions. This new species was given the name *B. irregularis* sp. nov. (anamorph *F. irregulare*). This study also indicated that *B. eucalyptorum* and *B. irregulare* are most likely native to *Eucalyptus* in Australia, but have been introduced to new environments on this host.

Chapter 6. *Botryosphaeria* spp. from southern hemisphere conifers

A number of rare coniferous tree species occur in the southern hemisphere, including *Wollemia nobilis* (Australia), *Widdringtonia* spp. (Africa) and some *Araucaria* spp. (widely distributed in the southern hemisphere). The small populations and limited genetic base of some of these species place them at high risk to diseases. Multiple gene genealogies and morphology were used to show that at least two Botryosphaeriaceous fungi infect these hosts. The first is most closely related to *B. ribis* and the second to *B. australis*. Both these fungi contain some unique DNA sequence polymorphism that necessitates further investigation.

Chapter 7. Taxonomy of *B. quercuum*, *B. stevensii*, *B. obtusa* and their anamorphs

The taxonomy of *B. quercuum*, *B. obtusa* and *B. stevensii* was thoroughly treated in 1964. Despite this study confusion has persisted regarding the identities of these species. Recent studies using modern molecular tools to study phylogeny of these fungi have also been hampered due to this confusion. In this study, DNA sequence data and morphological characteristics of the *Diplodia* anamorphs in culture are combined to address the questions relating to this group of species. It was possible to show that conidial size is inordinately variable amongst these species, to be used as an isolated taxonomic character. When these data were combined with data relating to cell wall thickness, patterns of conidial septation and color, as well as host

association, then *B. obtusa*, *B. stevensii* and *B. quercuum* could be more easily distinguished from each other.

Chapter 8. Development of SSR and RFLP markers for *Botryosphaeria* spp.

Simple sequence repeat (SSR) or micro-satellite markers are some of the most powerful modern tools to study populations and species. A set of eight polymorphic, co-dominant micro-satellite markers was developed for *B. parva*. In addition loci with varying restriction sites were also identified as a source of polymorphic markers. The primers developed to amplify these regions were tested on other *Botryosphaeria* species and were shown to be useful for at least nine other species.

Chapter 9. The *B. parva* – *B. ribis* complex

Botryosphaeria parva and *B. ribis* have been shown to be distinct species based on multiple gene genealogies, but their identification remains difficult. In this chapter, the genetic boundaries between these species were characterized by combining data from gene genealogies, SSR makers and PCR RFLP fingerprints. These data revealed variation within *B. parva* and *B. ribis* that are referred to as *sensu lato* and *sensu stricto* groups in these species. The RFLP profiles were particularly useful to provide a rapid and effective means to distinguish these species. Furthermore, it was shown that these fungi, especially *B. parva*, have been moved around the world on hosts such as *Eucalyptus*. Due to this movement, *B. parva* clearly represents a worldwide meta-population, where recombination is evident among the isolates from different hosts and continents. A number of clonal lines were, however, also identified that occurred across spatial and temporal boundaries. This indicates that asexual reproduction also plays an important role in structuring populations of this fungus.

Chapter 10. Species identification in *Botryosphaeria*, 1863-2003

The data presented in this thesis and other recent publications, make it clear that *Botryosphaeria* species can no longer be identified solely on morphological characters. DNA sequence data are most useful to distinguish most *Botryosphaeria* species. Not all other DNA based tools are, however, phylogenetically informative, or they are difficult to interpret in isolation. This is especially true when attempting to identify cryptic species. In these cases SSR markers and multiple gene DNA sequence data are needed to distinguish the species. Techniques such as PCR RFLP's and



species specific primers will facilitate more effective future identification of *Botryosphaeria* spp. These DNA data have also recently been successfully combined with phenotypic and ecological information to better understand and describe *Botryosphaeria* spp. I trust that the broader approach to the identification of *Botryosphaeria* spp. followed and advocated in this thesis will help to overcome problems relating to the systematics of this important group of fungi. I also hope that the information will promote accurate and reasonably rapid identification of species of *Botryosphaeria*.

