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**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  
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## **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 To the best of my knowledge, all work performed by others, published or unpublished, has been duly acknowledged.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 susceptibility 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.

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Ko Waitotara te awa

Ko Aotea te waka

Ko Ngā Rauru te iwi

Ko Ngāti Pourua te hapu

Ko Takirau te marae

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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.

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**“Ehara taku toa, he toa takitahi, he toa takitini”**

*“Success is not the work of one, but the work of many”*

## Table of Contents

<b>Declaration .....</b>	<b>i</b>
<b>Abstract.....</b>	<b>ii</b>
<b>Acknowledgements.....</b>	<b>iv</b>
<b>Table of contents .....</b>	<b>viii</b>
<b>List of Tables .....</b>	<b>xi</b>
<b>List of Figures.....</b>	<b>xii</b>
<b>Chapter 1. Introduction.....</b>	<b>1</b>
1.1 Overview.....	1
1.2 Kauri ( <i>Agathis australis</i> ).....	3
1.2.1 Kauri history .....	3
1.2.2 Present distribution .....	5
1.2.3 Botanical features .....	6
1.2.4 Cultural significance.....	9
1.3 <i>Phytophthora</i> diseases worldwide.....	10
1.4 Notable <i>Phytophthora</i> diseases .....	12
1.4.1 <i>Phytophthora cinnamomi</i> .....	12
1.4.2 <i>Phytophthora ramorum</i> .....	13
1.4.3 <i>Phytophthora lateralis</i> .....	14
1.5 Notable <i>Phytophthora</i> tree diseases in New Zealand .....	15
1.6 <i>Phytophthora agathidicida</i> .....	17
1.6.1 Impact of <i>Phytophthora agathidicida</i> on kauri .....	18
1.6.2 <i>Phytophthora agathidicida</i> isolation .....	18
1.6.3 Origin of <i>Phytophthora agathidicida</i> .....	19
1.6.4 Life-cycle and spread.....	20
1.7 Control measures.....	22
1.7.1 Kauri dieback management - quarantine .....	22
1.7.2 Kauri dieback management - resistance screening.....	23
1.7.3 Kauri dieback management - chemical control .....	24
1.8 Foliar resistance screening .....	25
1.8.1 Koch's postulates.....	26

1.9 Aims and objectives .....	27
<b>Chapter 2. Comparing foliar inoculation methods on detached <i>Agathis australis</i> (kauri) leaves with <i>Phytophthora agathidicida</i> .....</b>	<b>28</b>
2.1 Introduction .....	28
2.2 Materials and methods .....	31
2.2.1 Inoculation methods.....	37
2.2.2 Lesion assessments .....	40
2.2.3 Statistical analysis.....	41
2.3 Results .....	41
2.4 Discussion .....	45
2.5 Conclusion .....	48
<b>Chapter 3. Detached foliar inoculations on <i>Agathis australis</i> (kauri) saplings over 5 years of age with <i>Phytophthora agathidicida</i> .....</b>	<b>49</b>
3.1 Introduction .....	49
3.2 Materials and methods .....	51
3.2.1 Inoculation method .....	53
3.2.2 Lesion assessments .....	55
3.2.3 Statistical analysis.....	57
3.3 Results .....	57
3.4 Discussion .....	64
3.5 Conclusion .....	68
<b>Chapter 4. <i>Agathis australis</i> (kauri) foliar inoculations with three <i>Phytophthora</i> species to determine the effect of leaf age on lesion extension. ....</b>	<b>69</b>
4.1 Introduction .....	69
4.2 Materials and methods .....	70
4.2.1 Inoculation method .....	73
4.2.2 Lesion assessments .....	73
4.2.3 Statistical analysis.....	73
4.3 Results .....	74
4.4 Discussion .....	78
4.5 Conclusion .....	81

<b>Chapter 5. General Discussion.....</b>	<b>83</b>
5.1 Overview .....	83
5.2 Future research .....	85
5.2.1 Moving forward with Māori .....	88
5.3 Final conclusion .....	90
<b>Appendix A. Foliar inoculations on 50 <i>Agathis australis</i> (kauri) clones with <i>Phytophthora agathidicida</i>.....</b>	<b>93</b>
<b>Appendix B. Preparation of culture media.....</b>	<b>95</b>
<b>Appendix C. Preparation for <i>Phytophthora</i> zoospore production.....</b>	<b>98</b>
<b>References .....</b>	<b>99</b>

