Testing kauri for tolerance to *Phytophthora agathidicida*

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

<table>
<thead>
<tr>
<th>Trees</th>
<th>Proportion shoot length infected (%)</th>
<th>SE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>100b</td>
<td>0</td>
</tr>
<tr>
<td>2-2</td>
<td>50</td>
<td>17</td>
</tr>
<tr>
<td>2-3</td>
<td>50</td>
<td>17</td>
</tr>
<tr>
<td>2-4</td>
<td>0a</td>
<td>0</td>
</tr>
<tr>
<td>2-5</td>
<td>60</td>
<td>16</td>
</tr>
<tr>
<td>2-6</td>
<td>50</td>
<td>17</td>
</tr>
<tr>
<td>2-7</td>
<td>67b</td>
<td>33</td>
</tr>
<tr>
<td>5-1</td>
<td>0a</td>
<td>0</td>
</tr>
<tr>
<td>5-2</td>
<td>40</td>
<td>24</td>
</tr>
<tr>
<td>5-3</td>
<td>0a</td>
<td>0</td>
</tr>
<tr>
<td>5-4</td>
<td>40</td>
<td>24</td>
</tr>
<tr>
<td>5-5</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>5-6</td>
<td>0a</td>
<td>0</td>
</tr>
<tr>
<td>5-7</td>
<td>43</td>
<td>20</td>
</tr>
<tr>
<td>5-8</td>
<td>75b</td>
<td>25</td>
</tr>
<tr>
<td>5-9</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

- 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

**Family 2**
- Trees 1, 2, 3, 4, 5, 6, 7
- Frequency of recovery (%)
- Infection categories: 0 cm, 0.5 cm, 1 cm, 1.5 cm, 2 cm, 2.5 cm

**Family 5**
- Trees 1, 2, 3, 4, 5, 6, 7, 8, 9
- Frequency of recovery (%)
- Infection categories: 0 cm, 0.5 cm, 1 cm, 1.5 cm, 2 cm, 2.5 cm

**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

Visualisation under fluorescence microscope with UV light
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.