Phenotypic variation of two localised populations of *Phytophthora cinnamomi* from Western Australia and how they impact on *Eucalyptus marginata* resistance

by

Daniel Hüberli
Bachelor of Science (Honours)

This thesis is submitted
for the degree of
Doctor of Philosophy

School of Biological Sciences and Biotechnology
Murdoch University
Perth, Western Australia

November 2001

Declaration

The work described in this thesis was undertaken while I was an enrolled student for the degree of Doctor of Philosophy at Murdoch University, Western Australia. I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution. To the best of my knowledge, all work performed by others, published or unpublished, has been duly acknowledged.

Daniel Hüberli

November 2001

Abstract

Phytophthora cinnamomi is an introduced soilborne phytopathogen to Western Australia (WA) and impacts on 2000 of the approximately 9000 plant species indigenous in the southwest of WA. Amongst these is *Eucalyptus marginata* (jarrah), the dominant and economically important hardwood timber species of the jarrah forest. This thesis aimed to investigate the morphological, pathogenic and genotypic variation in two local WA populations of *P. cinnamomi* isolates. The populations were selected from areas where jarrah clonal lines selected for resistance to *P. cinnamomi* may be used in the rehabilitation of infested jarrah forest and rehabilitated bauxite minesites in the southwest of WA. Resistance against a range of isolates using different inoculation methods.

Seventy-three isolates of *P. cinnamomi* were collected from diseased jarrah and *Corymbia calophylla* (marri) trees from two populations located 70 km apart and these were examined for phenotypic and genotypic variation. Microsatellite DNA analysis showed that all isolates were of the same clonal lineage. In *P. cinnamomi* for the first time I show that there is a broad and continuous variation in the morphology and pathology between two populations of one clonal lineage, and that all phenotypes varied independently from one another. No relationship was found between morphological and pathogenic characters. The ability of isolates in both populations to cause deaths ranged from killing all plants within 59 days to plants being symptomless 182 days after inoculation.

Single and multiple paragynous antheridia formed along with amphigynous ones in mating studies with all WA isolates and a sample of worldwide isolates. Developmental studies and cytological examination showed fertilisation tubes developed asynchronously or synchronously from both antheridial types and indicated

that either antheridial type contributed a nucleus for fertilisation of the oosphere. This is the first report of paragynous antheridial associations in *P. cinnamomi*. Antheridial variation is a characteristic that needs to be adjusted in the taxonomic *Phytophthora* identification keys.

In underbark and zoospore stem inoculations of three 1.5-year-old jarrah clonal lines (two ranked as resistant (RR) and one as susceptible (SS) to *P. cinnamomi* in the original selection trials) at 15, 20, 25 and 30°C, it was found that the method of inoculation did not produce comparable results, particularly at 25 and 30°C. At these temperatures, all three clonal lines had 100% mortality when inoculated underbark, but when inoculated with zoospores, one RR line had 60% survival and the SS and remaining RR line had 100% mortality. Generally, the level of resistance of all clonal lines declined with increasing temperature. Lesion development was measured at 20, 25 and 30°C for 4 days in detached branches of an RR and SS clonal line inoculated underbark with four different *P. cinnamomi* isolates. Detached branches were found to be a potential screen for jarrah resistance to *P. cinnamomi* and to allow the identification of susceptible and resistant clonal lines at 30°C.

Lesion and colonisation development of *P. cinnamomi* isolates were assessed *in situ* (late autumn) of seed-grown and clonal lines of 3.5 to 4.5 year-old jarrah trees growing in a rehabilitated minesite jarrah forest in underbark inoculation of lateral branches (1995) or simultaneously in lateral branches and lateral roots (1996). Trees were underbark inoculated in lateral branches and lateral roots. Colonisation was more consistent as a measure of resistance than lesion length over the two trials because it accounted for the recovery of *P. cinnamomi* from macroscopically symptomless tissue beyond lesions, which on some occasions, was up to 6 cm. In the two trials, one RR clonal line consistently had small lesion and colonisation lengths in branches and roots. In contrast, the remaining two RR clonal lines had similar lesion and colonisation

lengths to the SS clonal line and may, therefore, not be suitable for use in the rehabilitation of *P. cinnamomi* infested areas. The relative rankings of the jarrah clonal lines by colonisation lengths were similar between branch and root inoculations. Branch inoculations are a valid option for testing resistance and susceptibility of young jarrah trees to *P. cinnamomi*.

