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

News

SCION forests · products · innovation

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Early outcomes in the fight against *Phytophthora*

Hi, I'm Nari Williams and it is my privilege to lead the 'Healthy trees, healthy future' programme. This is an exciting collaboration that is consolidating New Zealand's capacity in *Phytophthora*, plant breeding, molecular biology and analytical chemistry to tackle a group of pathogens that impacts plant systems across horticulture, forestry and our natural forest estates.

As we near the first anniversary of the programme, our understanding of New Zealand's key *Phytophthora* pathogens, the diseases they cause and their genetic characteristics has grown considerably.

The programme is using a multi-host-pathogen 'Systems Biology' model that is driving increased research focus on *Phytophthora* species nationally. We have been approached by researchers from outside the founding collaboration seeking to align their research with our enabling technology programme. Greater focus on these plant pathogens is building New Zealand's biosecurity capacity through increased awareness of *Phytophthora* diseases across research disciplines and to a growing network of industry stakeholders.

During the first period of the programme, we have strategically sent two of our emerging scientists to key international conferences to 'road-test' the project model with a broad international audience. Through these trips, the programme has been presented and discussed with world research leaders, as described in Dr Rebecca McDougal's article. In addition, Dr Ilena Isak attended the Metabolomics 2014 conference in Japan.

These visits have provided vital feedback from researchers working in host-pathogen systems biology, the most reassuring being that, while we have an ambitious programme, it's on a good course. Many of the tips received are now being considered within the programme.

At the Metabolomics 2014 conference, the multi-platform approach within the metabolomics component of the programme was confirmed as the leading one internationally. This is a great boost. Ilena has been subsequently invited by Prof. Ute Roessner (Metabolomics Australia) to attend a workshop on Metabolomics by GC-MS and LC-MS in Melbourne in October.

With experimental work now in full swing, the baseline data resources and experimental outcomes are flowing in, which as Science Leader, I find exciting. Some of our achievements to date include:

The sequencing of six *Phytophthora* genomes in collaboration with Exeter University with another six to be completed in this financial year. This resource will be used for the HTHF project and is seeding collaborations with other research parties such as the Canterbury University, Otago University and the University of British Columbia in Canada.

Work undertaken by our pathology and forest genetics teams has provided further evidence that resistance to red needle cast (RNC) is genetically controlled. 'Elite' clonal material developed by the Radiata Pine Breeding Company is being screened for resistance and as a benchmark for future work across the programme.

While early days, we're starting to see the real potential of using NMR as a tool for better describing the infection state of plant tissues, and the development of clonal propagation and root inoculation systems that enables parallel analysis with each host species. The proof of concept phase for both of these within the HTHF programme's system biology model is nearing completion and I look forward to updating you on these in our next newsletter.

Until then, it's back to the lab!

Nari

Exeter University

Phytophthora Diagnostics and Genomics Internationally

Scion's molecular biologist Rebecca McDougal spent three-weeks in Europe visiting current and potential collaborators, and attending international conferences. The trip was an opportunity to meet researchers involved in similar fields of work (plant pathology, molecular diagnostics and genomics) along with world-renowned *Phytophthora* researchers, and to introduce Scion's research to the international oomycete community.

Starting in the Netherlands, Rebecca visited Dr Peter Bonants at Plant Research International, at Wageningen University. Dr Bonants leads a team of molecular pathologists who are involved in developing diagnostic methods for plant pathologists and industry. Rebecca was introduced to the design and application of Luminex assays for multiplex detection of *Phytophthora* species from various types of samples, a technique that could have great potential here in New Zealand.

Other technologies discussed included LAMP assays, DNA extraction and storage, high-throughput real-time PCR, and the use of online diagnostic tools such as Q-Bank. In addition, discussions with genomics researchers about their experience with *Phytophthora infestans* and resistance breeding were very informative for some projects currently underway at Scion.

Next stop was Exeter University in the UK where Rebecca visited current collaborators

involved in genome sequencing for the *Phytophthora* 'Healthy trees, healthy future' (HTHF) programme. Valuable discussions were had around genetic diversity of *P. kernoviae,* effector repertoire and additional strains they could consider sequencing. A reciprocal visit to Scion by Prof. Murray Grant is likely in October this year to build on this collaboration.

Rebecca then attended the Oomycete Molecular Genetics Network Annual Meeting in Norwich. This attracted over 140 delegates, with 46 oral presentations and many poster presentations on offer. Rebecca's presentation on the HTHF programme was well received.

