

2016 wrap up and looking forward

What a year 2016 has been with some really significant phase-shifts in the Healthy Trees Healthy Future (HTHF) programme starting to take hold.

In the radiata pine we've stepped up to run parallel analyses of disease expression and severity on plants in the field and in our brand new custom built fog chamber. This inoculation facility is key in implementing the HTHF programme's systems biology model, for better understanding the infection cycle and environmental limits of red needle cast, and up-scaling the screening of germplasm for deployment to the field.

The recent field screening of industry relevant germplasm afforded an

opportunity to integrate the project work of our two mid-term PhD students Simren and Mireia. Simren will be using the samples she collected to confirm the gene expression of the *Phytophthora* species present while Mireia will be contrasting the sugars in needles of the more resistant and susceptible genotypes of radiata pine to assess the impact of infection on growth.

In the metabolomics work we now have confidence that liquid chromatography mass spectroscopy is providing a wealth of information pertaining to plant and pathogen interactions.

We now have a library of preliminarily identified chemicals extracted from radiata pine needles and will be following up on

those associated with *Phytophthora* infection and defence responses in the new year. This library, which will continue to be refined across the programme, is a key resource for understanding the biochemical interactions at play in pine needles challenged with *Phytophthora pluvialis* and *P. kernoviae* upon infection on resistant and susceptible pine needles.

Initial work screening across kauri families has commenced in partnership with mana whenua from regions affected by kauri dieback, and Landcare Research. We look forward to reporting these results in full in the new year.

Nari



Trees in Kinleith Forest showing symptoms of red needle cast

Testing for red needle cast susceptibility in the field

In our research to date, we have mainly used artificial systems to inoculate clones with red needle cast (RNC) to test their susceptibility.

To understand how this relates to a more natural infection process, we explored a number of alternative infection strategies.

In September we placed a selection of potted grafts underneath diseased trees in Kinleith Forest.

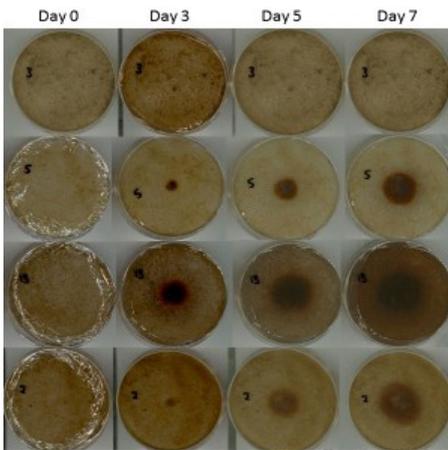
Under environmentally suitable weather conditions, rain splash from the infected canopy allows natural exposure of the plants to RNC inoculum. The wet weather

conditions over October were highly favourable for establishing RNC infection. These plants were assessed for disease expression and needle loss for six weeks.

This trial furthers our understanding of the relationship between field-based and laboratory infections and is necessary to

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Advances in metabolomic study techniques



Time series growth of *Phytophthora* species

Exciting progress has been made using cultured callus tissue for the metabolomics study of host-pathogen interaction. The metabolome (the total number of metabolites present within an organism, cell, or tissue) of a living organism responds very quickly to external influences, making metabolomic techniques an unparalleled tool for the diagnosis of a change in plant health status. However, a change in metabolism can be caused by numerous factors, from pathogen attack to a change in weather. Untangling the effects from one possible cause from another is a challenge. Ideally, a compound unique to the pathogen would be identified as a marker for infection, but the complexity of a tree make finding any pathogen related marker compounds comparable with looking for a needle in the haystack.

To address these challenges, we are starting with tissue cultured plant callus. These are plant cells at their most basic. Using this system we can establish a baseline of chemical and gene expression by the radiata pine and *Phytophthora* without the noise of environmental variability. This method is faster and requires far less sample preparation for analysis by nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry (MS) as compared with tree specimens.

The cultured tissue system is reproducible and provides a scalable system for ongoing research. Already initial NMR data has indicated that the tissue culture approach will fast track the development of diagnostic tools to be used in real trees.

Phytophthora study tour in Perth, Western Australia

The HTHF programme coordinated a *Phytophthora* study tour in Perth, Western Australia in alignment with the annual Dieback Information Group Conference “DIG” and Green Card hygiene training in July this year. DIG brings together the latest developments in *Phytophthora* dieback management, research, and on-ground projects from the perspective of speakers from government agencies, local government, industry groups, researchers and conservation groups. This year the DIG conference was preceded by a one-day Green Card hygiene / *Phytophthora* training course on best practice hygiene requirements to prevent the spread of *P. cinnamomi*.

The HTHF programme coordinated a three day study tour of dieback research and management in Perth and the surrounding areas. This tour covered *Phytophthora* disease interpretation, mapping, risk assessment, disease management planning, treatment and

hygiene along with the underpinning research that has led to best practice management.

The group visited the Western Australia Department of Parks and Wildlife (DPaW), Gaia Resources, Alcoa mine, Benara Nurseries and Kings Park. Our group of nine participants from the Kauri Dieback Programme (MPI), Department of Conservation, Northland Regional Council, Scion, Plant and Food Research and the Forest Owners Association were impressed by the size and scale of the efforts being made to contain *P. cinnamomi*. Western Australia is dealing with this *Phytophthora* infestation on a major scale, and we hope by increasing our understanding of the management practices used there, we can further strengthen New Zealand’s biosecurity capacity and management of *Phytophthora* pathogens.

For more information go to www.dwg.org.au



DIG Green Card trainees learning about best practices to decontaminate footwear



Green Card training group is shown the extent and destruction of *P. cinnamomi* in the field

Red needle cast susceptibility

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confirm that trial work completed under artificial conditions (in the lab and new fog chamber) is relative to what happens in the field.

Scion has commissioned two fog chambers for carrying out on-plant inoculations on trees up to 2m in height. The preliminary testing is complete and the first comparative inoculations are underway.

In pre-testing we observed lesions on the needles, and sporangia on the needle surface 10 days after inoculation, indicating that the pathogen life cycle is being completed and repeated infection may be achieved within the chamber. This is significant for our genetic screening and chemical control work as it provides a means of testing disease development in more realistic conditions than with detached needle assays or on-plant inoculation under sub-optimal environmental conditions. It also means we are now in a position to run samples from these on-plant inoculations through the suite of systems biology analyses to look at host-pathogen interactions that reflect forest conditions.

Whakapapa kauri lines work well underway

The first collection of kauri seed was completed in March this year in partnership with mana whenua.

After growing the seed on in the Scion nursery, the Scion team, along with Landcare colleagues Stan Bellgard and Chantal Probst, completed the second run of inoculations in late spring.

The results from the first inoculation run have been analysed and reports will be sent to mana whenua groups prior to the holiday break.

Planning for the second cohort is well underway and a more detailed report will be given in our next edition of HTHF News.



Whakapapa kauri lines being grown – nearly at first inoculation stage



Plants in Scion's new fog chambers. The growth lights emit red and blue light specific for photosynthesis, giving a pink effect in photos



Whakapapa kauri trees undergoing the second inoculation

To learn more about this programme

Contact Dr Nari Williams at nari.williams@scionresearch.com

Visit our website and subscribe to our quarterly newsletter

www.healthytrees.co.nz

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