The pathogen was recovered on *Phytophthora* selective agar 3–6 months after inoculation from 50% of samples with lesions and 30% of symptomless samples in a series of growth cabinet, glasshouse and field experiments. However, up to 11% of samples with and without lesions and from which *P. cinnamomi* was not initially isolated contained viable pathogen after leaching the plant material in water over 9 days. This indicates that the pathogen could be present as dormant structures, such as chlamydospores, where dormancy needs to be broken for germination to occur, or fungistatic compounds in the tissue need to be removed to allow the pathogen to grow, or both. These results have important implications for disease diagnosis and management, disease-free certification and quarantine clearance.

No clonal line of jarrah was found to be 100% resistant using different inoculation methods, environmental conditions and when challenged by individuals from a large range of *P. cinnamomi* isolates. Even the most promising RR line had individual replicates that were unable to contain lesions or died with time. This suggests that further screening work may be required using more isolates varying in their capacity to cause disease and a broader range of environmental conditions. Jarrah clonal lines that survive such rigorous screening could then be expected to survive planting out in a range of environments in the jarrah forest and rehabilitated bauxite minesites.

Authorities for species

The authorities of scientific names of pathogens and their plant hosts will not be presented in this thesis. The thesis adopts the policy of Mycological Research (Hawksworth, 2000: 124), whereby only taxonomic studies where species have been verified give authorities.

Table of contents

List of abbreviations

Universally accepted abbreviations and those listed by Hawksworth (2000: 122) are not listed below and are used throughout the thesis without explanation.

ANOVA	Analysis of variance
CMA	corn meal agar
EBL	extension of *Phytophthora cinnamomi* beyond the visible lesion
J1	Jarrah (*Eucalyptus marginata*) site 1 (Jarrahdale) isolates
J2	Jarrah (*E. marginata*) site 2 (Willowdale) isolates
LSD	Least Significant Difference test
M1	Marri (*Corymbia calophylla*) site 1 (Jarrahdale) isolates
M2	Marri (*C. calophylla*) site 2 (Willowdale) isolates
MV8A	modified vegetable-8 juice agar (Appendix 1)
NARPH	*Phytophthora*-selective agar (Appendix 1)
PDA	potato dextrose agar
r	correlation coefficient
ROI	region of inoculation (for zoospore inoculation)
RR	*E. marginata* resistant (to *P. cinnamomi*) clonal lines from resistant families as determined by McComb *et al*. (1990)
SOI	site of inoculation (for underbark inoculation)
SS	*E. marginata* susceptible (to *P. cinnamomi*) clonal lines from susceptible families as determined by McComb *et al*. (1990)
V8A	vegetable-8 juice agar (Appendix 1)
WA	Western Australia

***Eucalyptus marginata* clonal line abbreviations**

RR1	1J30
RR2	5J336
RR3	12J96
RR4	77C40
RR5	121E47
RR6	121E293 (or 121E29)

RR7	326J51
SS1	11J379
SS2	11J402

List of publications

The results of all five experimental chapters have been published or submitted as indicated below. Only minor changes have been made to these publications in the thesis. My co-authors included I. C. Tommerup and G. E. St J. Hardy (supervisors) and I. J. Colquhoun (industry supervisor), M. P. Dobrowolski provided expertise and guidance in microsatellite analysis, and M. C. Calver, provided assistance on experimental design and data analysis. This thesis outlines my individual role in each of these publications.

1. Chapter 2 published as follows:

Hüberli, D., Tommerup, I. C., Dobrowolski, M. P., Calver, M. C. & Hardy, G. E. St J. (2001) Phenotypic variation in a clonal lineage of two *Phytophthora cinnamomi* populations from Western Australia. *Mycological Research* **105**: 1053-1064.

2. Chapter 3 published as follows:

Hüberli, D., Tommerup, I. C. & Hardy, G. E. St J. (1997) The role of paragynous and amphigynous antheridia in sexual reproduction of *Phytophthora cinnamomi*. *Mycological Research* **101**: 1383-1388.

3. Chapter 4 published as follows:

Hüberli, D., Tommerup, I. C., Calver, M. C., Colquhoun, I. J. & Hardy, G. E. St J. (2002) Temperature and inoculation method influence disease phenotypes and mortality of *Eucalyptus marginata* clonal lines inoculated with *Phytophthora cinnamomi*. *Australasian Plant Pathology*, in press.

4. Chapter 5 submitted as follows:

Hüberli, D., Tommerup, I. C., Colquhoun, I. J. & Hardy, G. E. St J. Evaluation of resistance to *Phytophthora cinnamomi* in seed-grown trees and clonal lines of *Eucalyptus marginata* (jarrah) inoculated in lateral branches and roots. *Plant Pathology*.

