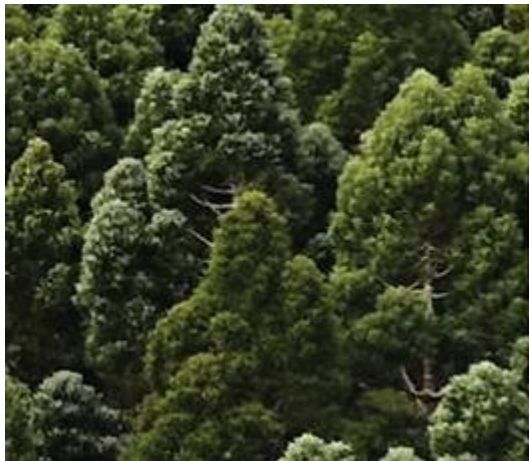


Testing kauri for tolerance to *Phytophthora agathidicida*

Stan Bellgard, Chantal Probst, Rose Williams, Chris Winks
and Nari Williams



Landcare Research
Manaaki Whenua

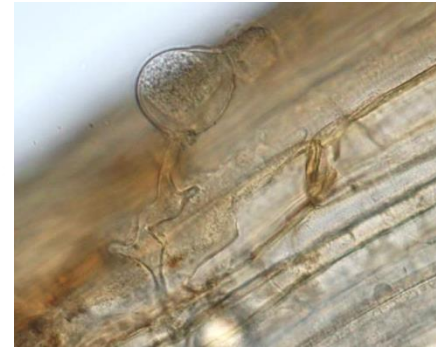
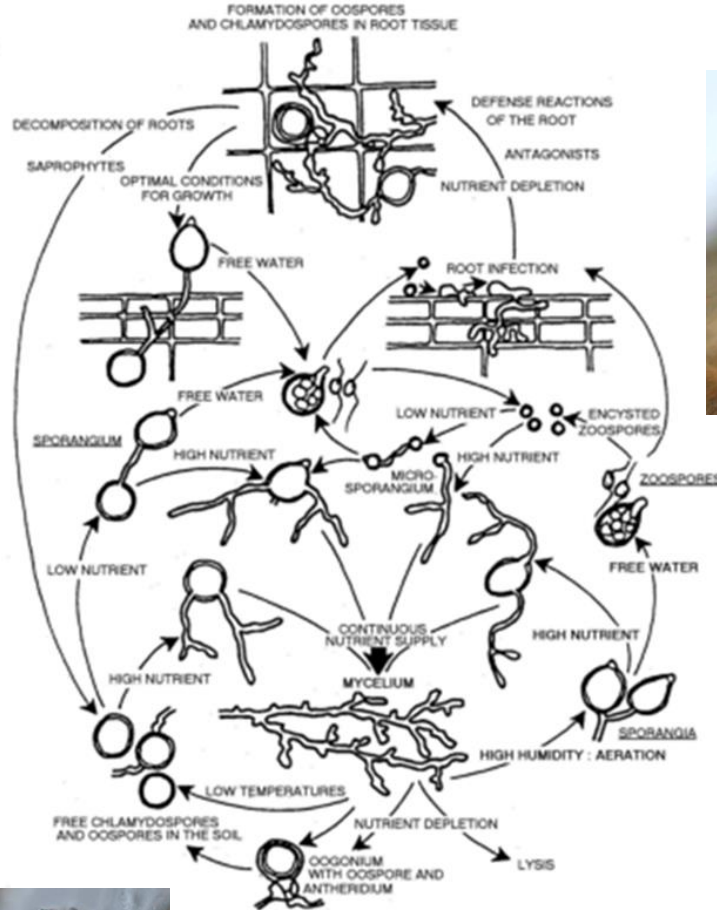
Kauri dieback

- First described in 1970s
- Dieback reported in 2006
- Symptoms include
 - foliage yellowing
 - canopy thinning
 - bleeding lesions on lower trunk
 - root rot
 - dysfunction of conducting vessels
 - crown decline
 - tree death



Causal agent *Phytophthora agathidicida*

Figure 1: Life cycle of soilborne *Phytophthora* species (adapted from Ribeiro 1978)

