

# **Diseases of *Eucalyptus* in Colombia**

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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.



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## PREFACE

Colombia is a tropical country located in South America. The size of Colombia is 114.1 million hectares, of which the potential forestry area has been estimated at 83 million hectares. Indiscriminate deforestation is a continuous problem and comprises of approximately 190 000 ha/year. To provide an alternative source of timber, 145 759 ha have been planted with different species of *Pinus*, *Cupressus* and *Eucalyptus* spp. during the course of the last 50 years. Private companies and government projects are developing reforestation programs in order to compensate for solid and pulpwood needs of the country. These plantations are particularly prone to damage by insect pests and diseases. Identification of diseases and subsequent management of pathogens is becoming essential in Colombia. The aim of this study was to develop a foundation for future research and particularly for management of *Eucalyptus* diseases in Colombia.

**Chapter One** of this dissertation is a report of the occurrence of *Eucalyptus* diseases in Colombia. This is based on three general surveys conducted in a variety of age classes of trees, represented by different seed sources and clones. A wide range of sites was also considered for plantations in Colombia, ranging from the low altitude tropics to those in the cooler Andean regions.

In **Chapter Two**, I characterise species of *Botryosphaeria* associated with cankers on *E. grandis* in plantations. This is achieved based on comparisons using morphological and DNA-based methods. Isolates were collected from three different and representative geographical zones, where *Eucalyptus grandis* has been planted in Colombia. Isolates of *Botryosphaeria* spp. identified in these studies are also tested for pathogenicity in greenhouse and field trials.

*Cryphonectria cubensis* is a well-known pathogen associated with basal cankers in *Eucalyptus* and considered a serious pathogen of *E. grandis* around the world. In **Chapter Three**, native Colombian Melastomataceae are reported as hosts of this important pathogen. These include *Miconia rubiginosa* and *M. theaezans*. The role of these trees as alternative hosts of *C. cubensis* is also considered.

*Cylindrocladium* blight of *Eucalyptus* is one of the most important diseases of *E. grandis* in young plantations. This disease has not been fully characterised in Colombia where it has previously been recognised as important. The aim of the study presented in **Chapter Four** is to identify the species of *Cylindrocladium* responsible for the disease. Isolates of the pathogen were, therefore, collected from three geographical zones and these are identified based on morphology and DNA based comparisons.

In **Chapter Five** of this dissertation, the presence of *Ceratocystis fimbriata* on *E. grandis* in Colombia is reported for the first time. The fungus is a serious pathogen of other crops such as coffee in the country, but it has not as yet been recognised as a threat to *Eucalyptus* in Colombia.

Studies that make up this dissertation were conducted both in Pretoria and in Colombia. This has to some extent limited the range of investigations that were possible. However, the overall aim of providing a background to the future studies of *Eucalyptus* diseases in Colombia was achieved. Chapters making up this document were developed over a period of two years and they should be read as entirely independent entities. This format has necessitated some duplication between chapters.



## SUMMARY

The forestry industry in Colombia comprises of 145 759 ha of commercial plantations. Of these, 47 700 ha are planted to *Eucalyptus* that is used mainly for the production of pulp, construction timber and paper. Eight years ago, an extensive breeding program to develop clones of *Eucalyptus* was started in Colombia. One of the objectives was to reduce the incidence of disease and insect pests. Little is, however, known regarding the diseases that occur on *Eucalyptus* spp. in Colombia, and this was a serious impediment to the breeding and tree improvement programme. The studies presented in this thesis were undertaken to improve our knowledge of *Eucalyptus* diseases in Colombia. Although most detailed experimentation has emerged during the course of the last two years, many collections, preliminary trials and observations extend back to 1995.

**Chapter One** of this thesis presents the results of extensive surveys on *Eucalyptus*, carried out to evaluate the diseases present in Colombia. A number of diseases were recorded. The most serious of these are Cryphonectria canker caused by *C. cubensis*, and Botryosphaeria canker, now known to be caused by *B. ribis* and *B. dothidea*. *Cylindrocladium* shoot and leaf blight disease was also important on *Eucalyptus* spp. This disease is associated with several *Cylindrocladium* species in Colombia, namely *C. reteaudii*, *C. candelabrum*, *C. parasiticum*, *C. gracile* and *C. spathulatum*. Other less important diseases present included Mycosphaerella leaf blotch disease (MLB) caused by *M. suberosa*, *M. parkii*, *M. africana*, *M. colombiensis*, *M. flexuosa* and *M. lateralis*. Phaeoseptoria leaf spot caused by *Phaeophleospora epicoccoides*, and corky leaf spot caused by *Aulographina eucalypti* also occurred on *Eucalyptus* leaves. *Puccinia psidii*, a serious rust pathogen on *Eucalyptus* spp. in Brazil and other countries in South America, was found on native *Eugenia jambos* and *Psidium guajava* (Myrtaceae) in Colombia, but never on *Eucalyptus*. Background literature pertaining to these diseases and *Eucalyptus* diseases in general is also presented in this chapter, which provides a foundation for many studies presented in subsequent parts of the thesis.

