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

News

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Hitting our stride

The HTHF programme has hit several key milestones in the first half of this year with lots of great highlights to be celebrated.

Firstly, we have seen the completion of a new facility to grow plants free of *Phytophthora* contaminants. This is fundamental to our research and will serve as a demonstration facility for hygienic plant production going forward.

Phytophthora species are a huge problem within nursery production. Consistent watering and succulent and abundant plant material combine to make the optimal conditions for pathogens to be harbored. Growing plants without nutrient or water stress, and in the presence of fungistatic chemicals, all serve to masks the presence of pathogens which is of real concern to us for our experiments but also for plant producers, plant industries and conservationists globally. The stark reality is that *Phytophthora*–free nursery production is a challenge for all nurseries, especially those with longstanding histories of plant production, such as our nursery at Scion's Rotorua campus.

Our new growth facility enables us to face the challenge of producing plants free of the pathogens by physically separating these plants in a dedicated facility in which work flows and hygiene protocols can be controlled. Key features include the elevation and curbing of the growth chamber to avoid water entering the growth area; a fully cemented and free draining floor; raised benches to get plants well off the ground; a dedicated forklift landing pad for receipt of potting mix once steam sterilized; an antechamber in which staff change into dedicated footwear and clothing and full enclosure with insect mesh to improve the management and control of nursery fly, which heavily impacted the germination of kauri last year. This has been a big project and was brought together in partnership with the Scion Nursery team, Vicky Hodder, Colin Faulds, Peter Harington and Simon Gey Van Pittius pulling out all stops to get it finished in time to receive the 2017 cohort of kauri collected under the whakapapa lines of kauri project.

A second exciting step forwards has been the recent appointments of data scientist Dr Ralf Gommers and bioinformatics expert Dr Tancred Frickey. Both Ralf and Tancred join us with extensive experience and expertise in complex data and dynamic systems. Along with considerable investment in Scion's data infrastructure and a partnership with Massey University's Al Rae Centre for Genetics and Biology, their appointment is enabling a considerable step-change in the analysis and integration of data across the programme that will start to bear fruit in the coming year.

The HTHF programme was externally reviewed in May by a panel of international scientists whose expertise spanned forest pathology, forest genetics and host-pathogen interactions in forest tree species. Professor Stephen Woodward (University of Aberdeen) chaired the panel with Dr Richard Sniezko (USDA), Professor Enrico Bonello (Ohio State University) and Dr Carl Gunnar Fossdal (Norwegian Forest and Landscape Institute). The panel spent a week with the team reviewing our science programme and direction and gave the team some invaluable feedback, food for thought, critique, and encouragement for the overall objectives and remaining two and a half years of the programme. The team did a great job in collating and presenting their work and it was a real honour to see how far we have come since 2013 and how much more we understand about our *Phytophthora* pathogens of greatest concern, the potential for identifying and selecting for resistance to kauri dieback and red needle cast, and better understanding the drivers for stem and collar rot in apple trees.

In the coming six months we will be performing the first long-term screening of kauri seedlings against *P. agathidicida* from the 2016 cohort of the whakapapa kauri lines, looking to up-scale the phenotyping of pine and kauri, and mapping out a HTHF data platform for integrating the pathology, genomic, gene expression and biochemical data sets across the programme.

HTHF Review Panel L to R: Richard Sniezko, Carl Gunnar Fossdal, Enrico Bonello, Steve Woodward

Students lead the charge collaborating with Oregon State University

With *P. pluvialis* having been recognized as being present in Oregon since 2008, it has always made strategic sense to collaborate strongly with the team at OSU. Our collaborations in the last 12 months, have started to bear fruit with PhD students Simren Brar and Mireia Gomez-Galleo having completed three month placements at OSU in 2016 and 2017, respectively.

Simren's visit enabled her to complete a population analysis of the *P. pluvialis* populations in both New Zealand and Oregon, providing further support for the introduction of the pathogen into New Zealand being recent. She is in the final stages of submitting this work for publication.

Mireia is currently completing her placement at OSU. She has performed a survey of Douglas-fir plantations to investigate the interaction between Swiss needle cast and red needle cast, to test the hypothesis that *P. pluvialis* may have a greater role in the casting of needles from Douglas-fir than previously realized.