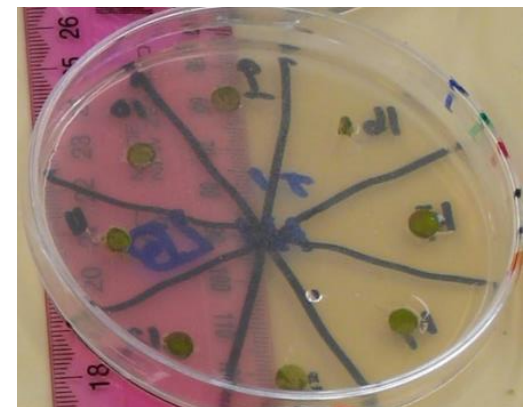
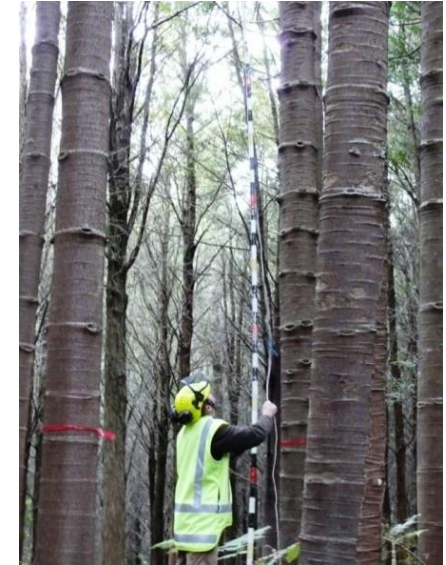


Testing for tolerance of kauri

- Search for kauri tolerance to *P. agathidicida* in forest remnants as part of long term management plan for kauri
- Sampling with Tangata Whenua across ecological range
- Critical that assessment protocols are non destructive
- Need to be able to trace back to parent tree and collect seeds

Ex-situ assays: detached shoots

- Shoots wounded at mid-point and inoculated with/ without *P. agathidicida* on millet seed
- Incubated for 21 days and lesion extension measured
- Pieces of shoot plated onto agar from set distances from p.o.i.



Shoot infection

Trees	Proportion shoot length infected (%)	SE (%)	
2-1	100b	0	←
2-2	50	17	
2-3	50	17	
2-4	0a	0	←
2-5	60	16	
2-6	50	17	
2-7	67b	33	←
5-1	0a	0	←
5-2	40	24	←
5-3	0a	0	←
5-4	40	24	
5-5	40	0	
5-6	0a	0	←
5-7	43	20	←
5-8	75b	25	←
5-9	0	0	←

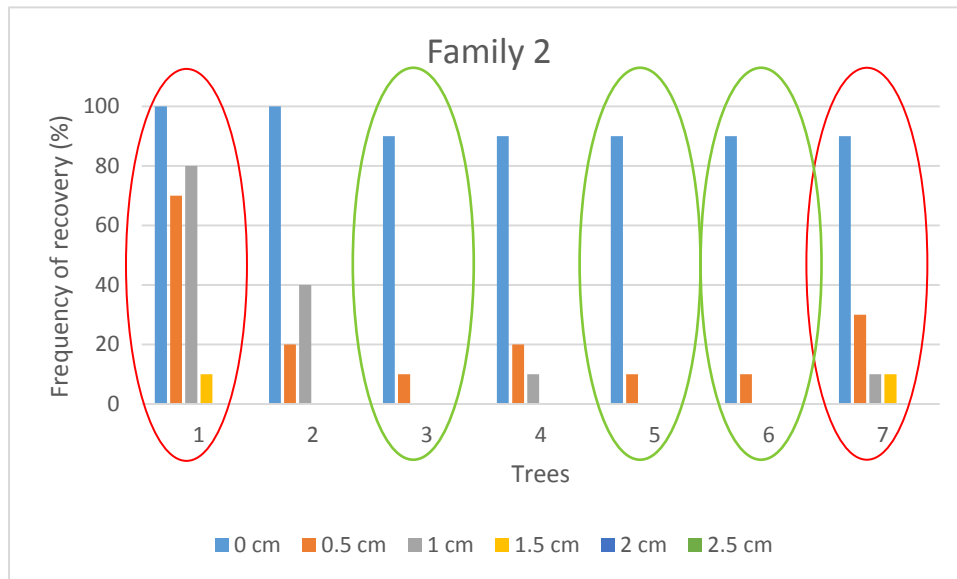
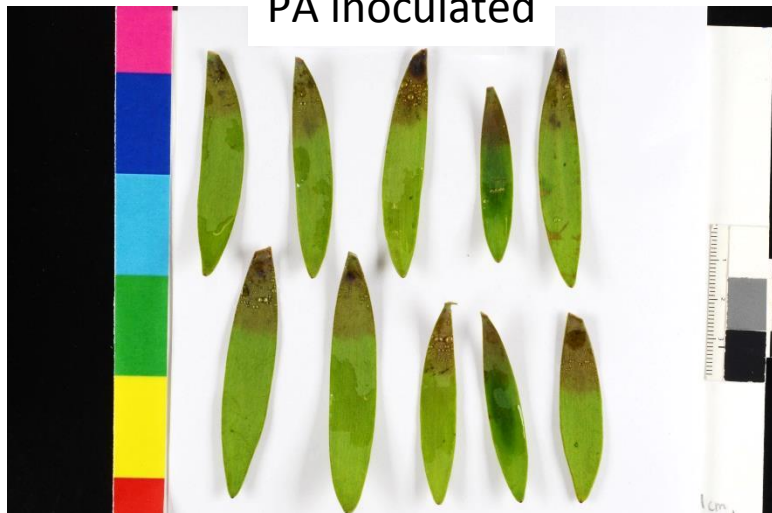
- Within family and between family differential responses
- Trees; 2-1, 2-7 and 5-8 were the most “susceptible”
- Trees; 2-4, 5-1, 5-3, 5-6 were the most “resistant”