In the chapters subsequent to the first, the identity and relative importance of the most important pathogens found in the disease surveys, was considered in greater detail. Isolates of these fungi were also used in pathogenicity trials. In **Chapter Two**, the

identity of the species associated with Botryosphaeria canker was considered. Previously, only the name *B. dothidea* had been used for collections from *Eucalyptus* in Colombia. In this study, however, it was shown that in addition to *B. dothidea*, *B. ribis* was also present. The latter species was also the more common. This was based on morphological comparisons and DNA sequence comparisons for the ITS1/2 region of the ribosomal operon (rDNA) and the elongation factor (EF) 1- $\alpha$  genes. In pathogenicity trials on *E. grandis*, *B. ribis* was found to be the more pathogenic species and clones were shown to vary considerably in their susceptibility to infection.

*Cryphonectria cubensis* is one of the most important canker pathogens of *Eucalyptus* world-wide. Recently this pathogen was discovered on native *Tibouchina* spp. in Colombia. These trees are members of the Melastomataceae, which is recognized as relatively closely related to the Myrtaceae. In **Chapter Three**, I report on additional native Melastomataceous hosts of *C. cubensis*. These include *Miconia theaezans* and *M. rubiginosa*. The identity of the fungus occurring on the native trees was confirmed based on comparisons of morphology and DNA sequences for the ITS1/2 rDNA regions and two regions of the  $\beta$ -tubulin gene. Pathogenicity trials were also conducted with isolates from *M. rubiginosa* and *M. theaezans* on *E. grandis* clones, *T. urvilleana*, *T. lepidota*, *T. semidecandra*, *M. theaezans* and *M. rubiginosa*. The isolates from the native hosts were mildly pathogenic on most of these tree species. Differences in resistance was also seen in various of these trees. This study provided further evidence that some pathogens of *Eucalyptus* in Colombia are probably native and have originated from Colombian Myrtaceae and Melastomataceae.

The aim of the studies presented in **Chapter Four** was to identify species associated with *Cylindrocladium* shoot and leaf blight disease. Based on morphological studies and DNA sequence comparisons for the  $\beta$ -tubulin gene, only one species, *C. spathulatum*, was found to occur in the regions sampled. A *Eucalyptus* clonal trial was also assessed for the presence of the disease. Results showed that different clones differed greatly in susceptibility to *C. spathulatum*.

*Ceratocystis fimbriata* is one of the most serious pathogens of coffee trees in Colombia. Recently, this pathogen has also begun to emerge as a serious threat to *Eucalyptus* trees

in Brazil, Uruguay and parts of Africa. This fungus has, however, not been found on *Eucalyptus* trees in Colombia. In **Chapter Five**, we considered whether *C. fimbriata* occurs on *Eucalyptus* spp. in Colombia. *Eucalyptus grandis* trees in plantations were artificially wounded, and checked for the presence of *Ceratocystis* spp. after eight weeks. Only two *C. fimbriata* isolates were obtained from two zones. The identification was based on morphology and DNA sequences of the ITS1/2 rDNA region. Two of the isolates from *E. grandis* and a known *C. fimbriata* isolate from *Schizolobium parahybum* (a native tree to Colombia), were inoculated into trees of two clones of *E. grandis* and a commonly grown *E. grandis* seed source. One of the *Eucalyptus* isolates was found to be highly pathogenic on all of the hosts. The second *Eucalyptus* isolate produced lesions that were not statistically different from those of the control inoculations. Differences in susceptibility were also found between the *E. grandis* clones. Our results confirm that *C. fimbriata* presents a serious threat to *Eucalyptus* in Colombia, but that breeding and selection of disease-tolerant clones and hybrids will reduce this risk.