OPSOMMING

Spesie-identifikasie is in baie groepe organismes kontroversieel. Dit geld ook vir *Botryosphaeria*-spesies. Een van die redes is dat fenotipiese en ekologiese karakters, wat tradisioneel gebruik is om spesiegrense te identifiseer, subjektief geïnterpreteer kan word. Moderne tegnieke, gebaseer op DNS-volgordeanalises kan meer objektief gebruik word om spesies te vergelyk. Die tekortkoming van DNS-gebaseerde tegnieke is dat die biologie van 'n organisme nie hierin te vinde is nie, maar wel in die fenotipiese uitvloeisels van die inligting vervat in die DNS en die uiteindelijke interaksie met die fisiese en biologiese omgewing. Daarom kan die kombinasie van DNS en fenotipiese data baie effektief gebruik word om spesiegrense te identifiseer en uiteindelik die populasies, waaruit die spesie bestaan, uit te beeld. Hierdie benadering is dus gevolg in die ontwikkeling van die proefskrif se hoofstukke wat fokus op die taksonomie en identifikasie van *Botryosphaeria*-spesies.

Hoofstuk 1. *Botryosphaeria dothidea* epitipifisering, *B. parva* en *B. ribis*

Botryosphaeria dothidea is die tipe en dus anker-spesie van die genus. Daar is deurlopende kontroversie oor die identiteit van hierdie takson, veral met verwysing na nabyverwante spesies soos *B. berengeriana*, *B. ribis* en *B. parva*. Om hierdie situasie op te los is epitipe-materiaal vir *B. dothidea* in 2001 van dieselfde areas (Italië en Switserland) en gashere as die oorspronklike versamelings in 1836 versamel. Materiaal van *B. ribis* is namens ons in New York versamel, waar hierdie spesie eerste in 1911 beskryf is. Materiaal van oorspronklike versamelings is ook vanaf herbaria in Rome, New York en Nieu-Seeland bekom. Deur die gebruik van hierdie versamelings en die kombinasie van morfologie en meervoudige DNS-geenfilogenie kon ons elk van die genoemde spesies identifiseer en beskryf. *Botryosphaeria dothidea* en *B. berengeriana* blyk sinoniem te wees. Daarenteen is *B. ribis* en *B. parva* afsonderlike spesies, alhoewel hulle baie naverwant is en kripties as individuele geenfilogene en morfologie bestudeer word.

Hoofstuk 2. *Botryosphaeria*-spesies vanaf *Mangifera indica*

Botryosphaeria-spesies veroorsaak terugsterwing van mangobome en ook ernstige voor- en na-oessiektes van die mangovrugte. Vir 'n paar jaar reeds word verskeie spesiename in die anamorfgenera *Dothiorella*, *Natrassia* en *Fisicocum* gebruik om

die swamme wat van hierdie bome geïsoleer word, te beskryf. Deur die gebruik van DNS-volgorde van twee geenareas kon ons vier spesies identifiseer wat siektes van mango veroorsaak. Hierdie spesies kan ook maklik op grond van hulle anamorfe morfologie onderskei word. Al vier spesies word saam met die *Botryosphaeria*-spesie geklassifiseer, maar spesifieke teleomorfnames kon slegs vir twee van hierdie spesies bepaal word. Hulle word dus as *B. parva*, *B. dothidea*, *F. mangiferun* (comb. nov.) en 'n onbekende *Fusicoccum*-spesie geïdentifiseer.

Hoofstuk 3. *Botryosphaeria australis* sp. nov. en *B. lutea*

Australië het 'n hele aantal inheemse *Acacia*-spesies. Sommige van hierdie spesies groei vinnig en is wêreldwyd 'n belangrike kommersiële en informele bosbougewas. Dit is bekend dat *Botryosphaeria*-spesies hierdie bome, in areas waar hulle uitheems groei, besmet. Daar is egter min bekend oor die patogene van hierdie bome in Australië. In hierdie studie word 'n groep isolate geïdentifiseer wat baie na *B. lutea* in morfologie en ITS rDNA-volgordes lyk. Ooreenstemming tussen die filogenie van die ITS rDNA en twee ander geenareas bevestig egter die filogenetiese skeiding van *B. lutea* en die isolate van Australiese *Acacia*. Hierdie voorheen onbekende spesie word as *B. australis* sp. nov. (anamorf *F. australe*) beskryf.