Ex-situ assays: detached leaves

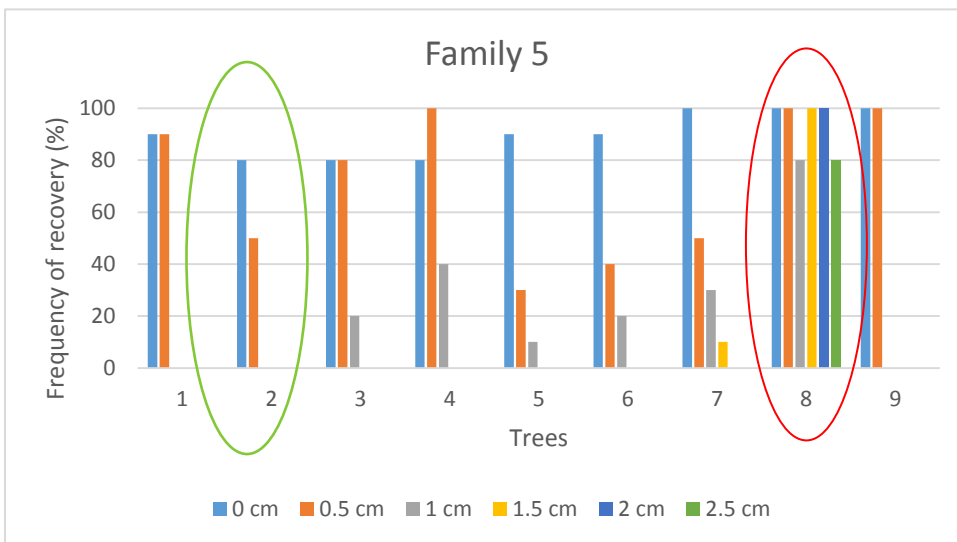
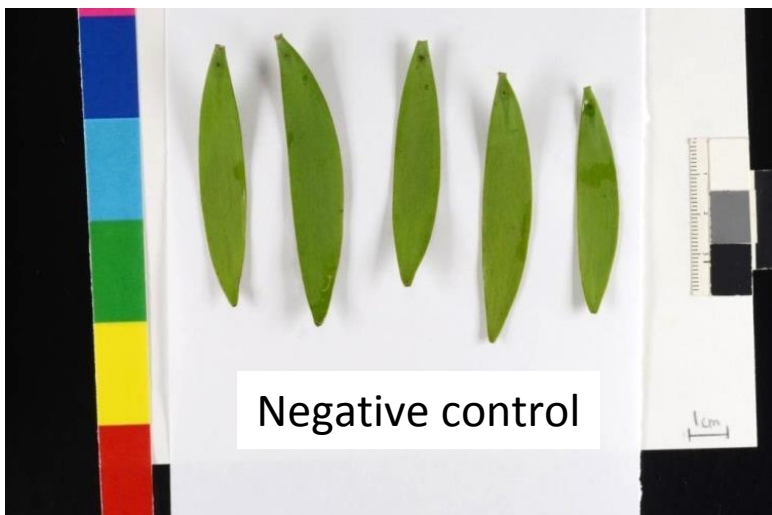
- Leaves wounded at the base with a needle
- Inoculated with agar plug with or without *P. agathidicida*
- Leaves placed in square boxes with moist filter paper
- Assessment after 7 days:
 - pictures taken of leaves for image analysis
 - leaf pieces taken at 0, 5, 10, 15, 20 and 25 mm from p.o.i and grown onto agar