It has been wonderful to see how well both students have made the most of the opportunity and experience of travelling during their candidature and building strong networks that will serve them well in their future careers. We look forward to reporting the results of their studies in full in the next issue.

Both Mireia and Simren have been generously hosted by the team at Oregon State University and based in Dr Jared LeBoldus' lab (OSU) with support from Professor Everett Hansen (Em. Prof OSU) and Dr Nik Grunwald (USDA/OSU) with whom we are furthering collaborations in the coming years. Jared will visit in September as we continue to further our collective understanding of the ecology and impact of aerial *Phytophthora* pathogens in forest systems.

Mireia collecting Douglas-fir needles in the field in Oregon

Simren Brar

Whakapapa kauri lines

The construction of the new *P. agathidicida*-free polyhouse environment at the Scion nursery was completed just in time for receiving the 2017 cohort of kauri seed from our kaurilands mana whenua partners. It has been a busy but exciting time in the nursery.

The collection programme started in February with a co-ordination and training day at the Auckland Botanic Gardens. Stan Bellgard (Landcare Research), Bec Stanley (Auckland Botanic Gardens) and Nari Williams (Scion) covered the objectives of the programme and collection, cone identification, seed viability, hygienic cone collection and drying.

Stan Bellgard and his team from Landcare Research, along with Waitangi Wood, have been instrumental in providing assistance and support for this years' collection, which has worked really well. Mana whenua groups were provided support in planning, scoping out suitable trees and seed collection where required.

Due to the narrow window of cone maturation, we set out to identify suitable trees ahead of time to allow the seed to be collected at the optimal point of maturity. All cone collection was done from the ground with long handled loppers as it is important that we can trace the seed back to the parent tree and ensure the seed remains free of *P. agathidicida*. This limited the collection to cones within reach of the loppers. The rampaging cyclones that crossed New Zealand during March hampered some collections with forest closures and early seed fall. However our mana whenua partners persisted and we were relieved to receive cones from 110 trees from Northland to Tauranga. They are now germinating well in the new environment with the first cohort due to go through challenge trials in 2018.

Colin Faulds pricking out 2017 kauri seedlings in the polyhouse environment

Functional analysis of RxLR effectors from the kauri dieback pathogen *Phytophthora agathidicida* kauri lines well underway

Phytophthora agathidicida is an oomycete that causes a serious dieback disease of kauri. Spores of *P. agathidicida* from the soil infect kauri roots and damage the tissues that carry nutrients within the tree. Nearly all infected kauri trees eventually die. Some infected kauri can be treated with phosphite to delay disease development but phosphite can be toxic at incorrect doses. Breeding for disease resistance provides hope for the survival of this iconic species, but in order to develop durable resistance it is important to understand how *P. agathidicida* infects kauri and how the host responds. So far, very little is known about these interactions at the molecular level.

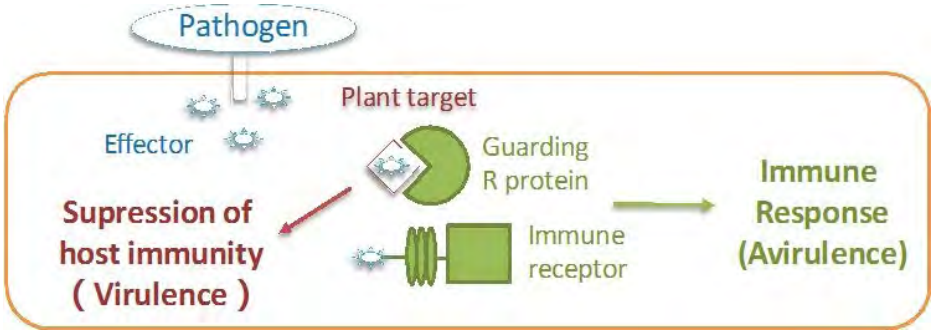

Figure 1. Schematic diagram of plant immunity to pathogens

The focus of this project is to identify proteins of *P. agathidicida* that are important in these interactions. Previous studies with related pathogens showed that, upon host entry, two classes of effector proteins, RxLRs and crinklers, are translocated into the host cell cytoplasm. Effectors are virulence factors that function to promote host colonization, typically by modulating host immune responses (Fig. 1). However, if the host has immune receptors that recognize these effectors, recognition will trigger plant defence responses; effectors that trigger defence in this way are called avirulence factors.

