Taxonomy and pathology of a new species of *Cryphonectria* from *Eucalyptus* in South Africa

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

MAGISTER SCIENTIAE

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

March 2000

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DECLARATION

I, the undersigned, hereby declare that the thesis submitted herewith for the degree Magister Scientiae to the University of Pretoria, contain my own independent work and has hitherto not been submitted for any degree at any other University.

M Venter

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March 2000

When wisdom enters your heart,

And knowledge is pleasant to your soul,

Discretion will preserve you,

Understanding will keep you.

PROVERBS 2:10-11

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ACKNOWLEDGEMENTS

This M.Sc. thesis could not have been completed without the help of several people. These people supported me morally, aided me with their expertise, and helped me with technical aspects. I would like to mention a few people, and thank them for what they meant to me.

The Lord and my Saviour, the real first author of this thesis, guided and supported me continuously. My parents were always in the background and prayed for me. Jean, my true friend in life, supported me and helped me many times. Dr. Hanlie Meyer shared her wisdom with me to enable me to better myself during the course of this thesis.

I would like to spend some time in thanking Teresa, Mike and Brenda for the excellent job they did in leading my study. I am grateful to Teresa for always being there, for being able to run into her office for small things, and all of the advice. Mike always manages to drag out the best from you, knows so much and always makes time for you. Brenda is also always willing, and gives such good advice!

I would especially like to thank Dr. Charles S. Hodges from North Carolina State University for sharing his knowledge and time so willingly, and taking the trouble of sending us specimens and papers. Professor Gerard Adams from Michigan State University unknowingly influenced my train of thought (and my work) and also answered many questions. We are grateful to Prof. R. Jay Stipes from the Virginia Polytechnic Institute for his advise and donating his precious culture collection containing different *Endothia* and *Cryphonectria* species, slides and most of his papers on *Endothia* and *Cryphonectria*. Dr. Paul Cannon from the Kew Botanical Institute also

counselled me on some taxonomical aspects. I am also grateful for the light micrographs (Fig. 3*a*, 3*b*) provided by Prof. Pedro W. Crous from the University of Stellenbosch.

Some people were actively involved in my project. Without the unselfish help of Dr. Ben Eisenberg with my statistical analyses, I would have been lost. Dr. Hugh Glen of the National Botanical Institute of Pretoria, provided me with the Latin descriptions in Chapter Three. I would also like to thank Anita Slabbert of the Academic Information Service for obtaining the most difficult and most needed references.

Members of the FABI team were also a rich source of help. Martin Coetzee helped me with the finer details of sequence analysis, and reviewed Chapter Two. Bernard Slippers and Dr. Jolanda Roux reviewed Chapter One and Four, respectively. Riana Jacobs and Dr. Karin Jacobs advised me on the various aspects of describing a new species. Various people also assisted me with my field trials and sampling.

Most projects would not have been possible without financial support. I am, therefore, thankful to the members of the Tree Pathology Co-operative Programme (TPCP) for their financial input. The National Research Foundation (NRF) and University of Pretoria also aided in the financing of this project. I also acknowledge the staff of the Mondi Nursery at KwaMbonambi for providing us with susceptible clones for field inoculations.

PREFACE

Endothia gyrosa is a canker pathogen of several tree genera and is best known for its association with pin oak blight in the USA. In the 1980s, investigations into the canker pathogens associated with *Eucalyptus* spp. in Australia led to the discovery of a similar fungus that was at the time also identified as *E. gyrosa*. This fungus was associated with cankers, die-back and in extreme cases, death of trees. Recently, this pathogen was also found on *Eucalyptus* spp. in South Africa. Slight morphological differences existed between the fungus called *E. gyrosa* on *Eucalyptus* in the Southern Hemisphere and the fungus from North America. It was, therefore, necessary to investigate the taxonomic relationships between *E. gyrosa* from North America, Australia and South Africa. Investigations into the pathogenicity of this fungus in South Africa has been considered important, since *Eucalyptus* forms an important part of the forestry industry in South Africa.

