Healthy trees, healthy future
Enabling technologies to combat *Phytophthora* diseases

HTHF Mid Program Symposium

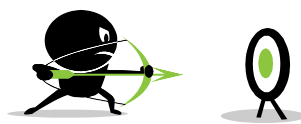
forestgrowers levy trust inc Funding Support Provided by the Forest Growers Levy Trust

FOREST OWNERS ASSOCIATION

KEEP KAURI STANDING

In the beginning…..

Hitting the target

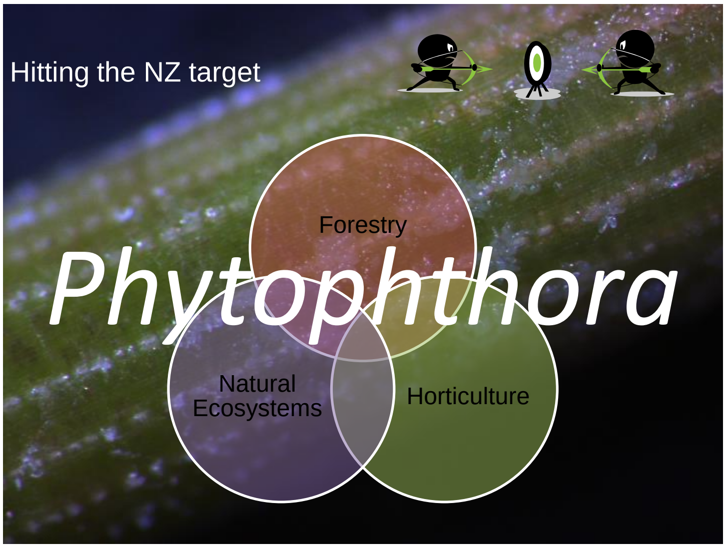


MBIE Research Question:

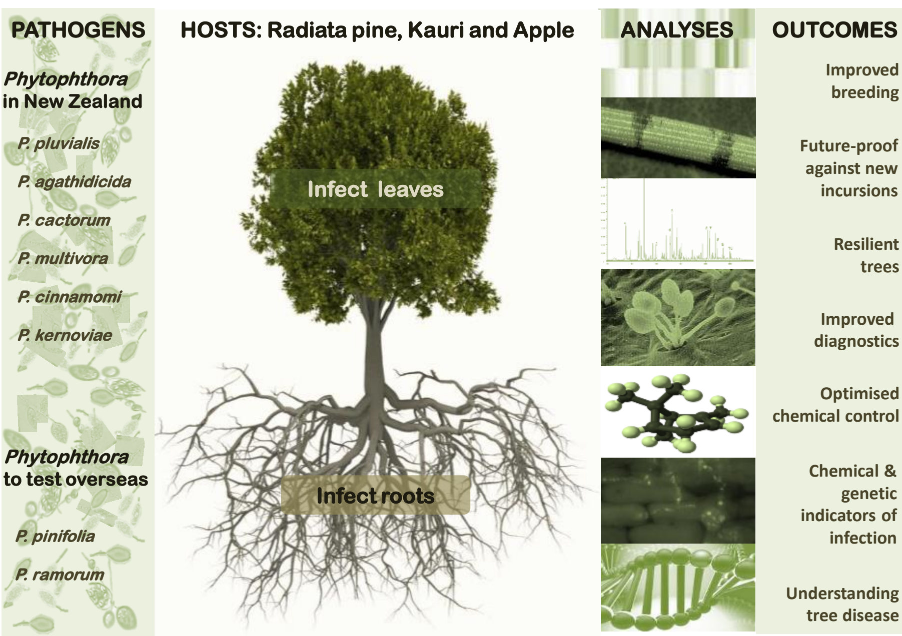
- How can we protect or mitigate risks to New Zealand's industries or improve access to export markets?