P. agathidicida effectors with potential virulence and avirulence functions were screened using non-host model plants *Nicotiana tabacum* and *Nicotiana benthamiana* using an Agrobacterium transformation system that delivers the RxLR effector gene candidates to the plant cells. Out of 78 predicted *P. agathidicida* RxLR effectors, eight of them triggered necrosis and are potential avirulence factors (Fig. 2), whilst two of them suppressed plant defence responses triggered by an elicitin INF1-1 and are potential virulence factors. For future work, purified *P. agathidicida* RxLR effector proteins will be screened using kauri tissue for virulence and avirulence activities. The results from this project are expected to help identify resistance traits in kauri that could be used in selection and breeding programmes.

Upon host entry, small secreted effector proteins are translocated into the host cell where they can suppress host immune responses, ie. the effectors have virulence functions. However, in some cases the host has immune receptors that can recognize the effectors and trigger downstream plant defence responses; in this case those effectors have avirulence functions. Ideally the effectors have both avirulence and virulence functions, so they can trigger defence in the plant, yet also be so important for the pathogen that the pathogen does not lose the effector gene to avoid recognition.

P. agathidicida RxLR effectors were screened for their ability to trigger non-host necrosis. 100 µl of cultured *A. tumefaciens* carrying an RXLR effector was infiltrated on the back of 5 week old *N. tabacum* leaves. The plants were incubated at 25° for 5-7 days. A defence response is indicated by plant cell death. INF1 was used as positive control, GFP protein was used as negative control. One example of a *P. agathidicida* RxLR effector that triggered necrosis on *N. tabacum* is PTA15012, a homologue of the well-characterised *P. sojae* virulence factor Avh238.

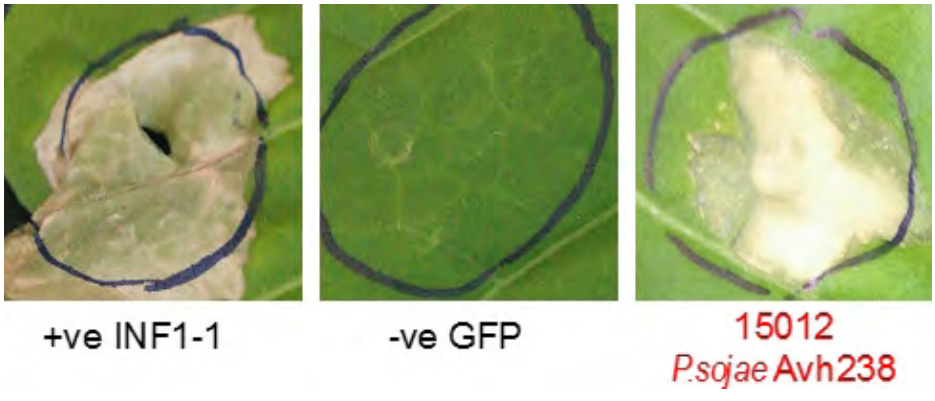

Figure 2. Agrobacterium transient transformation screening assay of P. agathidicida *RxLR effectors*

This work was funded by the Bio-Protection Research Centre in collaboration with the HTHF programme. Melissa Guo's work is a key implementation of the HTHF research and the first demonstration of the key pathogenicity related genes in *Phytophthora agathidicida*.

Contributors to this project are Rosie Bradshaw, Melissa Guo, Pierre-Yves Dupont, Carl Mesarich, Rebecca McDougal, and overseas collaborators in Exeter (University) and Norwich (John Innes Centre).

The HTHF review panel and team members on the Rotorua Redwoods Treewalk

To learn more about this programme

Contact Dr Nari Williams at nari.williams@scionresearch.com
Visit our website www.healthytrees.co.nz

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