UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Taxonomy and population genetics of *Teratosphaeria* causing stem cankers on *Eucalyptus* trees

by

María Noel Cortinas Irazabal

Submitted in partial fulfilment of the requirements for the degree

Philosophiae Doctor

In the faculty of Natural & Agricultural Science, Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria

© University of Pretoria

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

María Noel Cortinas

February 2011

TABLE OF CONTENT

First record of *Colletogloeopsis zuluense* comb. nov., causing a stem canker of *Eucalyptus* spp. in China

Chapter 3

Multi-gene gene phylogenies and phenotypic characters distinguish two species within the *Colletogloeopsis zuluensis* complex associated with *Eucalyptus* stem cankers

Chapter 4

Polymorphic microsatellite markers for the *Eucalyptus* fungal pathogen *Colletogloeopsis zuluensis*

Chapter 5

Microsatellite markers for the *Eucalyptus* stem canker fungal pathogen *Kirramyces gauchensis*

Chapter 6

Genetic diversity in the *Eucalyptus* stem pathogen *Teratosphaerizuluensis*

Chapter 7

Unexpected genetic diversity revealed in the *Eucalyptus* canker pathogen *Teratosphaeria gauchensis*

APPENDIX I

First record of the *Eucalyptus* stem canker pathogen, *Coniothyrium zuluense* from Hawaii

APPENDIX II

M - FIASCO at FABI

AKNOWLEDGEMENTS

I am sincerely grateful to all people and institutions that made possible the completion of this study.

To my supervisors, Brenda and Mike Wingfield, that offered me the opportunity to develop my doctoral studies at FABI. Thanks for their guidance and continuous support during my studies.

To the Genetic Department at the University of Pretoria, NRF organization, and the South African Forestry Industry, providing the facilities and financial support needed to complete this thesis.

To my family and friends in Uruguay that supported me in the decision to move to South Africa.

To Paullete Bloomer the first person that made possible my visit to the University of Pretoria, and who put me in contact with FABI.

To the people of the MEEP Laboratory. Thanks for receiving me with open arms, for your friendship and for your intellectual contributions.

To my dear colleagues at FABI, specially to my friends Irene Barnes, Joha Grobbelaar, Dina Paciura, Ryan Nadel, Draginja Pavlic, Wilhelm De Beer, Sonja De Beer, Guillermo Pérez, Renate Zipfel, Gavin Hunter and Grace Nakabonge and my friends in Australia Treena Burgess and Vera Andjic. Thank you all for your support, dedication and for your role as intellectual mentors and facilitators during my journey.

To Eva Müller, Rose Visser, Helen Doman, Jenny Hale, Heidi Fysh , Martha Mahlangu, Lydia Twala, Valentina Nkosi their friendship and proffessional dedication.

To Christoph, my husband for his love, continuous patience and “IT” support.

To South Africa, the marvellous rainbow country, teaching me so much about nature, science, people, love and friendship.

PREFACE

At the time of commencing this study, there were only five papers published on Coniothyrium canker disease of *Eucalyptus.* These studies included the formal description of the fungus causing the disease and some aspects of its biology and physiology were characterized. The fungus was described, at that time, as *Coniothyrium zuluense,* which had a very simple morphology, lacked sexual reproductive structures, had small nondescript conidia and it was slow growing in culture. Nevertheless, the taxonomic status of the Coniothyrium canker pathogens changed in several occasions during this study including placement in genera such as *Colletogloeopsis*, *Kirramyces* and *Teratosphaeria.*

After the first appearance of Coniothyrium canker in South Africa, the disease was found in many other parts of the world. DNA sequences from cultures of *C. zuluense* became easier to obtain and this made it possible to undertake phylogenetic comparisons of isolates from various areas. Such studies also showed that *C. zuluense* was closely related to *Mycosphaerella* species. The common appearance of Coniothyrium canker in new areas motivated further studies of this disease and it causal agent, particularly applying newly available rDNA-based techniques. This also provided the motivation for studies presented in this thesis.

The thesis is introduced by means of a literature review that treats Coniothyrium canker on *Eucalyptus.* Briefly, the general characteristics of the host species, *Eucalyptus,* are described. Furthermore, trends relating to emerging diseases in plantations of *Eucalyptus* during the past two decades are treated with particular focus being placed on *Mycosphaerella* diseases. The phylogenetic relationships between *Coniothyrium*, *Mycosphaerella* and its anamorphs are considered together with the population biology of related pathogens.

In chapter two of this thesis, DNA sequence comparisons were used to determine the phylogenetic position of *C. zuluense* related to other fungi. In particular, the question as to whether *C. zuluense* was correctly placed in the genus *Coniothyrium* and its relatedness to *Mycosphaerella* was considered. Comparisons with the type species of *Coniothyrium, C. palmarum* and a collection of sequences of *Mycosphaerella* species were also conducted. In addition, the identity of isolates

obtained from China with similarities in colony morphology to *C. zuluense* was considered.

The objective of the study presented in chapter three was to investigate whether all the available isolates in the FABI collection from different countries and associated with Coniothyrium canker represented a single phylogenetic species. An additional methodological objective of this chapter was to select the best DNA regions for phylogenetic studies on this fungus and its relatives. Four DNA regions were selected based on the informative content as well as ease and reproducibility for Polymerase Chain Reaction (PCR) amplification.

The studies presented in Chapter 3 of this thesis showed that two species cause Coniothyrium canker and these are now known as *Teratosphaeria zuluensis* and *Teratosphaeria gauchensis*. Therefore, the objectives of the studies presented in chapters four and five were to develop highly variable markers to study the genetic variability and population parameters of populations of both species. This included the development of a robust protocol to isolate microsatellites on both fungi and that would also be informative for related genera. The protocol finally developed and used is presented in Appendix 2 of this thesis.

In chapters six and seven, the microsatellite markers developed in the previous chapters were applied. The genetic structure of populations of *T. zuluensis* and *T. gauchensis* was thus studied. Analyses of the amplified alleles and their frequencies were used to determine the levels of genetic diversity, clonality and to draw preliminary conclusions regarding the origin and global movement of the pathogens.