

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

Submitted by

**Ronald Natale Heath**

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

**MAGISTER SCIENTIAE**

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

December 2003

Study leaders:

Prof. Michael J. Wingfield

Dr. Jolanda Roux

Prof. Brenda D. Wingfield

*This thesis is dedicated to my late grandfather  
Livio Scribante*

*Wissen ist Macht wie schief gedacht  
Wissen ist wenig  
Können ist König*

INDEX

	<b>Page</b>
<b>ACKNOWLEDGEMENTS</b>	i
<b>PREFACE</b>	ii
<b>CHAPTER 1</b>	
<b>THE MOVEMENT OF PLANT PATHOGENS ON A WORLDWIDE SCALE, THE IMPACT AND THREATS THEY POSE</b>	1
<b>Introduction</b>	2
<b>Means by which plant pathogens spread</b>	3
Wind dispersal	4
Spread of plant pathogens on germplasm	5
Spread of plant pathogens on seed	6
Spread of plant pathogens on alternate and alternative hosts	7
Spread of plant pathogens by insects	7
Spread of plant pathogens assisted by humans	9
Spread of plant pathogens by inconspicuous means	9
<b>Impact of non-indigenous pathogens</b>	10
Economic impacts	11
Environmental impacts	11
Social impacts	13
<b>Means to combat the spread of plant pathogens</b>	14
Quarantine measures	14
Molecular detection methods and diagnostic tools	17
<b>Determining the origin of introduced plant pathogens</b>	19
The study of biological patterns and historical records	19
The use of molecular techniques	19
<b>Possible threats of plant pathogens from native hosts to non-indigenous     plantation species</b>	22

<b>Conclusions</b>	23
<b>Literature cited</b>	25
<b>CHAPTER 2</b>	
<b>DISCOVERY OF <i>CRYPHONECTRIA CUBENSIS</i> ON NATIVE <i>SYZYGIUM</i> SPECIES IN SOUTH AFRICA</b>	40
<b>Abstract</b>	41
<b>Introduction</b>	42
<b>Materials and Methods</b>	43
Disease symptoms and collection of samples	43
Morphological comparisons	43
DNA isolation and amplification	44
DNA sequencing and analyses	44
Pathogenicity	45
<b>Results</b>	46
Disease symptoms and collection of samples	46
Morphological comparisons	46
DNA sequencing and analyses	47
Pathogenicity	48
<b>Discussion</b>	48
<b>Literature cited</b>	52
<b>CHAPTER 3</b>	
<b>GENETIC COMPARISON OF <i>CRYPHONECTRIA CUBENSIS</i> ISOLATES FROM NATIVE AND EXOTIC HOSTS IN SOUTH AFRICA</b>	79
<b>Abstract</b>	80

<b>Introduction</b>	81
<b>Material and Methods</b>	82
Isolates	82
Vegetative compatibility tests	83
Analyses using microsatellite markers	84
<i>DNA isolation and amplification</i>	84
<i>Genescan analysis</i>	85
<i>Statistical analyses</i>	85
<b>Results</b>	87
Isolates	87
Vegetative compatibility tests	87
Analysis using microsatellite markers	88
<i>Genescan analysis</i>	88
<i>Statistical analyses</i>	88
<b>Discussion</b>	89
<b>Literature cited</b>	93
<b>CHAPTER 4</b>	
<b>FIRST REPORT OF AN <i>ENDOTHIELLA</i> SP. ON <i>TIBOUCHINA URVILLEANA</i> IN AUSTRALIA</b>	112
<b>Abstract</b>	113
<b>Introduction</b>	114
<b>Materials and Methods</b>	115
Fungal isolates	115
Morphological characterisation	115
DNA isolation and amplification	115
DNA sequencing and analyses	116

Pathogenicity	117
<b>Results</b>	118
Fungal isolates	118
Morphological characterisation	118
DNA sequencing and analyses	119
Pathogenicity	119
<b>Discussion</b>	120
<b>Literature cited</b>	122
<b>CHAPTER 5</b>	
<b><i>BOTRYOSPHAERIA</i> SPECIES ON <i>TIBOUCHINA</i> IN SOUTH AFRICA, AUSTRALIA AND NEW ZEALAND</b>	150
<b>Abstract</b>	151
<b>Introduction</b>	152
<b>Materials and Methods</b>	154
Disease symptoms and collection of samples	154
Morphological characterisation	154
DNA isolation and amplification	155
Restriction fragment length polymorphisms (RFLPs)	156
DNA sequencing and analyses	156
Pathogenicity	157
<b>Results</b>	158
Disease symptoms and collection of samples	158
Morphological characterisation	158
Restriction fragment length polymorphisms (RFLPs)	158
DNA sequencing and analyses	159
Pathogenicity	159

<b>Discussion</b>	160
<b>Literature cited</b>	163
<b>SUMMARY</b>	187

## ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to the following people and institutions who have assisted me in preparing this thesis:

My study leaders, Dr. Jolanda Roux, Prof. Brenda Wingfield and Prof. Mike Wingfield for the critical review of this thesis and their valuable advice and support during my studies.

Albé van der Merwe, Marieka Gryzenhout and Dr. Bernard Slippers for their advice and assistance throughout my studies.

My dear friends Dr. Karin Jacobs, Marelize van Wyk, Lorenzo Lombard, Dr. Lawrie Wright, Riana Jacobs, Gavin Hunter and Hardus Hatting for their friendship and personal support.

My parents, brother and fiancé for their love, support, guidance and giving me courage, especially during the difficult times.

The fabulous FABI family and colleagues for sharing their knowledge and experience with me freely.

The Tree Protection Co-operative Programme and the Forestry and Agricultural Biotechnology Institute for providing facilities and financial support.

The National Research Foundation, South Africa and the University of Pretoria for providing facilities and financial support.

Natal parks board/Ezemvelo for assistance in the surveys conducted on *Syzygium* spp. in protected areas.

Our Creator, God Almighty



## PREFACE

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

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

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

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

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

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

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

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

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