## List of Tables

Table 2.1	Experimental design for experiment 1 .....	32
Table 2.2	Isolate details for all <i>Phytophthora</i> species used within this study .....	35
Table 3.1	A summary of mean foliar assessments for the three <i>Phytophthora agathidicida</i> isolates used in experiment 2 .....	62
Table 3.2	A summary of mean foliar lesion assessments for the six <i>Agathis australis</i> (kauri) saplings used in experiment 2 .....	63
Table 4.1	A summary of F and P values using the combined means of six <i>Phytophthora</i> isolates plus a control, for three lesion assessments .....	77
Table 4.2	A summary of data using the combined mean lesion assessments of six <i>Phytophthora isolates</i> and a control .....	77
Table 4.3	A summary data using the combined mean lesion assessments of three leaf age groups .....	78

## List of Figures

Figure 1.1	Map of the northern North Island of New Zealand showing where <i>Phytophthora agathidicida</i> has been detected (red) and undetected (yellow) in kauri stands .....	2
Figure 1.2	Giant kauri logs that were felled by Europeans in 1920.....	4
Figure 1.3	Tāne-Mahuta – Lord of the Forest.....	7
Figure 1.4	Kauri male cone (left) and female cone (right).....	8
Figure 1.5	Field expression of red needle cast disease on <i>Pinus radiata</i> trees caused by <i>Phytophthora pluvialis</i> .....	16
Figure 1.6	Kauri stand showing canopy thinning caused by <i>Phytophthora agathidicida</i> .....	17
Figure 1.7	A <i>Phytophthora agathidicida</i> sporangium containing biflagellate zoospores .....	21
Figure 2.1	One jar containing ramets (shoots) from a single kauri clone .....	33
Figure 2.2	Close-up image of three kauri ramets (shoots) from one single kauri clone .....	33
Figure 2.3	Average lesion length (mm) on <i>Agathis australis</i> (kauri) leaves .....	42
Figure 2.4	Average area infected (on a scale from 0-5) on <i>Agathis australis</i> (kauri) leaves .....	43
Figure 2.5	The possibility of recovering the pathogen (%) from <i>Agathis australis</i> (kauri) leaves.....	44
Figure 2.6	The best-fit correlation curve for the possibility of recovering the pathogen (%) from <i>Agathis australis</i> (kauri) leaves in relation lesion length (mm) or area infected (mm).....	44
Figure 3.1	A stand of <i>Agathis australis</i> (kauri) saplings over 5 years of age .....	52
Figure 3.2	A scanned image showing the randomised experimental layout for one replicate of leaves with necrotic leaf tissue, six days post-inoculation .....	54
Figure 3.3	Five 5 mm leaf tissue segments that were sliced from <i>Agathis australis</i> (kauri) leaves to measure infection length (mm) and to confirm the recovery of the pathogen from the leaf inoculation point.....	56
Figure 3.4	Average lesion length (mm) on detached leaves for six <i>Agathis australis</i> (kauri) saplings over 5 years of age .....	58

Figure 3.5	Average infection length (mm) on detached leaves from six <i>Agathis australis</i> (kauri) saplings over 5 years of age.....	59
Figure 3.6	Average asymptomatic infection length (mm) on detached leaves from six <i>Agathis australis</i> (kauri) saplings over 5 years of age.....	60
Figure 3.7	Average lesion area (mm <sup>2</sup> ) on detached leaves from six <i>Agathis australis</i> (kauri) saplings over 5 years of age.....	61
Figure 3.8	Average lesion area (%) on detached leaves from six <i>Agathis australis</i> (kauri) saplings over 5 years of age.....	61
Figure 3.9	Correlation and R <sup>2</sup> values for lesion measurement parameters .....	64
Figure 4.1	Experimental layout for experiment 3 .....	72
Figure 4.2	Average lesion length (mm) on young, middle-age, and old <i>Agathis australis</i> (kauri) leaves .....	74
Figure 4.3	Average lesion area in mm <sup>2</sup> on young, middle-age, and old <i>Agathis australis</i> (kauri) leaves .....	75
Figure 4.4	Average lesion area as a percentage of leaf area on young, middle-age, and old <i>Agathis australis</i> (kauri) leaves .....	75
Figure 4.5	The possibility of recovering the pathogen (%) from young, middle-age, and old <i>Agathis australis</i> (kauri) leaf tissue .....	76
Figure A1	Preliminary results showing average lesion length (mm) for a subset of <i>Agathis australis</i> (kauri) leaves from 50 different clonal lines infected with <i>Phytophthora agathidicida</i> , isolate 3118.....	93