The literature study presented in Chapter One of this thesis, aims to provide an understanding of the taxonomy of the closely related genera *Endothia* and *Cryphonectria*. This overview summarises the taxonomic history of *Endothia* and *Cryphonectria*, and compares key morphological features used in the distinction between members of these genera. The physiology, pathology, host range and geographical distribution of *E. gyrosa* are also discussed.

Phylogenetic comparisons, by means of molecular techniques, provide a reasonably unbiased method of determining the relationships between fungi. In Chapter Two, *E. gyrosa* isolates from North America are compared with isolates from Australia and South Africa at the molecular level. The comparison was carried out using Restriction Fragment Length Polymorphisms (RFLP) and DNA sequencing of Polymerase Chain Reaction (PCR) amplicons from the ITS1 (Internal Transcribed Spacer), ITS2 and 5.8S rRNA gene of the ribosomal DNA operon.

Chapter Three represents the outcome of a morphological study on *E. gyrosa* isolates and stromatal specimens from North America, Australia and South Africa. Bark specimens containing stromata of *E. gyrosa* from the various geographical areas were compared microscopically. Isolates of *E. gyrosa* from North America and South Africa were also compared in culture.

Assessing the pathogenicity of *E. gyrosa* on *Eucalyptus* in South Africa was the aim of studies presented in Chapter Four of this thesis. Fifteen isolates of the South African fungus were screened for their virulence on a clone of *E. grandis* (ZG14). A highly virulent isolate was subsequently chosen to screen different clones of *Eucalyptus* for levels of tolerance to this pathogen. It was believed that information from this trial would be useful in establishing a breeding programme against *E. gyrosa*.

The forestry industry in South Africa generates significant revenue and provides many employment opportunities. This thesis has been produced over a period of two years, and

was aimed at investigating the pathogenicity and correct identity of the canker pathogen known to us as *E. gyrosa*. Results of this thesis will hopefully also be of value in establishing potential control strategies against this pathogen in order to minimise losses. Some repetition between chapters has been unavoidable, because they are presented as independent entities suitable for publication. Chapters have been written according to the instructions of Mycological Research.

SUMMARY

The canker pathogen, *Endothia gyrosa*, was described at the beginning of the nineteenth century in North America. More recently, a similar fungus, also identified as *E. gyrosa*, was discovered in Australia and South Africa on *Eucalyptus* spp. that was able to cause tree death in severe cases. Slight morphological differences existed between the fungus from *Eucalyptus* and *E. gyrosa* from North America, raising questions as to the taxonomic position of *E. gyrosa* from *Eucalyptus*.

Eucalyptus makes up a substantial part of the forestry industry world-wide, as well as in South Africa. The pathogenicity of *E. gyrosa* on *Eucalyptus* in South Africa was investigated in this study to determine whether this pathogen poses a threat to *Eucalyptus* plantations. The correct identity of the South African and Australian fungus was also determined through molecular and morphological comparisons.

In Chapter One of this thesis, the history and morphology of *Endothia*, the closely related genus *Cryphonectria* and the Diaporthales, to which these genera belong, are considered in detail. This information aimed to provide background for the study on phylogenetic relationships between *E. gyrosa* from Northern America, and the similar fungus from Australia and South Africa. The pathology and physiology of *E. gyrosa* on all of its hosts were also considered.

Endothia gyrosa isolates from North America, Australia and South Africa were compared with each other at the molecular level in Chapter Two. This was achieved by means of

RFLPs and DNA sequencing of PCR amplicons of the ITS1, ITS2 and 5.8S rRNA gene. RFLP profiles of South African and Australian isolates were identical but different to those of the North American isolates. The Australian and South African isolates also grouped together in the phylogenetic tree based on the DNA sequences, but separately from the North American isolates. Moreover, the South African and Australian isolates grouped together with *C. parasitica*, and not in the same clade as *E. gyrosa* from North America. *Cryphonectria cubensis* grouped in a clade of its own and not together with *C. parasitica*, as expected.

