Pathogens in commercial *Eucalyptus* plantations in Chile, with special reference to *Mycosphaerella* and *Botryosphaeria* species

Submitted by

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

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CHAPTER 3

BOTRYOSPHERIA SPECIES CAUSING DIEBACK ON EUCALYPTUS SPP. IN CHILE

ABSTRACT
INTRODUCTION
MATERIALS AND METHODS
RESULTS
DISCUSSION
REFERENCES

SUMMARY

OPSOMMING
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>ACKNOWLEDGEMENTS</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREFACE</td>
<td>6</td>
</tr>
</tbody>
</table>

## CHAPTER 1

**PLANTATION FORESTRY IN CHILE WITH PARTICULAR REFERENCE TO DISEASE AND INSECT PROBLEMS**

1.0 INTRODUCTION                              | 9 |
2.0 FORESTRY PLANTATIONS IN CHILE            | 10 |
   2.1 *PINUS RADIATA*                        | 12 |
   2.2 *EUCALYPTUS* SPP.                      | 12 |
3.0 MAIN SANITARY PROBLEMS OCCURRING IN COMMERCIAL PLANTATIONS
   3.1 *PINUS* PLANTATIONS                    | 14 |
   3.2 *EUCALYPTUS* PLANTATIONS               | 21 |
4.0 CONCLUSIONS                              | 26 |
5.0 REFERENCES                               | 27 |

## CHAPTER 2

**MYCOSPHAERELLA SPECIES CAUSING LEAF BLOTCH DISEASE ON EUCALYPTUS SPP. IN CHILE**

ABSTRACT                                      | 53 |
INTRODUCTION                                   | 54 |
MATERIALS AND METHODS                          | 55 |
RESULTS                                        | 58 |
DISCUSSION                                     | 62 |
REFERENCES                                     | 65 |
I want to dedicate this thesis to my wonderful grandmother, with much love. At 96 years you are a great example in faith and optimism. Thanks “Mamita” you are great!

I want to thank the Lord for his constant love and guidance in the small and big things in my life. I really felt His support and care for both my family and myself.

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Commercial plantations in Chile are comprised of exotic *Pinus* and *Eucalyptus* species, which make up approximately 2.1 million ha. *Eucalyptus* spp. represent about 17% of the total, and the most widely planted species are *E. globulus* and *E. nitens*. Both species have been considered as relatively disease free in Chile, despite their susceptibility to several insects and diseases, elsewhere in the world. In Chile, leaf spot diseases are considered the most important sanitary problem associated with commercial *Eucalyptus* plantations and *Mycosphaerella* spp. appear to be the fungi causing the most significant damage. *Botryosphaeria* spp. have also been considered as a potential threat to *Eucalyptus* plantations, especially based on experience in other countries, where these fungi severely damage stressed trees. Very little research has been conducted on *Eucalyptus* diseases in Chile and the aim of this thesis was to expand our knowledge of this field. The more specific aim was to provide robust identifications for the species of *Mycosphaerella* and *Botryosphaeria* associated with disease symptoms in commercial *Eucalyptus* plantations in Chile.

In the first chapter of this thesis, a general review of forestry activities in Chile is presented. Special emphasis is placed on matters pertaining to tree health. Information is summarized separately for *Pinus* and *Eucalyptus* species and is organized in such a way that insect pests and disease problems are treated separately. A review of the available literature on tree pest and disease problems in Chile is provided to serve as a foundation for research undertaken in this thesis.

Prior to undertaking studies in this thesis, very little research had been conducted on forest pathogens. In the second chapter of the thesis, I have undertaken a study to identify the most common *Mycosphaerella* spp. associated with leaf spot diseases in Chile. The taxonomy of these fungi is known to be very complicated and it was previously based exclusively on morphological characteristics. However, recent studies have applied DNA sequencing techniques to this process. My aim has thus been to use DNA sequence data, together with morphological characters to identify *Mycosphaerella* spp. associated with leaf diseases of *Eucalyptus* in Chile. Ultimately, the intention has also been to gain some perspective on the relative importance of the various species of these fungi in Chile and to reduce losses caused by them. To achieve this objective, diseased adult and juvenile *E.*
*globulus* and *E. nitens* leaves were collected from plantations in Northern-central and Southern-central Chile. Species of *Mycosphaerella* were identified based on ascospore germination patterns, cultural characteristics and sequence data from the Internal Transcribed Spacer (ITS) region of the rRNA operon.

*Botryosphaeria* spp. have thus far not been considered as serious pathogens in *Eucalyptus* plantations in Chile. This is despite the fact that they can be highly damaging elsewhere in the world. However, very little is known regarding their presence or potential to cause disease. The rapid growth of commercial *Eucalyptus* plantations has resulted in an increase in new pests and diseases. Of these, *Botryosphaeria* dieback disease has begun to appear in young and adult trees more frequently in Chile. Therefore, the aim of studies presented in Chapter III of this thesis, was to identify the *Botryosphaeria* spp. associated with dieback disease of commercial *Eucalyptus* plantations. Symptomatic branches were thus collected from *E. globulus* and *E. nitens* plantations in Northern-central and Southern-central Chile. Species of *Botryosphaeria* were subsequently identified based on morphological characteristics and sequence data from the Internal Transcribe Spacer (ITS) and β-tubulin regions of the ribosomal RNA operon. A greenhouse pathogenicity trial was also conducted using the various *Botryosphaeria* spp. identified, to evaluate their relative importance in Chile.

The primary aim of this thesis is to provide a better understanding of *Mycosphaerella* and *Botryosphaeria* species that are currently damaging *Eucalyptus* spp. in commercial plantations in Chile. The study is the first of its kind and it will hopefully also form a foundation for further studies of *Eucalyptus* diseases in the country. Knowledge of contemporary techniques to identify pathogens has also been learned and this will also be useful to forestry researchers in Chile in the future.