MBIE Funding Mechanism - Enabling Technologies

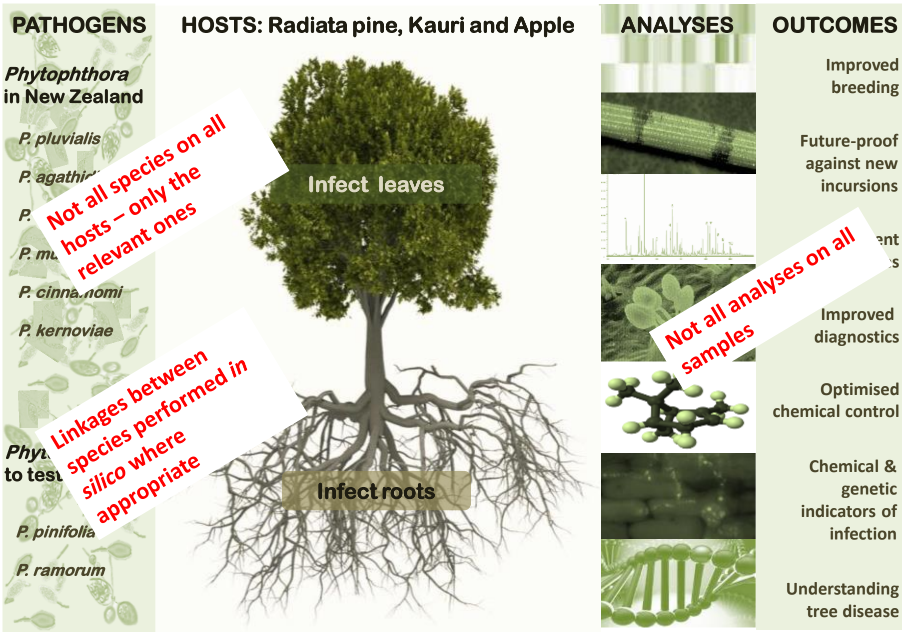
- Development of underpinning or generic enabling technologies that have potential application across multiple users, markets, or sectors.
- Strong common technology theme with multiple applications



