Development of an *in vitro* assay to screen *Agathis australis* (kauri) for resistance to *Phytophthora agathidicida*

A thesis presented in partial fulfilment of the requirements
for the degree of Master of Science

at
Massey University,
Manawatū, New Zealand

By
Echo Herewini

2017

Declaration

The work described in this thesis was undertaken while I was an enrolled student for the degree of Master of Science (Agriculture) at Massey University, Palmerston North. 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 To the best of my knowledge, all work performed by others, published or unpublished, has been duly acknowledged.

Echo Herewini

March 2017

Abstract

The iconic *Agathis australis* (kauri) of New Zealand, is under serious threat from kauri dieback disease caused by the soil-borne pathogen *Phytophthora agathidicida.* Infected kauri express symptoms of root and collar rot, bleeding resins at the base of the trunk, yellowing of foliage, canopy thinning, and tree mortality. *Phytophthora agathidicida* was first associated with kauri decline in 1972, where it was initially identified as *P. heveae* however, there was some uncertainty about its significance and taxonomy. The pathogen was officially identified as a new organism in 2008 and was called *Phytophthora* taxon Agathis until its formal description as *Phytophthora agathidicida* in 2015. This pathogen is easily vectored through root to root contact and mobile zoospores. Management and research has focused on mapping pathogen distribution, reducing spread, improving detection, *ex situ* conservation and clonal production using tissue culture techniques.

In order to gain better understanding of the disease epidemiology and to develop better breeding programmes, a reliable *in vitro* resistance screening assay is required. This research focused on the development of a screening assay using detached leaves from tissue culture material as a means of accelerating screening assays compared to the more labour-intensive root inoculation assays.

Foliar inoculations and assessment techniques were initially optimised on kauri leaves from tissue culture lines. The most successful inoculation method involved placing *P. agathidicida*-colonised agar plugs on wounded detached leaves. The assay was further tested on 2 year old kauri seedlings. Variation in susceptibility across kauri genotypes and leaf age, and variation in virulence among *P. agathidicida* isolates was observed. To further investigate the impact of leaf age on lesion extension, an assay was conducted on

detached leaves from six rooted kauri saplings over 5 years of age, across three leaf age groups with *P. agathidicida*, *P. multivora*, and *P. cinnamomi*. Variation in virulence among these *Phytophthora* species was observed. Leaf necrosis was most severe with young tissue and susceptibility tended to decrease with increasing leaf age. Preliminary studies with 50 kauri clones identified different levels of susceptibility and tolerance across the different genotypes to *P. agathidicida*.

The methods developed within this study have increased our understanding of the overall response of kauri to *P. agathidicida* foliar inoculations. This study demonstrated variation in the susceptability of kauri foliage to *Phytophthora* inoculation, although no complete resistance was observed. Further work is required to determine if there is a relationship between root and leaf responses which will help establish if *in vitro* genotypic variation can accurately predict natural genotypic variation seen within kauri forests.

Keywords: Kauri, *Agathis australis*, *Phytophthora agathidicida*, *Phytophthora*, Kauri dieback, Resistance, Susceptibility, Screening assay, Pathogenicity, Virulence, New Zealand taonga species.

Acknowledgements

Ko Taranaki te maunga

Ko Waitotara te awa

Ko Aotea te waka

Ko Ngā Rauru te iwi

Ko Ngāti Pourua te hapu

Ko Takirau te marae

Ko Rauru-Kītahi te tangata

Whakataka te hau ki te uru
Whakataka te hau ki te tonga
Kia mākinakina ki uta
Kia mātaratara ki tai
E hī ake ana te atakura
He tio, he huka, he hau hū
Tīhei mauri ora!

Cease the winds from the west,

Cease the winds from the south,

Let the breeze blow over the land,

Let the breeze blow over the ocean,

Let the red-tipped dawn come with a sharpened air,

A touch of frost, a promise of a glorious day.

I acknowledge my ancestors who I believe guided me in this path and gave me a tūrangawaewae or foundation to stand and position myself in this work. This entire journey has been a period of intense learning for me, not only in the scientific world, but

also on a personal level. I could have never reached the height or explored the depths of this thesis without the help and support of many people.

