Susceptibility of native plant species to *Phytophthora cinnamomi* and the spread of Phytophthora dieback in South Australia

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To my parents Mary Ong Lan Eng and Kueh Cheng Hai, for their unconditional love and patience and teaching me the value of education from an early age.

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Abstract

Phytophthora dieback, caused by *Phytophthora cinnamomi* Rands, affects a wide range of Australian native plants. In South Australia, the pathogen has affected large areas of native vegetation to threaten plant biodiversity. Lack of information on the disease in the local environment hampers management. The main objectives of this project were to: a) determine the rate of pathogen and disease spread in naturally infested native vegetation, b) assess the susceptibility of plant species native to South Australia to the disease and c) assess ability of antagonistic soil actinomycetes to protect susceptible species from Phytophthora dieback.

A confirmed *P. cinnamomi*-infested site, with gentle slope, at Mount Bold Reservoir Catchment Reserve in the Mount Lofty Ranges, was selected to assess pathogen and disease spread in native vegetation. The soil was loamy sand. The vegetation was open woodland dominated by *Eucalyptus obliqua* L'Hérit with an understorey dominated by *Xanthorrhoea semiplana* F. Muell, a highly susceptible species which was used as an indicator to assess disease spread. An area of 70 m x 70 m, extending from two disease fronts into the adjoining healthy vegetation, was marked into 10 m x 10 m quadrats. The number of dead and dying *X. semiplana* was counted and soil samples from each quadrat, collected every spring and autumn from 2008 to 2010, were baited for *P. cinnamomi* using cotyledons of *E. sieberi* L.A.S. Johnson. *P. cinnamomi* was regularly detected along the disease front. However, the pathogen did not spread across the slope into the adjoining healthy vegetation despite annual rainfall of 626 to 900 mm for three consecutive years (2008 to 2010). The slow spread of the pathogen was reflected in the small numbers of dead and dying *X. semiplana* observed in each quadrat at each assessment time. The limited spread of the pathogen may be due to unfavourable weather conditions. In winter (June to August), when the

precipitation was high (*ca*. 50% of the annual rainfall), soil temperature was generally too low (average temperature 9.3°C) for formation of sporangia. On the contrary when the temperature was warm (≥ 15°C) during spring (September to November) and autumn (March to May), the average soil water potential, ≤ -200 kPa, may have been too low for movement of zoospores. Further, sporadic distribution of *P. cinnamomi* and the patchiness of disease spread might have reflected the efficiency of the baiting technique.

Thirty-seven South Australian native plant species, including 15 threatened or locally endangered species, were assessed for susceptibility to Phytophthora dieback in a greenhouse from October 2009 to July 2010. Seedlings or cuttings were raised in potting mix for native species then transplanted to 15 cm-diameter pots filled with limed University of California mix or Bio Gro® (Bio Gro, South Australia). Plants were inoculated with *P. cinnamomi* via pine wood-inoculum plugs when up to 6 months old, maintained in moist conditions and monitored for disease symptoms for 3 to 6 months. Twenty-four of the 37 species studied, including 8 threatened species, were susceptible to the disease. Nine of these 24 species were ranked as highly susceptible. Another nine species were assessed as resistant. All species classed as susceptible were trees or shrubs while herbs were unaffected. In South Australia, where native vegetation has been extensively cleared or degraded, Phytophthora dieback represents an additional threat to the remnant native flora that might cause the extinction of native plant species, particularly the rare and endangered species, if not brought under control.

Actinomycetes were isolated from soil collected from roots of *Acacia pycnantha* Benth and young, healthy *X. semiplana* growing close to dead *X. semiplana* at the field site. Of 127 actinomycetes isolates selected, 78% inhibited *P. cinnamomi* in dual culture. Eight *Streptomyces* spp. which exhibited strong to weak antagonism, were

compared in the greenhouse for ability to protect 2-month old *E. sieberi*. One isolate delayed infection of *E. sieberi* by *P. cinnamomi*, although none prevented disease. The high soil moisture (≥ -10 kPa) required to induce disease was probably not conducive for the growth of the actinomycetes.

Knowledge generated in this project can be used in Phytophthora management to help prioritise threatened plant species in South Australia for protection, inform revegetation programs and to provide the basis for further research in the state.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Kueh Kiong Hook and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Publications and conference proceedings

The following publications and conference proceedings were produced during the PhD candidature:

Kueh KH, McKay SF, Facelli E, Facelli JM, Velzeboer RMA, Able AJ, Scott ES, 2012. Response of selected South Australian native plant species to *Phytophthora cinnamomi*. Manuscript published in Plant Pathology on 17 February 2012 (DOI: 10.1111/j.1365-3059.2012.02593.x) and presented in Chapter 4.

Kueh KH, Franco C, Able JA, Facelli J, Scott E, 2009. Screening for soil actinomycetes antagonistic to *Phytophthora cinnamomi* in a native ecosystem in South Australia. Abstract and oral presentation in Microbial Ecology Workshop: Concepts and techniques for disease control, a workshop of the 17th Australasian Plant Pathology Society Conference, held on the 27 September, at Newcastle Civic Centre, Newcastle, NSW, Australia.

McKay SF, Kueh KH, Able AJ, Velzeboer RMA, Facelli JM, Scott ES, 2009. Impact of *Phytophthora cinnamomi* on native vegetation in South Australia. Abstract in Proceedings of the 16th Australasian Plant Pathology Society Conference, held on 29 September to 1 October at New Civic Centre, Newcastle, NSW, Australia.

List of Abbreviations

ANOVA	analysis of variance
bp	base pair
CFU	colony forming unit
CGM	casein glycerol medium
CMA	cornmeal agar
CPSM	Centre for *Phytophthora* Science and Management
CTAB	hexadecyltrimethylammonium bromide
d	day
DNA	deoxyribonucleic acid
dNTP	2'-deoxyribonucleic acid
ETDA	ethylenediamine *tetra* acetic acid
h	hour
HA	humic acid-vitamin agar
ISP medium 2	International *Streptomyces* Project medium 2
LSD	least significant difference
min	minute
MS	mannitol-soy medium
OA	oatmeal agar
P_{10}ARPH	corn meal agar with antibiotics
PCNB	pentachloronitrobenzene
PCR	polymerase chain reaction
PDA	potato dextrose agar
rRNA	ribosomal ribonucleic acid

rDNA	ribosomal deoxyribonucleic acid
s	second
SARDI	South Australian Research and Development Institute
SDS	sodium dodecyl sulphate
UC	University of California
V8	V8 juice
WYE	water yeast extract medium
YCED	casamino-yeast extract-glucose agar