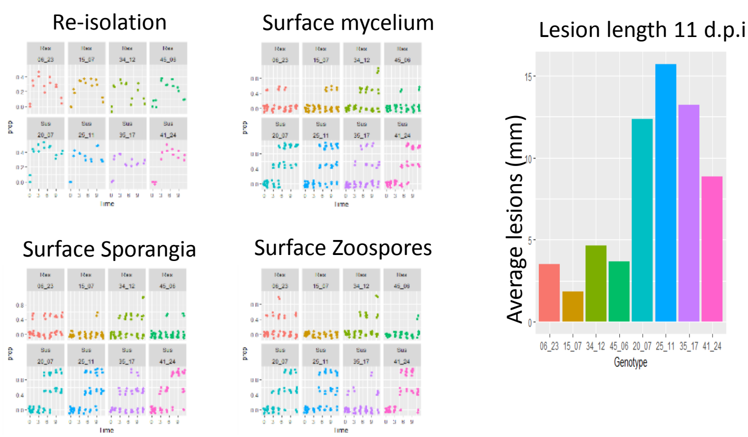
The HTHF Enabling Technology Platform



The HTHF Enabling Technology Platform

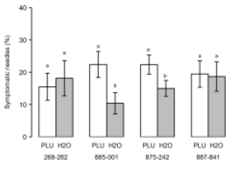


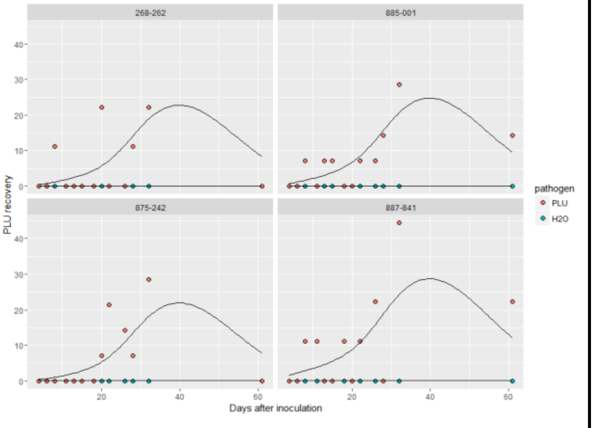
P. pluvialis time series pathology



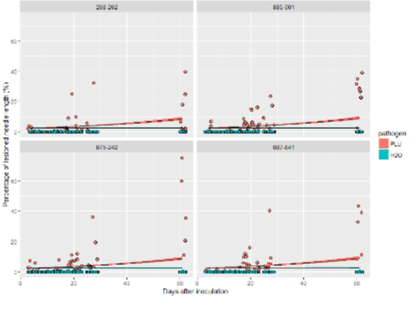
Physiological impacts of *P. pluvialis*

Mireia Gómez Gallego





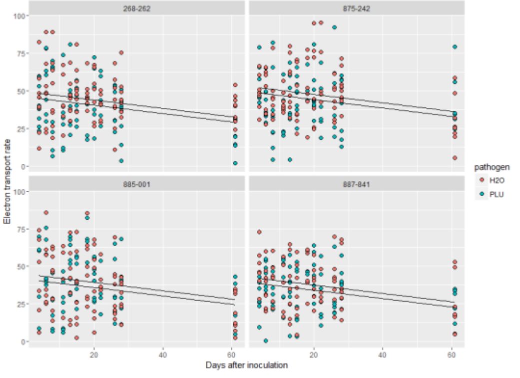
Results – Infection cycle



Pale-olive lesions

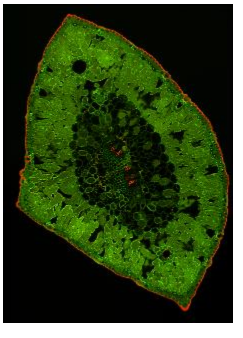
- Infection by *P. pluvialis* in fog chamber was successful
- *Phytophthora pluvialis* infection confirmed but limited number of lesions observed.
- Some characteristic RNC lesions produced
- Presence of other pathogens triggered by fog conditions in both rooms
- More necrotic needles in inoculated plants
- Peak of *P. pluvialis* isolates 40 days after inoculation
- Plants likely to have been physiologically stressed by ongoing wet conditions used to encourage infection.

Results – Photosynthetic activity



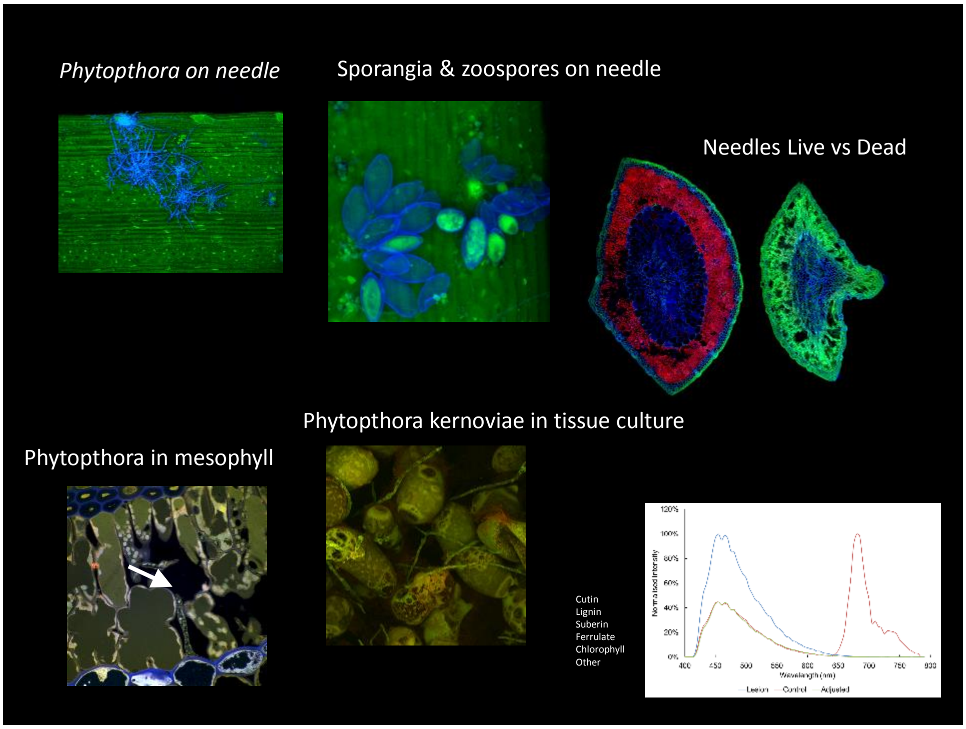
- General decrease in the electron transport rate in both control and inoculated plants
- No differences in photosynthetic activity between inoculated and control plants
- Difference between genotypes regardless inoculation with *P. pluvialis*
- Possibly due to presence of other pathogens or physiological impacts on the plants (seasonal shift, ongoing wet period)

Histology *Pinus radiata / P. pluvialis / P. kernoviae* histology - Lloyd Donaldson