Detached leaf infection

PA inoculated



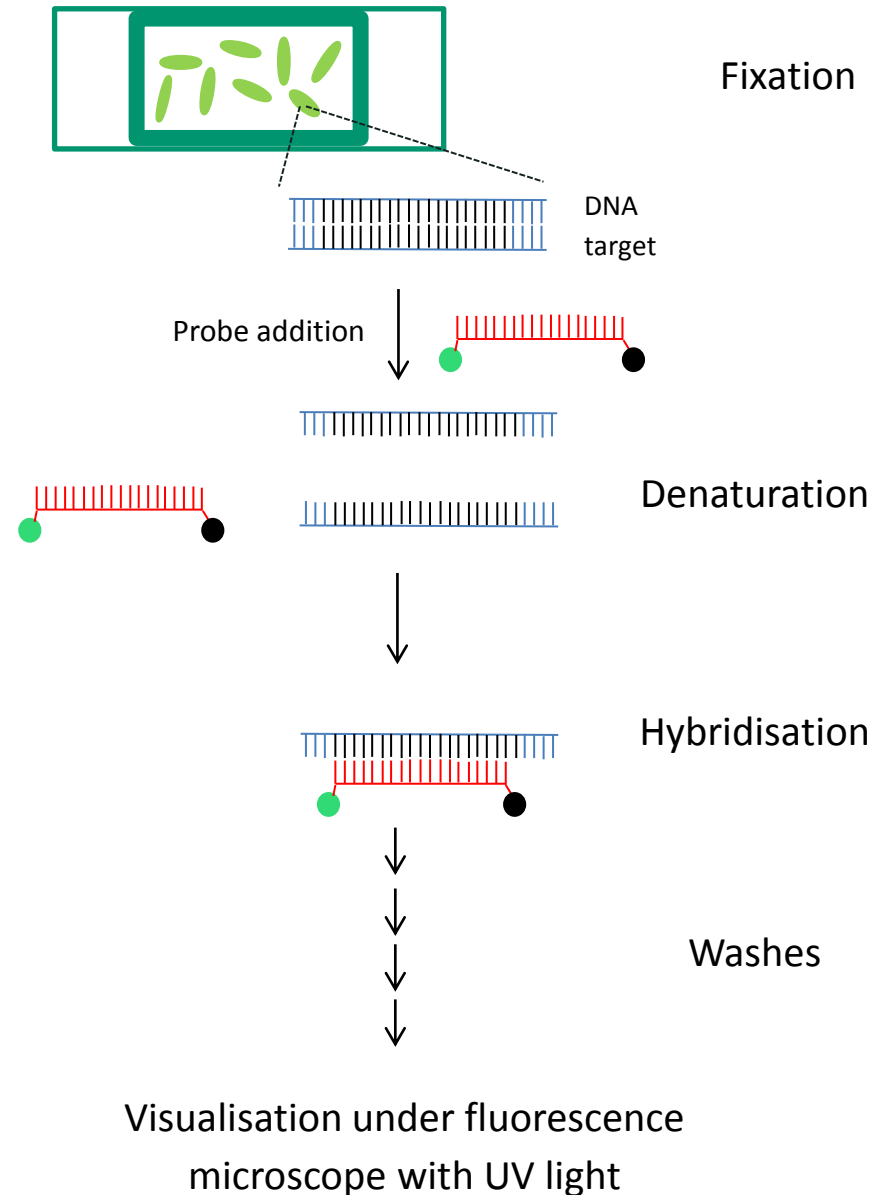
Negative control



Leaf histopathology

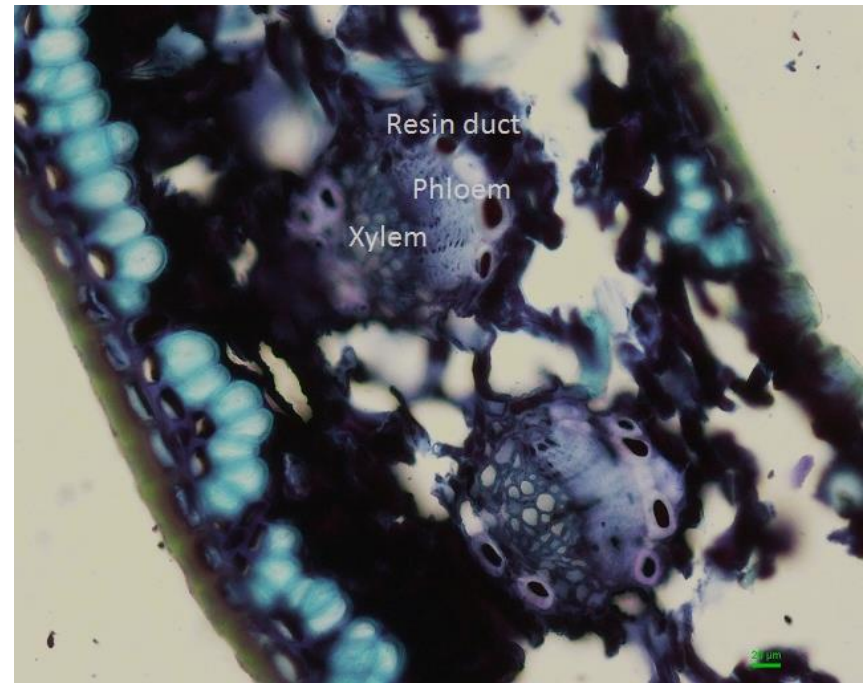
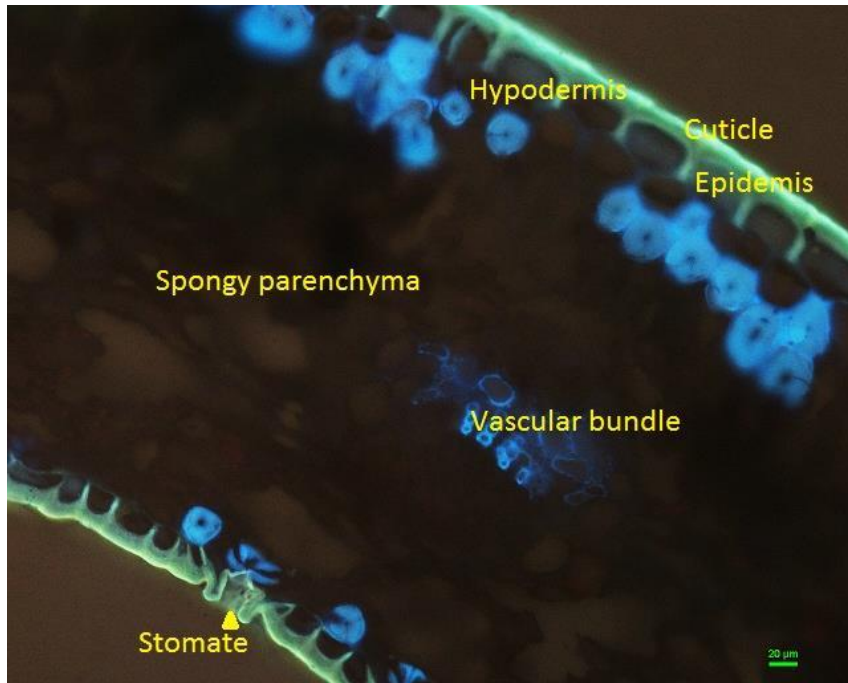
Fluorescence *in situ* hybridisation assay (FISH assay)

- Based upon protocol developed by Bellgard *et al.* 2016
- Sections before, across and post-lesion boundary for leaf samples from susceptible/resistant trees

