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

News

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Kauri dieback symposium

Over 90 participants from a cross-section of the community gathered in Hokianga last month to discuss the latest efforts in the fight against kauri dieback (caused by *Phytophthora* taxon Agathis, or PTA). Research, management, operational aspects and community engagement through the creative arts were all presented, along with a plea from the next generation to "work harder" to find a solution to the disease.

There was a real melting pot of views expressed and a very positive exchange of ideas. It gave scientists the opportunity to talk about what we are doing in the lab and field, and for us to hear how some of these findings are being applied by local communities actively involved in helping prevent the disease from spreading.

Presentations included the use of portable devices to speed up diagnosis of the disease in the field, PTA screening methods and identification of resistance, observations on how the pathogen moves inside tree roots and the use of phosphite to provide some short-term relief from disease symptoms.

Delegates also took a field trip to nearby Waipoua Forest, home to some of the country's iconic kauri, including Tane Mahuta, to see firsthand, the impact the disease is having on local forests and what the local community is doing to help contain its spread.

The symposium was a great success. Like all *Phytophthora* diseases, kauri dieback is a huge challenge which the HTHF team is endeavouring to tackle by determining what makes some species so virulent on specific hosts. With many key parts of the programme getting into full swing our first research objective is to determine if there is a level of resistance to PTA infection in the kauri population. This analysis will be pivotal to the long-term management of the species.

Nari

Targeting resistance to apple rots

Phytophthora cactorum and other *Phytophthora* species cause a root, crown or collar rot of apple trees. Although disease incidence is sporadic from season to season, losses can sometimes be high, particularly in young plantings.

Many of the commonly used apple rootstocks do not have good resistance to *Phytophthora*. As part of their long-term apple rootstock breeding programme, Plant and Food Research (PFR) screen all rootstock seedlings by challenging them with *P. cactorum* (Figure 1).

Figure 1. Screening of apple seedlings for resistance to Phytophthora cactorum.

This process is passive, merely ensuring that highly susceptible seedlings do not continue through the rootstock screening programme. However, it does not provide any information on the genetics of resistance required before we can breed for durable resistance. Within the HTHF programme, the plan is to have a more targeted approach to understanding resistance to *Phytophthora*, ultimately using information on metabolic responses to infection as an indicator of resistance and potentially building in resistance to multiple species of *Phytophthora*.

For the initial studies, apple rootstocks or varieties with known resistance ('Robusta 5', 'Aotea', 'Northern Spy', and 'M9') or susceptibility ('Cox's Orange Pippin', 'Braeburn', 'Gala' and 'MM106') to *Phytophthora* have been selected.

The first step is to develop controlled systems for propagating clean, *Phytophthora*-free apple material, similar to those being used for testing pine and kauri in other parts of the HTHF programme.

Apple rootstocks are normally propagated in stoolbeds, but such systems are unsuitable for the current work because of potential contamination by *Phytophthora*. Some varieties of apple are difficult to propagate by cuttings, but this possibility is currently being explored in experiments with the selected clones (Figure 2).

P. cactorum is potentially a variable species. Cultures in the PFR *Phytophthora* collection, bolstered by a few recent isolates collected from apple orchards around New Zealand, are currently being tested for their relative pathogenicity on apple clones. Following this testing, isolates will be selected for future studies of infection processes and metabolomic responses to identify resistance genes in the apple hosts.

Figure 2. Propagation of apple rootstock clones.

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An inside look at *Phytophthora agathidicida ined.:* Early infection in the roots of kauri seedlings

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Phytophthora agathidicida ined. (or PTA) causes a root and collar rot of *Agathis australis*. To date, the host range of this pathogen is restricted to kauri, and it is considered that early infection occurs through the fine roots. We studied how this pathogen infects the roots of two-year-old kauri plants 5, 10, 16, and 20 days after inoculation (d.a.i.) with *P. agathidicida*. We used light, scanning electron, and fluorescence microscopy to observe the way the pathogen progresses inside the roots.

We developed a fluorescent label to identify *P. agathidicida* cell structures within the kauri roots, and were able to observe cells of *P. agathidicida* within five d.a.i. Various forms of survival structures were observed for the first time in these artificially infected plants. This study has increased our understanding of the infection process of *P. agathidicida* in young kauri plants. We now know that this pathogen can grow within the root cells because of the specific structures that they form. We were also able to assess the rapidity with which these pathogens infect and kill the kauri seedlings.

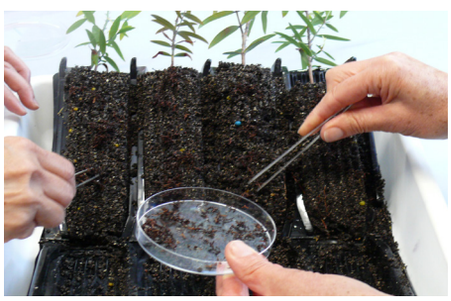
What we did

Used a culture to transfer infection to kauri grown on sterile soil.

Viewed nodulated roots using light and scanning electron microscopy.

Observed 5, 10, 16, 20 days after infection.

Fluorescence test to look at *P. agathidicida* hyphae/cells in roots.