Aims:

- Understand the infection process
- Understand the host response to pathogen



Summary of RNC histology observations

- Infection involves stomata but no cuticle penetration
- No cell penetration in needles but some evidence in tissue culture (cell walls are different !)
- Endodermal barrier restricts infection to mesophyll
- No obvious cell wall modification post infection
- Major change in autofluorescence during needle death, healthy, chlorotic, necrotic stages

GC-MS Results (Scion) – all time points

- Gentiobiose 8TMS Results
- Gluconic Acid 6TMS_01 Results
- Glucopyranose 5TMS Results
- Glucopyranose -H2O 4TMS Results
- Glucose 1,6-anhydro Results
- Glucose, 1,6-anhydro-beta Results
- Glutamic Acid 3TMS Results
- Glyceric Acid 3TMS Results
- Glycerol 3 phosphate 4TMS Results
- Glycine 3TMS Results
- Glycolic Acid TMS Results
- hexadecanoic Acid TMS Results
- Hydroquinone 2TMS Results
- Hydroquinone bis(trimethylsilyl) ether Results
- Inositol Myo 6TMS Results
- Isopimaric Acid TMS Results
- Linoleic Acid TMS Results
- Linolenic Acid TMS Results
- Lumichrome 2MeOX Results
- Lysine 3 TMS Results
- Saccharic Acid 6TMS Results
- Salicin 5TMS Results
- Serine 3TMS Results
- Shikimic Acid 4TMS Results
- Sitosterolo beta 1TMS Results
- Sorbitol 13C ISTD Results
- Sorbose MeOX 5TMS Results
- Stearic acid TMS Results
- Sterol TMS Results
- Succinic Acid 2TMS Results
- Sucrose 8TMS Results
- Taxifolin 5TMS Results
- Tetradecanoic Acid 1 TMS Results
- Tetradecanoin Acid 1 TMS Results
- Threitol 4TMS Results
- Threonic Acid 4TMS_01 Results
- Tocopherol alpha 1TMS Results
- Lyxose 1MeOX 4TMS Results
- Malic Acid 3TMS Results
- Mannose 1MeOX 5TMS Results
- Mannose-6-phosphate 6TMS Results
- monopalmitoyl glycerol 2TMS Results
- myo inositol 1 phosphate 7 TMS Results
- Neoabietic Acid 1TMS Results
- Ononitol 5TMS Results
- Oxalic Acid 2TMS Results
- Pentamethyldisiloxybutane Results
- Phenanthrene CAS 26549-04-2 Results
- Phenol TMS Results
- Phosphoric Acid 3TMS Results
- Phytol 1TMS Results
- Pimaric Acid TMS Results
- Pinitol 5TMS Results
- Putrescine 4TMS Results
- Pyridine 2-hydroxy 1TMS Results
- Quinic Acid 5TMS Results
- Raffinose 11TMS Results
- Ribonic Acid 5TMS Results
- 1,2,3, propanetriol 1 4 hydro Results
- 1-Pyrroline-3-hydroxy-5-carbox Results
- 2(3H)-Furanone, dihydro-3,4-bis[(trimethylsilyl)oxy]-, trans- Results
- 2-Keto-l-gluconic acid, penta(O-trimethylsilyl)- Results
- 2-propenoic Acid 2TMS Results
- 4-Ketoglucose 4TMS Results
- 6-deoxy-Mannopyranose 4TMS Results
- Abietic Acid TMS Results
- Acetic Acid 2TMS Results
- alpha-D-Galactopyranosyl-1,4 Results
- Ampelopsin 6TMS Results
- Anthracene DB Results
- ArabinoHexos2ulose 4TMS Results
- Ascorbic acid 4TMS Results
- Aspartic Acid 3TMS Results
- Benzoic acid, 4-hydroxy 2TMS Results
- Butanoic Acid 4 Amino 3TMS Results
- Campsterol 1TMS Results
- Catechin 5TMS Results
- Cellobiose 8TMS Results
- Cinnamic acid 4 hydroxy Results
- Citric Acid 4TMS Results
- Cytosine 2TMS Results
- Dehydroabietic Acid TMS Results
- Dehydroascorbic Acid dimer 2MeOX Results
- D-Limonene Results
- Docosahexaenoic Acid TMS - cis 4,7,10,13,16,19 Results
- Dodecanoic Acid 1TMS Results
- Epigallocatechin 6TMS Results
- Erythro-Pentonic acid 3TMS Results
- Erythrose 1MeOX 3TMS Results
- Ethanolamine 3TMS Results
- Farnesol 1TMS Results
- ferulic acid 2TMS Results
- Fructose 1MeOX 5TMS Results
- Fucitol 5TMS Results
- Galactaric Acid 6TMS Results
- Galactinol 9TMS Results
- Galactopyranoside Results
- Galactose 5TMS Results
- Gallic Acid TMS Results