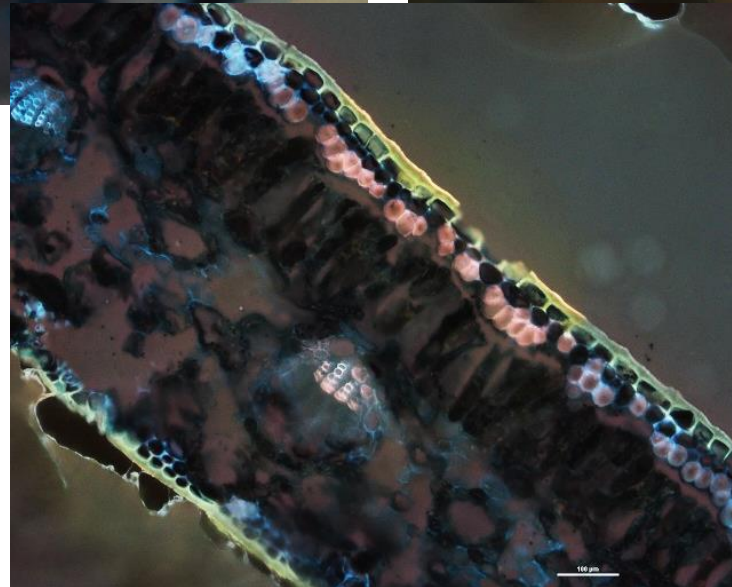
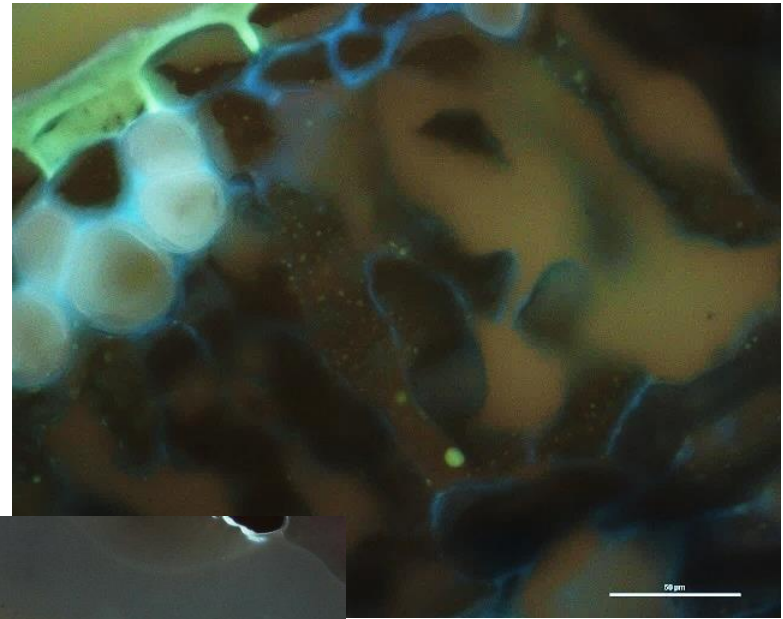
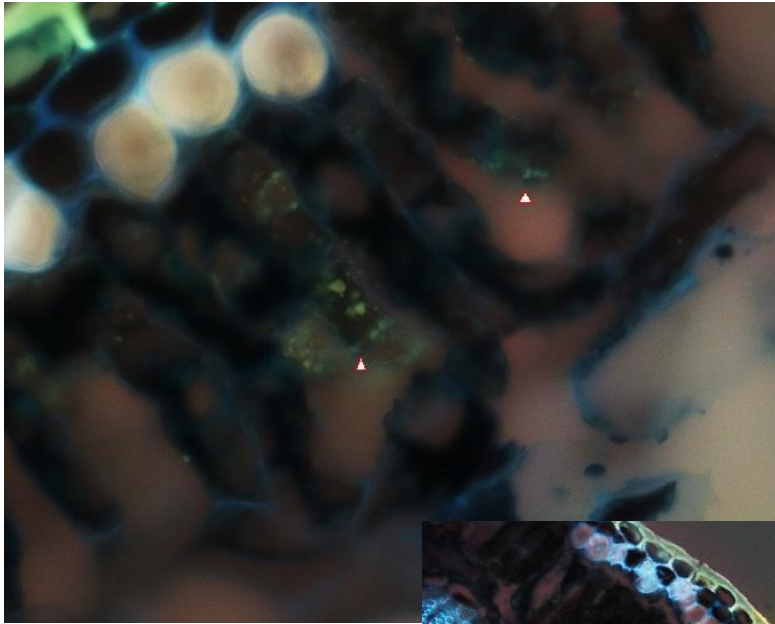