Diseases of *Eucalyptus* trees can result in serious economic losses to the forestry industry in Colombia. Such experiences are being felt elsewhere in the world where these trees are used in exotic plantation forestry. Through the studies presented in this thesis, I hope to have established a firm understanding of the diseases present on *Eucalyptus* trees in Colombia. This was based on disease surveys, as well as more detailed taxonomic studies to confirm identifications. In the various studies, it was shown that some of these pathogens have originated on native plants and have adapted to infect *Eucalyptus* trees. It was also evident from the various pathogenicity trials that differences in resistance against these fungal pathogens exist in currently used *E. grandis* clones. This is encouraging as these differences can now be exploited in breeding programs aimed at reducing losses due to disease.

## OPSOMMING

Die bosbou industrie in Colombië beslaan 145 759 ha kommersiële plantasies. Hiervan is 47 700 ha beplant met *Eucalyptus* wat hoofsaaklik gebruik word vir die produksie van pulp, konstruksiehout en papier. Agt jaar gelede is 'n omvangryke telingsprogram begin om *Eucalyptus* klone te ontwikkel. Een van die doelwitte was om die voorkoms van siektes en insekpeste te verminder. Min is egter bekend oor die siektes wat op *Eucalyptus* spp. in Colombië voorkom, en dit was 'n ernstige hindernis in die telings- en boomveredelingsprogram. Die studies voorgedra in hierdie tesis was onderneem om ons kennis oor *Eucalyptus* siektes in Colombië te vermeerder. Alhoewel die meeste werk die afgelope twee jaar gedoen is, dateer baie versamelings, voorbereidende proewe en waarnemings terug tot 1995.

**Hoofstuk Een** van die tesis bevat die resultate van uitgebreide opnames op *Eucalyptus* om vas te stel watter siektes voorkom in Colombië. Heelwat siektes is gevind. Die ernstigste siektes is *Cryphonectria* kanker wat veroorsaak word deur *C. cubensis*, en *Botryosphaeria* kanker, wat ons nou weet veroorsaak word deur *B. ribis* en *B. dothidea*. *Cylindrocladium*-loot-en-blaar-skimmelsiekte was ook belangrik op *Eucalyptus* spp. Hierdie siekte word geassosieer met verskeie *Cylindrocladium* spesies in Colombië, naamlik *C. reteaudii*, *C. candelabrum*, *C. parasiticum*, *C. gracile* en *C. spathulatum*. Ander minder belangrike siektes wat teenwoordig is, is *Mycosphaerella* blaarvleksiekte (MLB) wat veroorsaak word deur *M. suberosa*, *M. parkii*, *M. africana*, *M. colombiensis*, *M. flexuosa* en *M. lateralis*. *Phaeoseptoria* blaarvlek veroorsaak deur *Phaeophleospora epicoccoides*, en kurkblaarvlek veroorsaak deur *Aulographina eucalypti* kom ook voor op *Eucalyptus* blare. *Puccinia psidii*, 'n ernstige roespatogeen van *Eucalyptus* spp. in Brazil en ander lande in Suid Amerika, was gevind op inheemse *Eugenia jambos* en *Psidium guajava* (Myrtaceae) in Colombië, maar nooit op *Eucalyptus* nie. Agtergrondliteratuur oor hierdie siektes en oor *Eucalyptus* siektes in die algemeen, is ook opgeneem in die hoofstuk, wat 'n fondasie lê vir baie studies voorgedra in opvolgende dele van die tesis.

In die daaropvolgende hoofstukke, is die identiteit en relatiewe belangrikheid van die belangrikste patogene wat gevind is in die siekteopnames, in groter detail ondersoek. Isolate van hierdie swamme was ook in patogenisiteitstoetse gebruik. In **Hoofstuk Twee**, is die identiteit van die spesies verbind met *Botryosphaeria* kanker, ondersoek. Voorheen

is slegs die naam *B. dothidea* gebruik vir versamelings vanaf *Eucalyptus* in Colombië. In hierdie studie, het ek egter gevind dat behalwe *B. dothidea*, ook *B. ribis* teenwoordig is. Laasgenoemde spesie kom ook die algemeenste voor. Hierdie bevindinge was gebaseer op morfologiese vergelykings en DNS volgordebepalings van die ITS1/2 gebied van die ribosomale operon (rDNA) en die 'elongation factor' (EF) 1- $\alpha$  geen. In die patogenisiteitsproewe op *E. grandis*, is daar gevind dat *B. ribis* die meer patogeniese spesie was, en dat klone heelwat gevarieer het ten opsigte van hul vatbaarheid vir infeksie.