GC-MS Results – comparison between resistant needles at the time of infection to those 11 days later identified the chemicals that may be associated with resistance or susceptibility.

MCF method

Name	06-23 (t0)	06-23 (H2O t11)	06-23 (PLUt11)	diff	%diff(H2O)	41-24 (T0)	41-24 H20(t11)	41-24 (PLUt11)	diff	%diff(H2O)
Benzoic acid	0.37	0.50	5.62	5.11	1015.0%	0.53	1.32	0.92	-0.40	-30.4%
trans-Cinnamic acid	0.12	0.12	0.27	0.15	131.7%	0.07	0.09	0.07	-0.02	-20.4%
4-Hydroxyphenylacetic acid	0.01	0.01	0.03	0.02	126.4%	0.02	0.25	0.04	-0.21	-84.2%
2,2'-Dimethyl-6,6'-dinitroazobenzene	0.55	0.74	0.60	-0.14	-18.8%	0.33	0.10	0.29	0.19	195.5%

TMS method

Name	06-23 (t0)	06-23 (H2O t11)	06-23 (PLUt11)	diff	%diff(H2O)	41-24 (T0)	41-24 H20(t11)	41-24 (PLUt11)	diff	%diff(H2O)
Acetin	0.50	0.17	0.02	-0.15	-87.2%	0.17	0.02	0.09	0.07	374.8%
1,2,3-Butanetriol	0.49	0.16	0.01	-0.15	-92.1%	0.28	0.02	0.05	0.03	140.0%
Benzoic Acid	0.00	0.10	0.15	0.05	54.8%	0.00	0.02	0.04	0.02	147.5%
Hydroquinone	1.07	0.25	0.01	-0.25	-97.9%	0.35	0.01	0.02	0.01	159.2%
D-(-)-Fructofuranose	0.98	0.28	1.28	1.00	351.1%	0.39	0.13	0.09	-0.04	-28.6%
Shikimic acid	14.30	14.69	29.41	14.72	100.2%	14.38	31.36	29.47	-1.89	-6.0%
Cyanuric acid	0.00	0.09	2.40	2.31	2652.2%	0.20	3.60	0.93	-2.68	-74.2%
Quininic acid	1.53	0.88	1.78	0.90	101.7%	0.77	2.92	0.98	-1.94	-66.3%
Myristic acid	3.62	1.17	6.69	5.52	471.3%	1.70	7.37	1.70	-5.68	-77.0%
Methyl .alpha.-D-ribofuranoside	0.60	0.50	1.47	0.97	195.5%	0.45	0.35	0.21	-0.14	-39.5%
Glucopyranose	1.27	0.80	2.32	1.52	189.2%	0.97	0.94	0.79	-0.14	-15.2%
D-Allopyranose	0.49	0.56	4.04	3.48	620.6%	0.25	0.21	0.09	-0.12	-56.6%
2-Hydroxycyclohexane-1-carboxylic acid	0.58	0.21	0.06	-0.15	-71.1%	0.57	0.09	0.54	0.45	482.5%
2-Mono-isobutyrin	1.84	0.58	0.16	-0.41	-71.8%	1.13	0.43	1.53	1.10	253.3%
Cyclohexene	0.23	0.08	0.05	-0.03	-36.6%	0.24	0.11	0.46	0.35	315.1%
Uridine	1.49	0.48	0.27	-0.21	-44.2%	1.17	0.54	2.01	1.46	270.0%
Methyl(methyl 4-O-methyl-.alpha.-d-mannopyranoside)uronate	0.38	2.01	0.01	-2.00	-99.3%	1.04	0.04	0.47	0.43	1000.4%
23-acetoxy-3,6,9,12,15,18,21-heptaoxatricosan-1-oate	0.20	0.59	0.01	-0.57	-98.0%	0.20	0.04	0.47	0.43	1000.4%
Quinic acid	0.23	7.61	0.05	-7.56	-99.3%	1.30	0.08	0.75	0.67	853.8%
Ethanone, 1-cyclopentyl-	0.48	6.18	0.04	-6.14	-99.3%	0.90	0.06	0.57	0.51	859.9%
D-(-)-Ribopyranose	0.77	0.46	0.40	-0.06	-13.4%	0.28	0.10	0.11	0.01	7.5%
Meso-inositol	0.00	0.04	0.39	0.36	1020.5%	0.00	0.02	0.00	-0.02	-80.2%