The visit provided Rebecca with the opportunity to meet researchers with a long history and excellent track-record in oomycete research, and see the diverse range of research from fossilised oospores and herbarium-derived genome sequences, to comparative genomics, effector delivery and heterologous hosts.

The final stop was the XVI International Congress on Molecular Plant-Microbe Interactions in Rhodes, Greece. This four-day conference attracted over 1400 delegates, 32 plenary speakers, 190 speakers in concurrent sessions and 755 posters. Rebecca presented research on detection of *Phytophthora* from herbarium samples.

Highlights from this meeting included talks on diagnostic methods for use in the field, and ways in which genomics can provide clues about how pathogens switch hosts and evade recognition.

In summing things up, Rebecca says "there is no silver bullet. Several strategies are needed to overcome plant disease, and pathogen variability will drive the deployment of resistance genes. Population genomics, effector repertoire and evolution in the field require constant monitoring, using large numbers of isolates".

Many of the researchers Rebecca met are keen to remain in touch and potentially collaborate on similar types of *Phytophthora* research.

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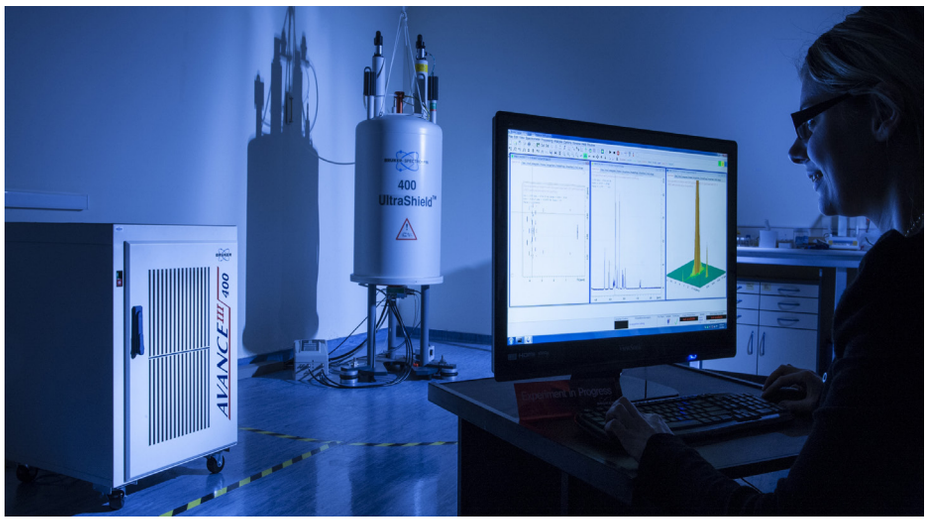

Nuclear magnetic resonance (NMR) provides a characteristic chemical footprint of plant metabolites.

Metabolite screening of woody plants using nuclear magnetic resonance and mass spectroscopy

Work is underway to develop high throughput screening methods for *Phytophthora* species infection in radiata pine (red needle cast), apple species, and kauri (kauri dieback).

Metabolomics is one such method, and is used to screen plant tissue extracts for changes in the metabolites that may indicate pathogen infection. This approach combines Mass Spectrometry and Nuclear Magnetic Resonance (NMR) to identify indicator chemicals, and changes to these chemicals within plants, for the rapid and cost-effective diagnosis of *Phytophthora* species infection.

NMR data of plant extracts provides a characteristic chemical fingerprint that is used to evaluate if a plant is in a healthy state or is being challenged. A challenge may be a response to a pathogen (e.g. *Phytophthora spp.*) or may be a stress response to environmental factors (e.g. lack of water). This data can detect the class of compounds that are changing and be explored further by adding in data from Mass Spectrometry to identify the compounds individually.

Scion has recently expanded its sample throughput capability by acquiring an automatic sample changer for the NMR spectrometer with a 60 sample capacity, and the purchase of cryogenic grinders. These grinders can pulverise leaves, needles, roots and wood tissue samples rapidly, making it possible to analyse around 50 NMR samples daily.

Recent development has involved expanding Scion's current metabolomics platform to include needles and roots from radiata pine and leaves from apple trees. Current research is based on refining extraction methods to analyse metabolites from these plant tissues. Experiments have shown that the metabolite extractions and NMR analyses are highly robust and reproducible.

By using NMR to screen the metabolites in both healthy and infected tissues, markers for infection will be identified that can be used to detect infection before a tree shows any outward signs of disease.

Ultimately, combined with genetic studies this new knowledge will help tree breeders select for greater *Phytophthora* resistance.

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To learn more about this programme

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