5. Chapter 6 published as follows:

Hüberli, D., Tommerup, I. C. & Hardy, G. E. St J. (2000) False-negative isolations or absence of lesions may cause mis-diagnosis of diseased plants infected with *Phytophthora cinnamomi*. *Australasian Plant Pathology* **29**: 164-169.

List of conference proceedings

1. Oral Presentation

Hüberli, D. (1997) Environmental effects on *Phytophthora cinnamomi* disease expression in jarrah. *Phytophthora* and Other Plant Diseases of Natural Ecosystems Workshop, Eleventh Biennial Conference of the Australasian Plant Pathology Society. Perth, Western Australia.

2. Poster Presentation

Hüberli, D., Hardy, G. E. St J. & Tommerup, I. C. (1997) Morphological and physiological characteristics of two *Phytophthora cinnamomi* populations. In *Eleventh Biennial Conference of the Australasian Plant Pathology Society,* Abstract p. 307. Perth, Western Australia.

3. Poster presentation

Hüberli, D., Tommerup, I. C., Hardy, G. E. St J. & Colquhoun, I. J. (1998) Temperature changes resistance of clonal *Eucalyptus marginata* to *Phytophthora cinnamomi*. In *Seventh International Congress of Plant Pathology,* Abstract p. 2.5.4. Edinburgh, Scotland.

4. Oral presentation

Hüberli, D., Tommerup, I. C., Colquhoun, I. J. & Hardy, G. E. St J. (2001) Measuring resistance in jarrah, *Eucalyptus marginata*, to *Phytophthora cinnamomi*: What factors change disease expression? In *Second International IUFRO Meeting on Phytophthora in Forests and Natural Ecosystems*, p. 30. Albany, Western Australia.

Acknowledgements

I am grateful to many people and admit openly that without their help, advice and encouragement this project would not have been plausible. I appreciated their moral support, friendship, humour and valuable ideas. The following people deserve a mention for their efforts.

First and foremostly, I would like to thank my supervisors, Dr. Giles Hardy and Dr. Inez Tommerup. We have had some exciting and very animated discussions in dingy corridors, stuffy offices and crowded cars that I have found both stimulating and rewarding. Your enthusiasm, guidance, support and boundless ideas was much appreciated. I hope those long hours of proof reading were not the source of too many nightmares! Thank you also for those uplifting comments in times when I doubted that I would ever see the light at the end of the PhD tunnel.

I thank Alcoa World Alumina Australia for their inkind support in providing travel for field trips, access to field sites, keen workers and the jarrah clonal lines. The jarrah clonal lines were important, and without them, this thesis would not have been possible. In particular, I like to thank Dr. Ian Colquhoun who helped in so many ways during the project, and to all the Marrinup Nursery Personnel and Mattiske and Associates who helped out with some of the big plating-out sessions.

A big thank you to all the individual people who showed me new techniques and to all the volunteers who braved the storms, early rises and late finishes with me. Without this wonderful team and knowledge base my project would not have been possible. We have shared some great laughs in what might have been otherwise tedious and boring work. So a standing ovation is due to Janet Box, Libby Burgess, Dr. Mark Dobrowolski, Nola D'Souza, Dr. Khaled El-Tarabily, Dr. Meredith Fairbanks, Dr. Morag Glen, Angela Hollomes, Dr. Kay Howard, Anne Lucas, Julie Mahony, Dr. Emer

O’Gara, Ros Pilbeam, Kelli Sargent, Carla Wilkinson and Diane White. I extend my gratitude also to the Murdoch University staff from the School of Biology who put up with me for the last 5 years, and in particular, to the technical staff, Max Dawson, Ian McKernan and Kim Tan, who made all the glasshouse and cabinet trials run smoothly.

I am indebted to all the suggestions provided by the internal reviewers and co-authors on my manuscripts. They included Dr. Mike Calver, Dr. Ian Colquhoun, Dr. Mark Dobrowolski, Mark Dudzinski, Dr. Caroline Mohammed, Dr. Ken Old, and A. Prof. Jen McComb. I would also like to thank all the reviewers of the journal papers, who although anonymous, have provided valuable positive and negative criticisms.

Thank you to Dr. Meredith Fairbanks, Dr. Kay Howard and Ros Pilbeam that formed the PhD discussion group. I loved our little lunchtime discussions and the criticisms you scribbled on my drafts of chapters and papers. I found it rewarding to gain insight into your projects. Thank you to Tonia Jones who emailed me regularly with support and advice. I hope the five of us can continue our friendships despite that we are no longer fellow suffers in PhDs!

And last, but by no means least, I thank my parents, family and friends for their continued love, support and prayers. They have been a great stability factor and undoubtedly kept me sane through it all.