*Cryphonectria cubensis* is een van die belangrikste kankerpatogene van *Eucalyptus* wêreldwyd. Hierdie patoogeen is onlangs op inheemse *Tibouchina* spp. in Colombië ontdek. Hierdie bome is lid van die Melastomataceae, wat relatief naby verwant is aan die Myrtaceae waarbinne *Eucalyptus* resorteer. In **Hoofstuk Drie**, rapporteer ek addisionele Melastomataceae gashere vir *C. cubensis*. Hierdie sluit in *Miconia theaezans* en *M. rubiginosa*. Die identiteit van die swam op hierdie inheemse bome was bevestig met morfologie en vergelykings van DNS volgordes van die ITS1/2 rDNA gebied en twee dele van die  $\beta$ -tubulin gene. Patogenisiteitsproewe was ook met isolate van *M. rubiginosa* and *M. theaezans* gedoen op *E. grandis* klone, *T. urvilleana*, *T. lepidota*, *T. semidecandra*, *M. theaezans* en *M. rubiginosa*. Die isolate van die inheemse gashere was matig patogenies op die meeste van hierdie bome. Verskille in weerstand was ook waargeneem in verskeie van hierdie bome. Hierdie studie het verder bewys dat sommige patogene van *Eucalyptus* moontlik in Colombië inheems kan wees en ontstaan het op Colombiaanse Myrtaceae en Melastomataceae.

Die doelwit van die studies in **Hoofstuk Vier** was om spesies geassosieer met *Cylindrocladium*-loot-en-blaar-skimmelsiekte, te identifiseer. Morfologiese studies en DNS volgordebepaling van die  $\beta$ -tubulin gene, het gewys dat slegs een spesie, *C. spathulatum*, voorkom in die areas waar opnames gedoen was. 'n *Eucalyptus* klonale proef was ook ge-evalueer vir die teenwoordigheid van die siekte. Resultate het gewys dat die onderskeie klone heelwat verskil ten opsigte van hul vatbaarheid vir *C. spathulatum*.

*Ceratocystis fimbriata* is een van die ernstigste patogene van koffieboome in Colombië. Hierdie patoogeen het ook onlangs 'n ernstige bedreiging begin word vir *Eucalyptus* bome

in Brazil, Uruguay en dele van Afrika. Die swam is egter nie gevind op *Eucalyptus* bome in Colombië nie. In **Hoofstuk Vyf**, het ons probeer vasstel of *C. fimbriata* op *Eucalyptus* spp. in Colombië voorkom. *Eucalyptus grandis* bome in plantasies was kunsmatig gewond, en spesifieke isolasies vir *Ceratocystis* spp. was na agt weke gemaak. Slegs twee *C. fimbriata* isolate is verkry vanaf twee areas in Colombië. Hierdie identifikasie was gebaseer op morfologie en DNS volgordes van die ITS1/2 rDNA gebied. Twee van die isolate van *E. grandis* en 'n bekende *C. fimbriata* isolaat van *Schizolobium parahybum* ('n inheemse boom in Colombië), was geïnkuleer in twee klone van *E. grandis* en bome van saad wat algemeen gebruik word. Die tweede *Eucalyptus* isolaat het egter letsels geprodusier wat nie van die kontrole inokulasies verskil het nie. Vatbaarheidsverskille was ook tussen die *E. grandis* klone gekry. Ons resultate bevestig dat *C. fimbriata* 'n ernstige bedreiging inhou vir *Eucalyptus* in Colombië, maar dat teling en seleksie van siekteweerstandbiedende klone en hibriede die risiko sal verminder.

Siektes van *Eucalyptus* bome kan ernstige ekonomiese verliese veroorsaak vir die bosbou industrie in Colombië. Soortgelyke bevindings word ook in ander dele van die wêreld ondervind waar die bome in uitheemse plantasië bosbou gebruik word. Ek hoop om deur die studies opgevat in hierdie tesis, 'n waardevolle bydrae te lewer oor die teenwoordigheid van siektes op *Eucalyptus* bome in Colombië. Hierdie inligting is verkry deur siekteopnames, asook meer gedetailleerde taksonomiese studies om die identifikasie van patogene te bevestig. Ek het ook in die onderskeie studies ontdek dat sommige van hierdie patogene ontstaan het op inheemse plante en aangepas het om *Eucalyptus* bome te infekteer. Dit was verder duidelik uit die verskeie patogenisiteitsproewe dat verskille in weerstand in *E. grandis* klone teen hierdie swampatogene bestaan. Dit is bemoedigend, omdat hierdie verskille gebruik kan word in telingsprogramme wat ontwikkel word om verliese as gevolg van siektes te verminder.