To my supervisor, Peter Scott, it has been an honour and an absolute pleasure to be able to work with kauri under your supervision at Scion. Words cannot describe how appreciative I am for your constant support, guidance, motivation, and the encouragement you gave me throughout this entire experience. Without you, this thesis would not have been possible. Your enthusiasm, passion for conservation and spirit of adventure has been inspiring. You have instilled in me the qualities of a good scientist and you have provided me with a wealth of knowledge. As I battled my way through this thesis and the challenges of motherhood, your understanding and compassion as both a scientist and a father meant the world to me. Thank you, Peter, for believing in me.

To my lovely supervisor, Rosie Bradshaw, thank you for giving me this amazing opportunity. From start to finish, you have been there every step of the way. Your warm, loving nature and compassion towards juggling student life and motherhood has meant a lot to me. I can't thank you enough for being so approachable, understanding, patient, and kind towards me and my family. You were only a phone call away whenever I needed to talk. Thank you for always proof reading my chapters, even when you were half way across the world. I appreciate everything you have done for me and I hope you know how much you are valued.

To my supervisor, Nari Williams, I have looked up to you as a role model ever since you took me under your wing at Scion. For the past three years, you have been kind, generous, and supportive of me. Your knowledge and expertise working with *Phytophthora* pathogens has been invaluable. Thank you for your endless help with my research and for allowing me to grow as a research scientist. Your patience,

understanding, and uplifting comments were always appreciated. You welcomed me into your home with open arms and kindly looked after Aria just so I could write. You are a kind person with a heart of gold.

Ki a koe Phillip Wilcox, tōku kaiwhakahaere. Tēna koe mō tō tautāwhi, mō ōu kōrero e pāana ki te ao Māori me tēnei kaupapa. He tangata tino mōhio koe, he tangata tino mīharo koe. He hoa koe mō āke tonu āke. Ngā mihi māhana ki a koe.

To my co-supervisor, Terry Stewart, I would not have started down this path if it wasn't for your enjoyable lectures which sparked my interest in the plant world. Thank you for all your time, help, and input towards this thesis and for putting my name forward for this project.

I would like to acknowledge all the staff at Scion, Rotorua. Thank you to the Scion tissue culture team for dedicating your time to grow, care, and nurture the precious kauri seedlings used within my experiments. My dear friend Keiko Gough, thank you for your constant help and support. You will forever be a true friend.

I owe a mountain of gratitude to the Scion Forest Protection team including Pam Taylor, Catherine Banham, Rita Tetenburg, Sarah Orton, Shannon Hunter, Judy Gardner, Debra Bly, Andrew Pugh, and Tomoko Pearson. Thank you all for your hard labour and involvement with planning and conducting my experiments. The set of skills and knowledge I learnt from everyone will always be appreciated. To my friend, Rose O'Brien Gardner, thank you for consistently being on top of my administration work and for simply being a friend. We always had the best conversations in your office.

I would like to extend a big thank you to the Scion nursery team for accommodating table space requirements for my experiments and for all the advice and

assistance I received. To the Scion herbarium team, thank you for generously letting me use your equipment and room. I owe a big thank you to the talented statisticians Martin Bader and Zhao Xing for their help with all the statistical analyses conducted in this thesis. Thank you to the staff of Massey University for being on top of my administration work.

I would like to especially thank Stan Bellgard, Ian Horner, and Nick Waipara for giving me the opportunity to gain experience in plant pathology and for sharing your knowledge on kauri dieback and *Phytophthora* pathogens. It has been a real pleasure to know you all and I look forward to crossing paths in the future.

I would like to thank everyone who assists in the conservation of kauri, including the Kauri dieback management team. Ngā mihi mahana ki te Tāngata whenua rōpu, Te Rōroa iwi, me te Mana whenua o Waipoua ngāhere.

A big thank you to the Bio-Protection Research Centre for providing funding for this research and to the Māori Education Trust for awarding me a scholarship grant.

Lastly, I could have never completed this thesis without the love and support of my close friends, and family. I am grateful for my Aunty Tracey and Nana Joan for being a constant source of motivation. Thank you both for your wise counsel and sympathetic ear. To Mum and Dad, thank you for believing in me and supporting me in every way possible. My beloved partner Liam, thanks for being my rock through it all. To my precious daughter, Aria, you are an absolute blessing in my life. This is all for you.

"Ehara taku toa, he toa takitahi, he toa takitini"

"Success is not the work of one, but the work of many"

Table of Contents

List of Tables

List of Figures