Persistence of *Phytophthora cinnamomi* in nature: Biotrophic growth and presence of stromata, oospores and chlamydosporoles in annual and herbaceous perennial plant species

by

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This thesis is submitted for the degree of Doctor of Philosophy

Murdoch University
Perth, Western Australia

November 2012
DECLARATION

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.

Michael Crone

November 2012
ACKNOWLEDGEMENTS

 ―Wie die Narren laufen wir bisjetzt umher; in den ersten drei Tagen können wir nichts bestimmen, da man immer einen Gegenstand wegwirft, um einen anderen zu ergreifen. Bonpland versichert, dass er von Sinnen kommen werde, wenn die Wunder nicht bald aufhören."  

Alexander von Humboldt 1799

―Like fools we were wondering around; in the first three days we could not determine anything, as we had to continuously throw away one object to be able to pick up another. Bonpland assured me, he will turn insane, if these wonders do not cease soon.‘

When the botanists Humboldt and Bonpland arrived in the New World in 1799 to explore the tropical rainforest of Venezuela, their breath was taken away by the experience. So was I when I arrived in the world of Phytophthora. Even though linked with plant diversity of the jarrah forest interacting with the pathogen - in difference to Humboldt and Bonpland - my task was ultimately bound to the study of only one species. However, on a microscopic scale within examined roots, there was a fascinating abundance of associated organisms I had never seen before. I soon realised as well that Phytophthora cinnamomi also evoked in me a sense of never ceasing wonders by displaying a plasticity greater than ever experienced when studying the morphology and life cycle of single species. Certainly, my fascination with this single organism was inevitably linked with the extent of damage it is able to cause to biodiversity, resulting in ambiguity of the overall experience.

Like Humboldt and Bonpland I had to let go of many other species I came across (such as members of Pythium) as well as interesting aspects within my topic to be able to manage my task.
I am appreciative that just like Bonpland, I was able to turn to more experienced people, namely my supervisors, Murdoch staff, various helpful members of the research community and fellow students.

Without tracing Humboldt’s and Bonpland’s circumstances, I am certain that they also received sponsorship to enable their research and that they were just as thankful as I am to my sponsors.

Like me, they would have also had a support network of family, friends and community, whose influences were certainly treasured by me during this exiting journey.

**Funding**

**Australian Research Council** (ARC): Australian Postgraduate Award Industry (APAI) scholarship, project LP0776740

**Alcoa Australia**

**Supervision**

**Murdoch University**

Principal supervisor: Prof. Giles E. St J. Hardy BAgric. PhD

Supervisor: Em. Prof. Jen A. McComb AM. BSc. PhD

Supervisor: Dr. Philip A. O’Brien BSc. PhD

**Alcoa Australia**

Supervisor: Dr. Ian J. Colquhoun

Supervisor: Dr. Vicki Stokes
Whilst my supervisors have contributed enormously and fit in almost every listed category below, I would like to give additional acknowledgements of the following influential people:

**Scientific advice and assistance:** Dr. Barbara Bowen, Dr. Treena Burgess, A/Prof. Elaine Davison, Prof. Bernie Dell, Dr. Chris Dunne, Dr. Bill Dunstan, Dr. Thomas Jung, Dr. Andrew Li, Dr. Trudy Paap, Dr. Peter Scott, Amy Smith, Dr. Mike Stukely, Gordon Thomson, Diane White, Dr. Nari Williams

**Practical help, various:** Sonia Aghighi, Papor Barua, Janet Box, Lily Ishaq, Murray Lindau, Dr. George Matusik, Dr. Wayne Reeve, Agnes Simamora, Amy Smith, Rebecca Swift

**Practical help, specified:**
- Assistance with the summer 2009/10 sampling: Alvaro Durán
- Assistance with the 30th August 2011 fieldtrip: Dang Nhu Quynh
- Checking for phosphite: Patsy Stasikowski
- DNA sequencing: Dr. Treena Burgess, Diane White
- FISH assay: Dr. Andrew Li
- Glasshouse, propagation area: Ian McKernan, Jose Minetto
- Statistics: Dr. Ian Wright
- Support with plant identification: Dr. Alex George, Dr. Penny Hollick
- Transfer of germinated oospores and hyphal tips: Dr. Endah Yulia

**Certainly thank you to the following teams:** Murdoch University: administration, health and counselling services, IT, librarians, science store; Alcoa: security, environmental crew, Derek – a volunteer in 2009 and unseen people pulling the strings in the background; DEC: Ian Freeman and crew assisting with the herbicide application

**How it all started:** Thank you to Pro-Vice Chancellor Prof. David Macey for his encouraging speech in April 2009 at my daughter’s former school, who won me over (instead of my daughter) to study at Murdoch.