Yellow – associated with resistant genotype
Red – associated with susceptible genotype

Outcomes (GC-MS)

- GC-MS has proved a useful tool for exploring the volatile compounds found in the extractive from pine needles.
- Attempting to statistically explore all the controls and time points in a single go yielded no useful results and may actively mask information.
- While a vital tool for understanding targeted volatile compounds GC-MS has been discontinued as a screening tool as LC-MS is proving to be considerably more informative.

The 21,000 chemical entities separated from the LC-MS have ben identified, where possible against the Plant Metabolic Mass Spec Database

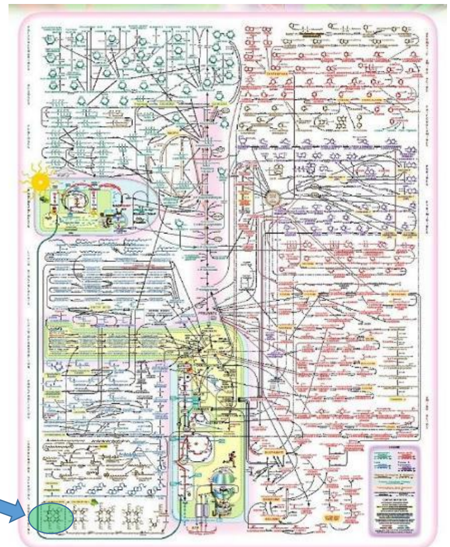
- 350 plant species including:
  - Pinus banksiana (jack pine)
  - Pinus contorta
  - Pinus densiflora (tanyosho pine, Japanese red pine)
  - Pinus strobus
  - Pinus sylvestris (Scotch pine)
  - Pinus taeda (loblolly pine)

- 1,213 Pathways
- 140,581 Enzymes
- 6,051 Reactions
- 5,128 Compounds

PMN
Plant Metabolic Network

Analysis between the water and *P. pluvialis* infected samples 7 days post inoculation identified:

- 4288 compounds detected
- ~100 compounds tentatively assigned
- Now associating these to metabolic pathways and putative pathogen responses



For example…

Example compounds (tentative)

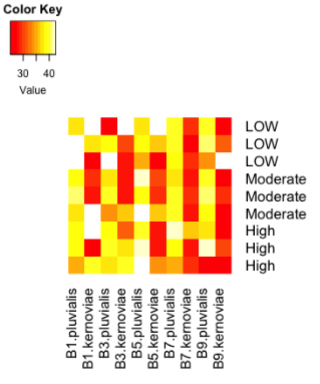
- Acifluorfen
  - Inhibitor for *protoporphyrinogen oxidase*
- Protoporphyrinogen oxidase
  - Protoporphyrinogen oxidase is responsible for the seventh step in biosynthesis of protoporphyrin IX. This porphyrin is the precursor to chlorophyll in plants. The enzyme catalyzes the dehydrogenation (removal of hydrogen atoms) of protoporphyrinogen IX (the product of the sixth step in the production of heme) to form protoporphyrin IX. One additional enzyme must modify protoporphyrin IX before it becomes heme. **Inhibition of this enzyme is a strategy used in certain herbicides.**

COULD THIS BE A COMPOUND PRODUCED BY THE PATHOGEN???

Outcomes (LC-MS)

- LC-MS provides a vast number of compounds.
- My utilising cloud computing large datasets can be processed in a working day.
- LC-MS is the tool that will provide the detail needed to identify specific compounds that have an infection related response.
- Use of online databases will allow the placement of identified compounds in the Metabolomic Pathways.
- This information will be feed back into the NMR databases to reduce 'false positives' encountered during NMR screening.