The fungus from *Eucalyptus* in the Southern Hemisphere is distinct from *E. gyrosa* in Northern America. Furthermore, it appears to belong to the genus *Cryphonectria*. This hypothesis was supported by morphological comparisons and cultural characteristics presented in Chapter Three. Stromatal specimens on bark from South Africa and Australia had a similar morphology to that of *Cryphonectria*, in that the stromata were semi-immersed and consisted of erumpent ectostromatal discs and immersed entostromatal discs, containing the perithecia. In contrast, *Endothia* had widely erumpent, predominantly entostromatic stromata with perithecia borne in the fungal tissue above the bark surface. Different cultural characteristics and different growth characteristics at 10 °C and 15 °C, provided further evidence that the fungus from *Eucalyptus* is distinct from *E. gyrosa* from North America. The fungus from *Eucalyptus* was, therefore, described as a new species belonging in *Cryphonectria*, and the name *C. eucalypti* was proposed for it.

The pathogenicity of the new species, *C. eucalypti*, was assessed through a series of field inoculations presented in Chapter Four. Fifteen isolates were inoculated into *E. grandis* clone

ZG14 and lesions of varying sizes were produced after seven weeks. Statistical analyses of the data showed that isolates of *C. eucalypti* exhibit significant isolate-environment interaction. Differing degrees of pathogenicity will, therefore, be exhibited under different conditions by a particular isolate. This indicates that *C. eucalypti* could play a role in causing disease when host trees are stressed. Levels of tolerance to *C. eucalypti* were also detected in the 42 clones tested, indicating that a breeding programme for resistance to *C. eucalypti* would be possible.

The outcome of this thesis is twofold. The true identity of the fungus on *Eucalyptus* in South Africa and Australia has been established, and its pathology on *Eucalyptus* clones in South Africa has been determined. Based on these results, we now know that *C. eucalypti* has the potential to cause serious disease under conditions favourable for disease development. Canker caused by *C. eucalypti*, however, does not pose a serious enough threat to warrant the establishment of a large scale breeding programme. The pathogen should, however, be monitored closely in the field.

OPSOMMING

Die kanker patogeen, *Endothia gyrosa*, is beskryf aan die begin van die negentiende eeu in Noord-Amerika. 'n Kanker patogeen wat boomsterfte kan veroorsaak in ernstige gevalle, en soortgelyk is aan *E. gyrosa*, is onlangs ontdek in Australië en Suid-Afrika op *Eucalyptus* spesies. Klein morfologiese verskille is waargeneem tussen die fungus van *Eucalyptus* en *E. gyrosa* van Noord-Amerika. Dit het vrae laat ontstaan rakende die taksonomiese posisie van *E. gyrosa* op *Eucalyptus*.

Eucalyptus vorm 'n aansienlike deel van die bosbou industrie wêreldwyd asook in Suid-Afrika. Die patogenisiteit van *E. gyrosa* op *Eucalyptus* in Suid-Afrika is ondersoek in hierdie studie om vas te stel of hierdie patogeen 'n bedreiging inhou vir *Eucalyptus* plantasies. Die regte identiteit van die Suid-Afrikaanse en Australiese fungus is ook ondersoek deur middel van molekulêre and morfologiese vergelykings.

In Hoofstuk Een van hierdie tesis, word die geskiedenis en morfologie van *Endothia*, die naby-verwante genus *Cryphonectria* en die Diaportales in detail behandel. Die doel met hierdie inligting is om agtergrond te verskaf vir hierdie studie wat handel oor die filogenetiese verhoudings tussen *E. gyrosa* van Noord-Amerika, Australië en Suid-Afrika. Die patologie en fisiologie van *E. gyrosa* op al sy gashere is ook beskryf.