**Shaping of my academic background:** Prof. Dr. Regine Classen-Bockhoff, Prof. Dr. Albrecht Siegert

**Private support:** My wife Mariany (a listing of her influence would fill a whole book), my children Marlene and Michael (who play pieces of the great baroque, classical, romantic and modern composers and inspired my studies) and my parents Helga and Siegfried (who nurtured my interest in nature from the time I could walk). Our family friends and supporters and various members of our informal Perth Fruit Tree Club, who provided welcoming distraction from the studies and some of the finest grafting materials.
ABSTRACT

*Phytophthora cinnamomi* is a broad host range pathogen responsible for the degradation of many natural and cultivated ecosystems worldwide. For most biomes, numerous woody plant species are susceptible hosts and are killed after infection. Two unresolved phenomena with regards to the life-cycle of *P. cinnamomi* were addressed in this study. Firstly, an investigation was conducted to determine how this poorly saprophytic pathogen persists in the long-term in the absence of susceptible hosts. Secondly, it is not known how *P. cinnamomi* survives the long hot and dry summers of Mediterranean environments. Infested black gravel landscapes within the *Eucalyptus marginata* (jarrah) forest of South-West Western Australia were suitable areas to address both knowledge gaps.

Native annual and herbaceous perennial plant species common to severely infested black gravel sites were screened as possible hosts by controlled inoculation experiments and by sampling plants from the natural environment. Secondly, it was tested how long *P. cinnamomi* can survive on naturally infested black gravel sites in the absence of any living plant material. Vegetation was removed by herbicides, and pathogen survival monitored over 28 months.

Based on the sampling of plants from the natural environment and from controlled inoculation experiments, it was evident that the majority of annual and herbaceous perennial plant species screened were infected by *P. cinnamomi* but most were asymptomatic hosts. These species are clearly a reason why *P. cinnamomi* can survive indefinitely on infested sites in the absence of susceptible woody host species. Transmission electron microscopic examination demonstrated that haustoria were produced in some annual and herbaceous perennial species and indicated for the first time that *P. cinnamomi* can colonise plants as a biotroph, without becoming necrotrophic. This is the first time haustoria have been shown to be definitively produced by *P. cinnamomi* in continuously asymptomatic plants. Survival propagules, in the
form of oospores, thick-walled chlamydospores and stromata were also observed in these naturally infected species. Mating tests showed that the oospores were selfed by the A2 mating type, and were produced in abundance with as many as 400 mm$^2$ of plant tissue. Selfed oospores have not been observed previously in naturally infected plants. Thick-walled chlamydospores were also produced in abundance, and both propagules types were shown to survive the environmentally adverse summer months and germinate when conditions were conducive the following autumn. Finally, stromata were observed in the majority of asymptomatic hosts, and have never been observed previously for P. cinnamomi. These stromata were shown to provide the nutrients for the prolific oospores numbers observed.

28 months after vegetation was killed by the herbicide treatments no P. cinnamomi was recovered from the soil, indicating that the pathogen can be eradicated from a site, when no living host material is present.

This study has made major and significant contributions to our understanding of the life cycle of P. cinnamomi under natural conditions. The ability of the pathogen to grow as a necrotroph or endophytic biotroph indicates that the pathogen has evolved a range of growth modes allowing it to persist in an ecosystem regardless of whether susceptible hosts are present or not. It is likely, that similar studies in other natural ecosystems in Australia or elsewhere will provide similar results. The ability to eradicate the pathogen in a relatively short time period through the removal of host material provides many opportunities for the control of this pathogen in managed and natural ecosystems.
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