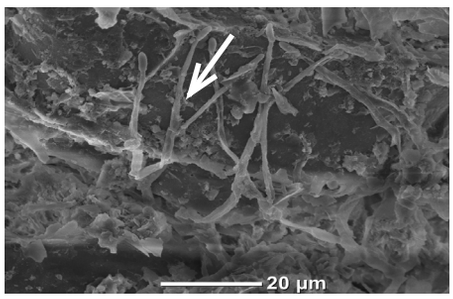
Cultivation of PTA-innoculated kauri.

What we saw 5 days after inoculation

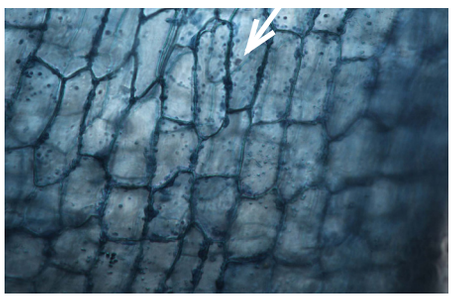
Haustoria (cells) of *P. agathidicida* inside nodules and roots.

Specialised structures formed by cell invaginations for absorption of food by pathogen inside root cells and hyphae within cells.

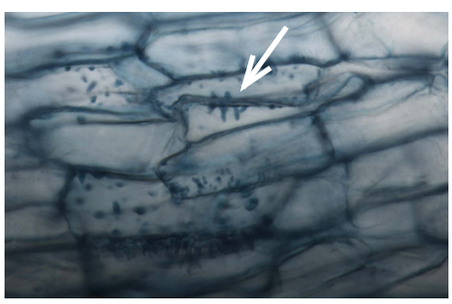
Hyphae and hyphal nets of *P. agathidicida* that showed the rapidity of colonisation of kauri by the pathogen.



Long branching hyphae (cells) of PTA.



Haustoria (cells) of PTA.



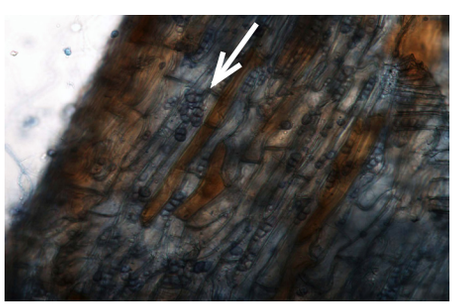
Cell invagination.

What we saw 20 days after inoculation

Aggregation of pathogen cells (stromata) that are separated by walls (so unlike hyphae).

Structures that may help pathogen survive in unfavourable conditions.

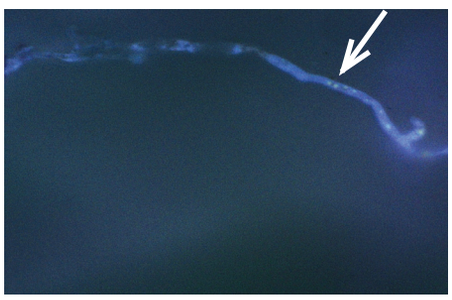
First time we have observed these structures, which are produced when the seedlings are dying.



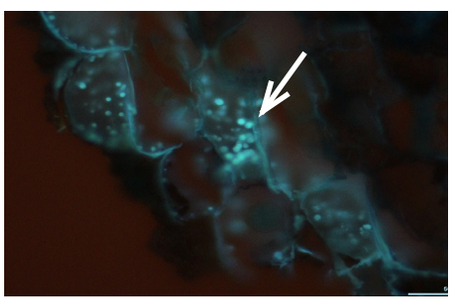
Aggregation of pathogen cells (stromata).

Fluorescent in situ hybridisation (FISH) testing

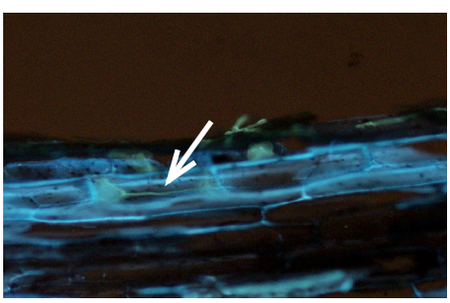
Kauri may have other fungi inside their roots - arbuscular mycorrhizae, which are beneficial to kauri. We wanted to distinguish *P. agathidicida* from these helpful fungi, and to have a quick method to see if the pathogen was present in the roots especially from field-collected material. We developed a fluorescent probe based on a *P. agathidicida*-specific DNA sequence, designed to only bind to *P. agathidicida*.



PTA nuclei in hypha.



Aggregation of pathogen cells.



Hyphae in roots 20 d.a.i.

What we learned

Phytophthora agathidicida is an extremely efficient pathogen and kills two-year-old kauri seedlings 20 days after infection. This species produces copious hyphae within the roots and nodules as well as possible storage structures. The fluorescent probe that we developed allows us to view the pathogen within the roots, which may be used as a rapid survey tool for field material and to observe interactions with the mycorrhizae in the kauri roots.

We would like to acknowledge the support of MBIE, the Forest Growers Levy Trust and the Kauri Dieback Programme in the funding of this programme.