Endothia gyrosa isolate van Noord-Amerika, Australië en Suid-Afrika is vergelyk met mekaar op die molekulêre vlak in Hoofstuk Twee. Dit is gedoen deur middel van 'n RFLP analise en DNA basispaar-opeenvolgingsbepaling van PKR fragmente van die ITS1, ITS2 en

5.8S rRNA geen. RFLP profiele van die Suid-Afrikaanse en Australiese isolate was identies, maar verskillend van die profiele van die Noord-Amerikaanse isolate. Die Australiese en Suid-Afrikaanse isolate het ook bymekaar gegroepeer in die filogenetiese boom gebaseer op die DNA volgordes, maar apart van die Noord-Amerikaanse isolate. Meer nog, die Suid-Afrikaanse en Australiese isolate het saammet *C. parasitica* gegroepeer, en nie in dieselfde groep as *E. gyrosa* van Noord-Amerika nie. *Cryphonectria cubensis* het in 'n aparte groep gegroepeer en nie, soos verwag, saammet *C. parasitica* nie.

Die fungus van *Eucalyptus* in die Suidelike Halfrond verskil van *E. gyrosa* van Noord-Amerika. Dit blyk ook om tot die genus *Cryphonectria* te behoort. Hierdie hipotese was ondersteun deur die morfologiese vergelykings en kulturele eienskappe bespreek in Hoofstuk Drie. Die morfologie van stromata op bas van Suid Afrika en Australië was soortgelyk aan die morfologie van *Cryphonectria*, naamlik dat die stromata semi-ingesonke was en bestaan het uit 'n uitstaande ektostromatale skyf en ingesonke entostromatale skyf wat die peritesia bevat. In kontras, het *Endothia* wyd uitstaande, grotendeels entostromatiese stromata gehad, met peritesia wat voorgekom het in die fungale weefsel bo die bas oppervlak. Verskillende kulturele en groei eienskappe by 10 °C en 15 °C, het verder bewys dat die fungus van *Eucalyptus* verskillend is van *E. gyrosa* van Noord Amerika. Die fungus van *Eucalyptus* is dus beskryf as 'n nuwe spesie wat behoort in *Cryphonectria*, en die naam *C. eucalypti* is voorgestel.

Die patogenisiteit van die nuwe spesie, *C. eucalypti*, is ondersoek deur middel van 'n reeks veld inokulasies voorgelê in Hoofstuk Vier. Vyftien isolate is geïnokuleer in 'n *E. grandis* kloon (ZG14) en letsels van varieerbare groottes is na sewe weke gevorm. Statistiese

ontleding van die data het gewys dat isolate van *C. eucalypti* aansienlike isolaat-omgewing interaksie getoon het. Wisselende vlakke van patogenisiteit sal dus onder verskillende toestande deur 'n betrokke isolaat getoon word. Dit impliseer dat *C. eucalypti* moontlik 'n rol kan speel in die veroorsaking van siekte wanneer die gasheer onder stres is. Vlakke van toleransie tot *C. eucalypti* is waargeneem in die 42 klone wat getoets is. Dit is 'n aanduiding dat 'n telingsprogram vir weerstand teen *C. eucalypti* moontlik is.

Die uitslag van hierdie tesis is tweevoudig. Die ware identiteit van die fungus op *Eucalyptus* in Suid-Afrika en Australië is vasgestel, en die patologie daarvan op *Eucalyptus* klone in Suid-Afrika is bepaal. Ons weet dus nou dat *C. eucalypti* die potensiaal besit om ernstige siekte te veroorsaak in toestande gunstig daartoe. Kanker veroorsaak deur *C. eucalypti* hou egter nie so 'n ernstige gevaar in vir die bosbou bedryf in Suid-Afrika, om die daarstelling van 'n grootskaalse telingsprogram te regverdig nie. Die patogeen moet egter noukeurig in veld toestande gemonitor word.