2.2.1. Industry Relevant Clones (Field trial)



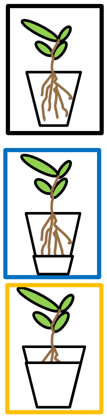
- Both *P. pluvialis* and *P. kernoviae* isolated and identified in IR clones field trial.
- A total of 45 samples will be RNA sequenced (9 trees from 5 blocks)
- Determine the abundance of both *P. pluvialis* and *P. kernoviae* in each sample using qPCR
- Interested in *P. pluvialis* and *P. kernoviae* gene expression when present in vary abandance in needles

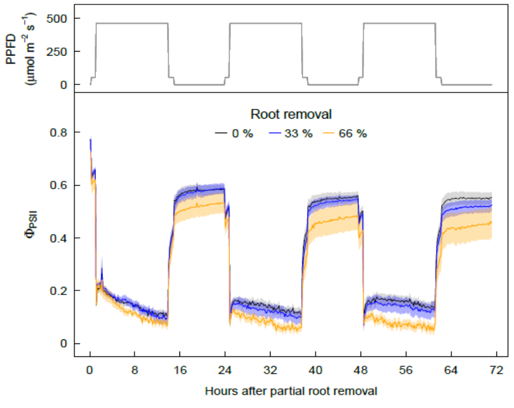
Assessment of kauri root health using Chlorophyll fluorescence

No Root Removal

33% Root Removal

66% Root Removal





Impact of *P. agathidicida* root infection on kauri Chlorophyll fluorescence

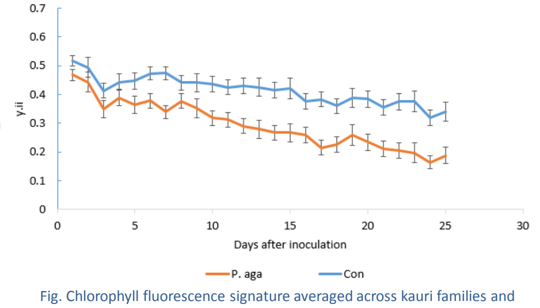
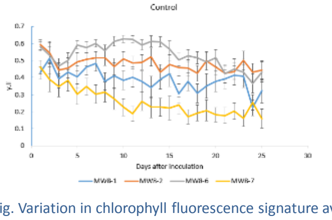


Fig. Chlorophyll fluorescence signature averaged across kauri families and assessment periods (three assessments per day).



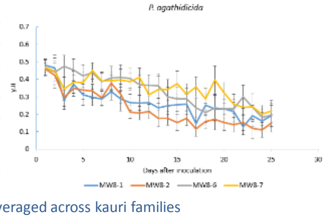


Fig. Variation in chlorophyll fluorescence signature averaged across kauri families

Healthy trees, healthy future
Enabling technologies to combat *Phytophthora* diseases

Publication Pipeline

forestgrowers levy trust inc — Funding Support Provided by the Forest Growers Levy Trust
FOREST OWNERS ASSOCIATION
KEEP KAURI STANDING

Publication pipeline

Paper	Programme	Authors	Progress/Notes	Draft	Submitted	In review	Published
Visualisation of early infection by Phytophthora "taxon Agathis" in the roots of 2-year old kauri Agathis australis plants	HTHF	Bellgard, SE, Williams, SE, Probst, C, Padamsee, M Lebel, T	100%	☺	☺	☺	2016 Forest Pathology
Genome sequences of six Phytophthora species associated with forests in New Zealand	HTHF	Studholme, DJ McDougal, RL Sambles, C Hansen, E Hardy, G Grant, M Ganley, RJ Williams, NM	100%	☺	☺	☺	2016 Genomic Data
Chemical control of two Phytophthora species infecting the canopy of Monterey pine (Pinus radiata)	NDS - aligned	Carol A Rolando*, Nari M Williams, Margaret A Dick, Judy Gardner, Martin K-F Bader, Nari M Williams	100%	☺	☺	☺	2017 Forest Pathology
The emerging science of linked plant-fungal invasions	HTHF-BPRC (aligned)	Dickie, Ian A., Bufford, Jennifer L., Cobb, Richard, Desprez-Loustau, Marie-Laure, Grelet, Gwen, Hulme, Philip E., Klironomos, John, Makiola, Andreas, Nuñez, Martin A., Pringle, Anne, Thrall, Peter H., Tourtellot, Samuel G., Waller, Lauren, Williams, Nari M.,	95%	☺	☺	☺	To be published as a New Phytologist Tansley Review In 2017.
Book Chapter: Red needle cast on Pinus radiata. Compendium of Forest Diseases	HTHF-NDS	Williams N., Hansen Ed.	100%	☺	☺	☺	In press
Kauri dieback. In the compendium of Conifer and Christmas tree diseases APS.	HTHF	Bellgard, Weir, Pennycook	100%	☺	☺	☺	*In Press*
Phytophthora agathidicida.	HTHF	Bellgard, Weir, Pennycook, Ho and Waipara	100%	☺	☺	☺	Forest Phytophthora. 6(1). Do I 10.5399/osufp. 1.3748.
Phytophthora pluvialis studies on Douglas fir require Swiss needle cast suppression	HTHF:NDS	Mireia Gomez-Gallego1, 2; Martin Karl-Friedrich Bader2; Sebastian Leuzinger1; Peter Scott.2; Nari Williams2	95%	☺	☺	☺	Accepted Feb 2017