Hoofstuk 4. *Botryosphaeria*-spesies wat vrugtebome infekteer

'n Aantal *Botryosphaeria*-spesies veroorsaak wêreldwyd siektes van appel- en steenvrugbome. Die verwarring oor name van hierdie spesies bemoeilik die vergelyking van resultate tussen verskillende studies en uiteindelijke beheer van die siektes. DNA-volgordedata en morfologie word in hierdie studie gebruik om Suid-Afrikaanse versamelings met data van ander wêrelddele te vergelyk. *Botryosphaeria obtusa* word as die vernaamste spesie wat hierdie gasheerplante in Suid-Afrika beïnvloed, geïdentifiseer. Die nuutbeskryfde *B. australis* word ook vir die eerste keer op vrugtebome geïdentifiseer. Gebaseer op data van GenBank word *B. dothidea*, *B. stevensii* en die *B. ribis* – *B. parva*-kompleks ook algemeen op vrugtebome van ander wêrelddele gevind. 'n Vinnige PKR RFLP-tegniek is ontwikkel om die *Botryosphaeria*-spesies effektief van mekaar te onderskei.

Hoofstuk 5. *Botryosphaeria*-spesies vanaf *Eucalyptus*

Eucalyptus-bome is van die belangrikste veselbronne vir die vervaardiging van papier

in Suid-Afrika en ander lande in die trope en suidelike halfmond. *Botryosphaeria*-spesies is van die belangrikste patogene van *Eucalyptus*-bome in die areas waar hulle uitheems geplant word. In hierdie hoofstuk word isolate van inheemse *Eucalyptus*-bome in Australië en uitheemse bome in Suid-Afrika, Hawaii en Uruguay op grond van morfologie en DNA-data met mekaar vergelyk. 'n PKR RFLP-tegniek is ontwikkel om die swamme van hierdie gasheer te identifiseer. Daar word aangetoon dat *B. dothidea* wat voorheen algemeen vanaf *Eucalyptus* beskryf is, eintlik raar op hierdie gasheer aangetref word. Hierdie swam en *B. ribis* is voorheen met *B. parva* verwar, wat algemeen op *Eucalyptus* voorkom. 'n Nuwe, kriptiese spesie wat naverwant aan *B. eucalyptorum* is, word geïdentifiseer deurdat ooreenstemming tussen drie geenareas gewys word. Hierdie nuwe spesie word as *B. irregularis* sp. nov. (anamorf *F. irregulare*) benoem. Die navorsing toon dat *B. eucalyptorum* en *B. irregularis* inheems op *Eucalyptus* in Australië is, maar dat hulle via hierdie gasheer na verskeie ander lande versprei het.

Hoofstuk 6. *Botryosphaeria*-spesies vanaf suidelike halfmond keëldraende bome

Daar is heelparty skaars, keëldraende bome in die suidelike halfmond, insluitende *Wollemia nobilis* (Australië), *Widdringtonia*-spesies (Afrika) en sommige *Araucaria*-spesies (wydversprei in die suidelike halfmond). Die klein populasies en beperkte genetiese basis van sommige van hierdie spesies veroorsaak dat daar 'n hoë risiko bestaan om deur siektes uitgewis te word. Meervoudige geenfilogenie en morfologie word gebruik om aan te toon dat daar ten minstens twee *Botryosphaeria*-agtige swamme is wat hierdie gasheer besmet. Hulle is onderskeidelik na aan *B. ribis* en *B. australis* verwant. Beide hierdie spesies bevat egter unieke DNS-volgorde polimorfismes wat verdere ondersoek vereis.