Observations

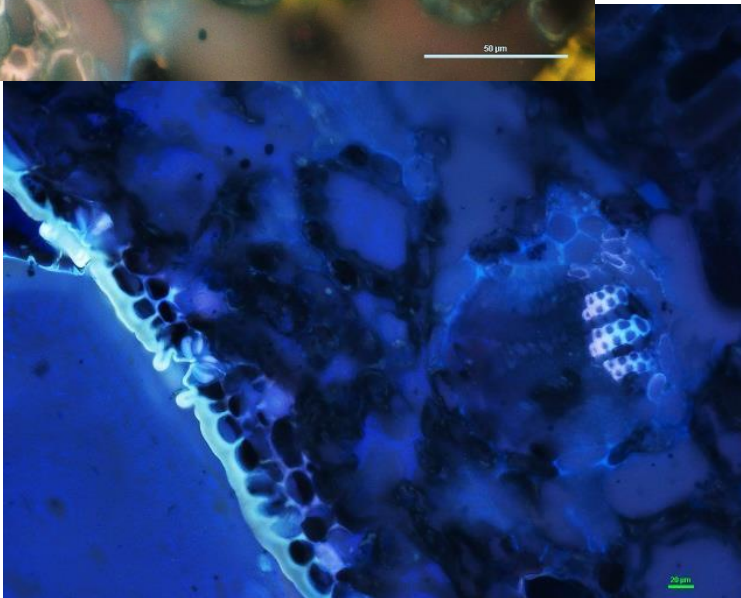
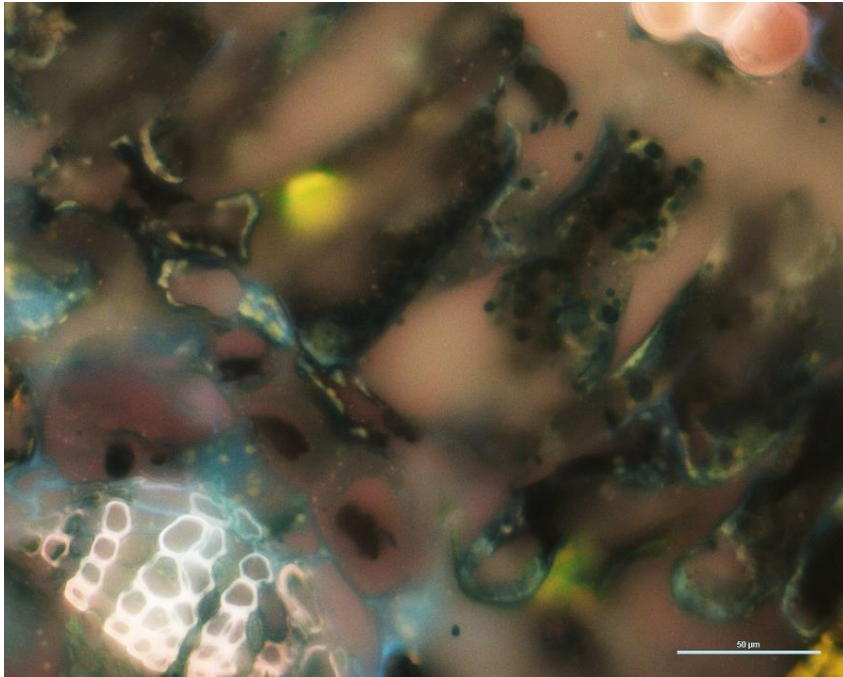
Un-infected negative controls



Inoculated - lesion-margin



Inoculated - lesion



- Deposition of “granular” (tannin) material in palisade parenchyma
- Thickening of palisade parenchyma
- Hyphal-thickening within and around spongy parenchyma

Establishing whakapapa lines

- First cohort of seed collected in partnership with Mana Whenua in 2016
- Seed from up to 10 lines per Mana Whenua group
- Family lines established

Screening pipeline:

Feb-April: seed collection



April-June: seed drying and maturation



June-July: seed germination



July-September: seedlings pricked out



September: first screening assay



Screening whakapapa lines

Screening assay testing the rate of infection

Each seedling assigned a barcode

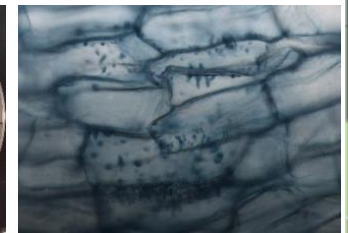
Root inoculation

Inspecting the root health after infection

Sections plated and stored for microscopy

Plating tells us infection progress

Microscopy shows damage caused at the cellular level



Plants to be screened again at

6 Months

18 months

Field trials



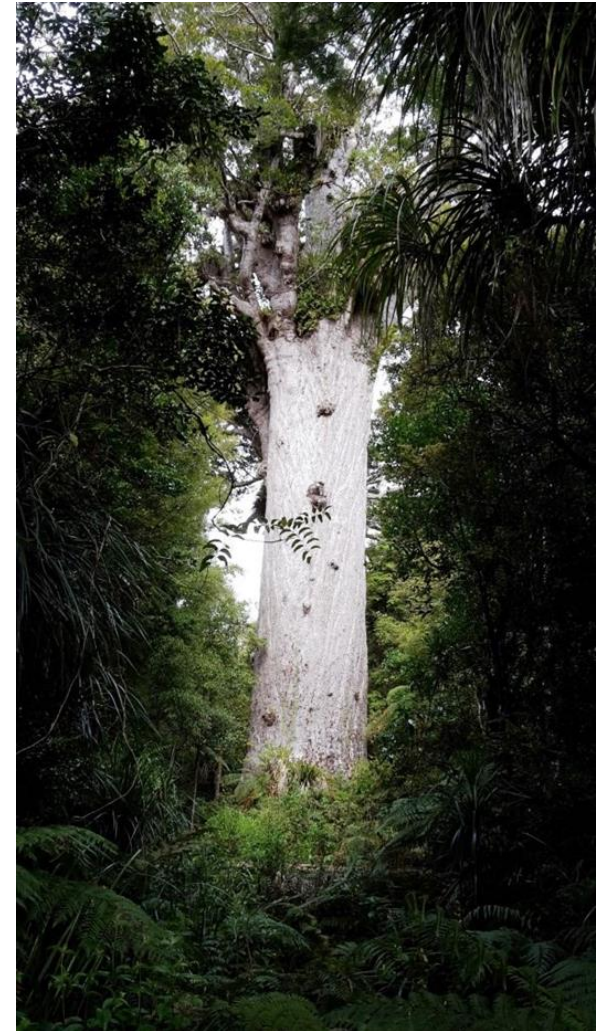
Samples removed for gene expression /biochemical analyses

Achievements towards finding tolerance in kauri

- Establishment of Mana Whenua partnerships
- Development of whakapapa lines of kauri from seeds across kauri range with Mana Whenua
- Observation of variations in phenotypic responses of kauri to infection by *P. agathidicida* in leaves and shoots – need to correlate with root inoculations
- Progressive curation of plant material for parallel transcriptomic and biochemical analysis, which will provide information regarding the genetic and chemical signals related to the different responses observed

Achievements towards finding tolerance in kauri

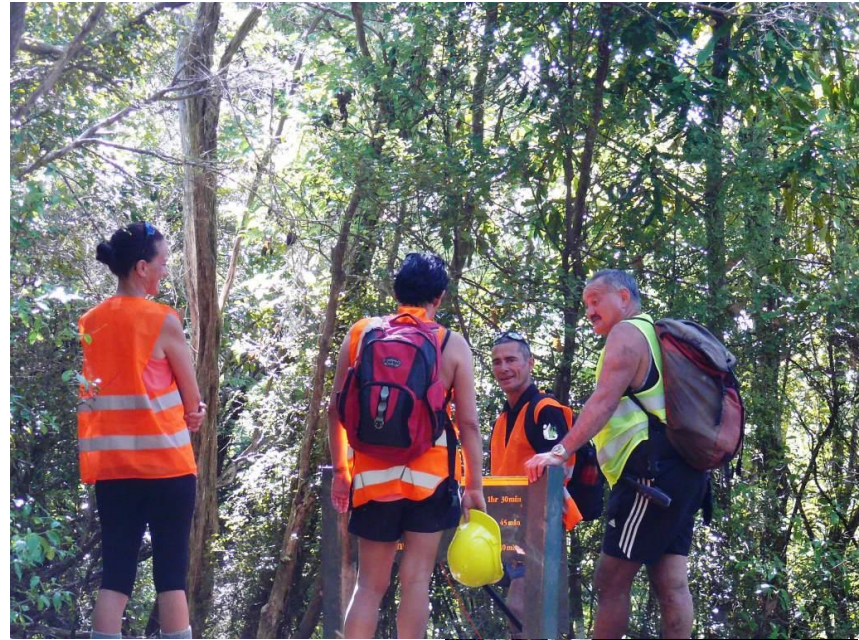
- Development of a species specific tool (FISH assay) to help enable the visualisation of the interaction between host and *P. agathidicida*
- Development of a set of criteria which will enable us to find tolerant individuals, based upon composite indices which indicate durable, tolerance of kauri to *P. agathidicida*



Acknowledgments

- Vicky Hodder and Colin Faulds (Scion)
- Tangata Whenua Roopu (Waitangi Wood), KDP and Mana Whenua Associations
- Quentin Paynter, Lynn Booth and Daile Hendry (LR)

“Kia toitu he kauri”



This research is inspired and dedicated to the late, Dr Ross E. Beever MSc Auckland, PhD Leeds, FRSNZ, FNZIAHS, FAPPS.