Publication pipeline cont…..

Paper	Programme	Authors	Progress/Notes	Draft	Submitted	In review	Published
Genetic diversity of Phytophthora pluvialis in New Zealand and USA	HTHF	S. Brar, R.L. McDougal, N.M. Williams , R.C. Hamelin, N. Feau, N.J. Grunwald, J.M. LeBoldus, J.F. Tabima and R.E. Bradshaw	65%	March 2017			
Genome sequences of Phytophthora kernoviae isolated from South America reveal relationships to isolates from Europe and New Zealand	HTHF	D.J. Studholme, R.L. McDougal, E. Sanfuentes, Hill, Rowena, C. Sambles, M. Grant, N.M. Williams	50%	30 June			
Pluvialis epidemiology and infection paper	FOA-NDS	BG, PS, NW	90%	15 Jan			
Breeding	HTHF	NG, NW ++	80%	15 Feb			
Wild Solanum species in Sweden and New Zealand as reservoirs of Phytophthora pathogens	HTHF-FD aligned	Ramesh Vetukuri, Laura Masini, Rebecca McDougal, Preeti Panda, Levine de Zinger, Maja Brus, Erik Andreasson, Åsa Lankinen, Laura Grenville-Briggs.	70%				
The relationship between sporulation potential and host susceptibility	HTHF	NW, JG, LD	50 %	30 June			
Transcriptomics RAD X PLU & KER (Effectors)	HTHF	RM, PP, LM, NW		30 June			
Tissue Culture Callus	HTHF	NW, KG, CH	40%	15 March			
Metabolomics	HTHF	SH, LR, II, NW	30%	31 March			
Biological Variation & Optimisation	HTHF	RM, LM, NG, NW, ET		TBD			
P aff cactorum Description	HTHF/Path	PS, RM, NW, Ian Horner, Sarah Addison, BG	80 %	June 30			
Conveyance Review	HTHF	PS, MB, TB, GH, NW		TBD			
Diagnostics Review	HTHF/Path	RV, RM, LG, EA, PS, NW		TBD			
HTHF Position Paper	HTHF	NW, RM, Richard Hamelin, Nik Grunwald, David Studholm	15 %	TBD			
RAD x PLU physiology paper	HTHF	MG MB, PS, NW SL					

Key non-research projects

Project	Key People	Progress	Complete
Data Management	Melissa Evans, Dean Christie, Alison Chick, Ralph Gomers, Wayne Schou	Ongoing	
2. Growing Phytophthora free kauri • **Nursery protocols and procedures** • **Seed collection and propagation**	Vicky Hodder, Peter Harington, Colin Faulds, Nari Williams, Bob Shula, Heidi Dungey	On track	☺
3. Room 8 and 7 Re-fit	Marcel van Leeuwen, Keith Walker, Lisa Stanbra, Nari Williams	Currently operational. Further room testing and optimisation to be scheduled between inoculation runs.	☺
4. Kauri Breeding and Maori Partnerships **- 2017 Whakapapa lines of kauri** • **TWR** • **Te Roroa** • **Identifying a site for *P. agathidicida* field testing**	Nari Williams, Vicky Hodder, Stan Bellgard, Waitangi Wood	To commence 2017	☺