Hoofstuk 7. Taksonomie van *B. quercuum*, *B. stevensii*, *B. obtusa* en hul anamorwe

Die taksonomie van *B. quercuum*, *B. obtusa* en *B. stevensii* is deeglik in 1964 ondersoek. Ten spyte van hierdie studie het verwarring aangaande die identiteit van hierdie spesies voortgeduur. Onlangse studies wat moderne tegnieke gebruik om die filogenie van hierdie swamme te bestudeer, is deur hierdie verwarring gekortwiek. In hierdie studie word DNS-volgorde data en die morfologiese karaktereienskappe van *Diplodia*-anamorwe in kultuur gebruik om die vrae aangaande hierdie groep spesies te beantwoord. Daar word getoon dat die konidium grootte te veel varieer om in

isolasie as taksonomies onderskeidende karakter gebruik te word. As hierdie data egter gekombineer word met data van selwanddikte, die patroon van die konidium septering en -verkleuring en ook gasheerassosiasie kan *B. quercuum*, *B. obtusa* en *B. stevensii* meer akkuraat van mekaar onderskei word.

Hoofstuk 8. Ontwikkeling van SSR en RFLP merkers vir *Botryosphaeria*-spesies

Mikrosateliemerkers is van die mees kragtige, moderne molekulêre instrumente om spesies en populasies van organismes te bestudeer. 'n Stel van agt polimorfiese, ko-dominante mikrosateliemerkers word vir *B. parva* ontwikkel. In ander loki word polimorfiese restruksiesnydingspunte, wat as bykomende merkers dien, geïdentifiseer. Dit word getoon dat die voorvoeders wat ontwikkel is om die verskillende loki te vermenigvuldig ook in nege ander *Botryosphaeria*-spesies benut kan word.

Hoofstuk 9. Die *B. parva* - *B. ribis*-kompleks

Daar is vroeër aangetoon dat *B. parva* en *B. ribis* afsonderlike spesies is, maar hulle roetine-identifikasie bly problematies. In die hoofstuk word die genetiese grense tussen hierdie spesies deur die kombinasie van meervoudige geenfilogenie, mikrosateliemerkers en PKR RFLP-patrone uitgebeeld. Hierdie data het variasie binne *B. parva* en *B. ribis* uitgewys wat as die *sensu lato* en *sensu stricto* groepe vir hierdie spesies beskryf word. Die RFLP-patrone was veral nuttig as vinnige en effektiewe manier om die spesies te onderskei. Verder word getoon dat hierdie swamme, veral *B. parva*, oor die wêreld op gashere, soos *Eucalyptus*, versprei is. As gevolg van hierdie verspreiding vorm *B. parva* 'n wêreldwye metapopulasie, waar genetiese herkombinasie tussen isolate van verskillende gashere en lande plaasvind. 'n Aantal klonale lyne word oor ruimtelike en tydsgrense geïdentifiseer. Hieruit kan afgelei word dat a-seksuele voortplanting 'n belangrike rol in die opbou van populasies van hierdie swam speel.

Hoofstuk 10. Identifikasie van spesies in *Botryosphaeria*, 1863-2003

Die resultate van hierdie proefskrif en ander onlangse publikasies maak dit duidelik dat *Botryosphaeria*-spesies nie slegs op grond van morfologie geïdentifiseer kan word nie. DNS-volgordes is die mees bruikbare data om *Botryosphaeria*-spesies te onderskei. Nie alle ander DNS gebaseerde instrumente is egter filogeneties insiggewend nie, of hulle is moeilik om in isolasie te vertolk. Dit is veral die geval

met die identifisering van kriptiese spesies. In hierdie gevalle word mikrosatellietmerkers en DNS-volgorde van meervoudige geenloki-data benodig om die spesies te onderskei. Tegnieke soos PKR RFLP en spesie spesifieke voorloperfragmente is ook nuttig as vinnige en effektiewe manier om spesies te identifiseer. Hierdie DNS-data is onlangs suksesvol met fenotipiese en ekologiese inligting gekombineer om *Botryosphaeria*-spesies te identifiseer en te omskryf. Ek vertrou dat dié breër benadering tot die identifisering van *Botryosphaeria*-spesies, wat in hierdie proefskrif gevolg en aanbeveel is, sal bydra om probleme in verband met die sistematiek van hierdie groep swamme te oorkom. Daarmee saam hoop ek dat die inligting met die akkurate en redelik vinnige identifisering van *Botryosphaeria*-spesies sal help.