8th Meeting
IUFRO
Working Party 7.02.09
Phytophthora in Forests and Natural Ecosystems

**Proceedings of the 8th Meeting of the**
**International Union of Forestry Research Organisations**

## IUFRO Working Party S07-02-09

# *Phytophthora* in Forests and Natural Ecosystems

**Hanoi-Sapa**
**Viet Nam**

**18 - 25 March 2017**

**www.iufrophytophthora2017.org**

*Version 2: Revised June 2017*

# Index:

Dear Participants,

We are delighted to have you here to participate and share in the 8th IUFRO meeting *Phytophthora* in Forest and Natural Ecosystems. Thank you very much for coming.

I know that many of you have traveled from quite a distance and I'd like to acknowledge participants from Australia, Belgium, Bhutan, Canada, France, Germany, India, Ireland, Iran, Iraq, Italy, Libya, Nepal, Netherland, Pakistan, Serbia, South Africa, Spain, Czech Republic, Sweden, Switzerland, United Kingdom, and United State of America who have made this long trip.

The diverse forest types and plant species of Viet Nam provide much needed resources for over 25 million people. Unfortunately, this need has led to unsustainable forestry practices including a long history of deforestation. Economic development, climate change, and pest and disease factors as well have already impacted diversity of Viet Nam's forests, and the problem has great potential to increase over time.

We hope very much that this meeting can bring to us valuable scientific information on *Phytophthora* and the role it plays in natural ecosystems, especially pathways to and from other land uses such as agricultural, forestry and the urban environment. Such information is crucial for proper policy development on protection and development at the national scale of our natural forests and forest plantation.

To all of you, thank you for being here, welcome, and enjoy the conference!

**Prof. Vo Dai Hai**
General Doctor of Vietnamese Academy of Forest Sciences

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# Conference Program

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|---|---|
| | **Saturday 18th March 2017** |
| 17.30 | Registration opens in Foyer of Army Hotel, Hanoi |
| 18.00 | Opening ceremony - Army Hotel Hanoi |
| | **Sunday 19th March 2017** |
| 8.00-17.00 | FIELD TRIP: commence from Army Hotel in Hanoi |
| | **Monday 20th March 2017** |
| 8.30-17.00 | FIELD TRIP: Finish in Sapa |

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|---|---|---|
| | **Tuesday 21st March 2017** | |
| 8.00 | Welcome to Sapa | |
| | **1. Management and control** | |
| 8.15 | Williams, N | Workshop Briefing Plan |
| 8.25 | Scott, P | Predicting global and national *Phytophthora* diversity and biosecurity risk |
| 9.00 | Navarro, S | Sudden Oak Death in Oregon Forests: Disease intensification and changes in disease management in the wake of a new clonal lineage |
| 9.15 | Dunstan, W | Survival of *Phytophthora cinnamomi* (Pc) in mine haul roads, road bunds and soil stockpiles. |
| 9.30 | Horner,I | Overview of phosphite treatment trials in forests with kauri dieback, and potential strategies for deployment |
| 9.45 | Belhaj, R | The in vitro tolerance of some recently described *Phytophthora* species to phosphite in solid and liquid media |
| 10.00 | Williams, N | Developing a chemical control programme for management of red needle cast in planted Pinus radiata forests in New Zealand |
| 10.15 | **MORNING TEA** | |
| 10.45 | Hardy, G | From 'then to now'- *Phytophthora* science and management in Western Australia |
| 11.30 | Brown, K. | Kauri Dieback (*Phytophthora agathidicida*) Cryptic challenges of disease detection and long term management. |
| 11.45 | Chastagner, C | Update on *Phytophthora ramorum* mitigation at the Bloedel Reserve in Western Washington |
| 12.00 | Cobb, R | Practical solutions to ecosystem changes from sudden oak death |
| 12.15 | Williams, N | Nari Williams: Do alternate land use boundaries present as realistic barriers to the spread of *Phytophthora agathidicida?* |
| 12.30 | **LUNCH** | |

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|---|---|---|
| | **Tuesday 21st March 2017** | |
| | **2. Community engagement** | |
| 13.30 | Williams N | A case study for culturally relevant *Phytophthora* research: beyond engagement and working together to cross inform indigenous and modern science knowledge systems |
| 14.00 | Horner, I | Citizen science project to accelerate research on control tools for kauri dieback |
| 14.15 | Goheen, E | Managing sudden oak death on Federally-administered lands in Southwest Oregon, USA: past, present, and future |
| 14.30 | Frankel, S | Responding to *Phytophthora* introductions in California restoration areas: The Phytophthoras in Native Habitats Work Group |
| 14.45 | Massenbauer, T | Sharing plant disease information for better biodiversity outcomes using online technology - "Dieback Information Delivery Management System" - DIDMS |
| 15.00 | Massenbauer, T | An Investment Framework for Managing *Phytophthora* Dieback in south-west Western Australia. |
| 15.15 | **AFTERNOON TEA** | |
| 15.45 | Hulbert, J | Cape Citizen Science: public engagement to survey *Phytophthora* in a developing country |
| 16.00 | Bellgard, S | "Unlocking a Nation of Curious Minds": a science participatory platform for the next generation of *Phytophthora* scientists. |
| 16.15 | Tuffnell G | *Phytophthora* Control in Western Australian Backyards and Bushland, where the solution isn't the only solution! |
| 16.30 | Sambrooks, K | Green Card: A new training standard for biosecurity hygiene management |
| 16.45 | Bhandari, J. | The challenges of *Phytophthora* study and management in the forest of Nepal (Review study). |
| 17.00 | | Discussion/Planning for final workshop |
| 17.15 | **SELF ORGANISED DINNER** | |
| 19.00 - 21.00 | **POSTER SESSION; options for a 1 slide, 3 minute presentation** | |

| | | |
|---|---|---|
| | **Wednesday 22nd March 2017** | |
| | **3. Diversity** | |
| 8.15 | Legeay, J | A study of Phytophthora communities in the soils of two French Guiana forest sites, using three different identification methods. |
| 8.30 | Prospero S | Distribution and causal agents of ink disease of *Castanea sativa* in Southern Switzerland |
| 8.45 | Henricot, B | Metabarcoding of *Phytophthora* communities in Scottish soils. |
| 9.00 | Blomquist, M | *Phytophthora* affecting protected beech forests across Southern Sweden |
| 9.15 | Scanu, B | Multiple new cryptic Phytophthora species from Fagaceae forests in Europe |
| 9.30 | Gonzalez, M | A third *Phytophthora* species causing wild olive decline in Spanish forests |
| 9.45 | Jung, T | Diversity of *Phytophthora* species in forests, forest nurseries and riparian ecosystems of Portugal |
| 10.15 | **MORNING TEA** | |
| | **4. Ecology** | |
| 10.45 | Scanu, B | The role of Phytophthora in the lack of seedling recruitment syndrome in Mediterranean oak forests |
| 11.00 | Shaw, C | Damping-off of native southwest Australian plant species by *Phytophthora* and *Pythium* species. |
| 11.15 | Burgess T | Native soil-borne pathogens equalize differences in competitive ability between plants of contrasting nutrient-acquisition strategies. |
| 11.30 | Hansen, E | Current status of *Phytophthora pluvialis* in western North America |
| 11.45 | Bose, T | Does *Phytophthora* species migrate from natural forest to plantations of non-native tree in South Africa? |
| 12.00 | Webber, J | Shifting disease dynamic of *Phytophthora ramorum* causing localized epidemics on sweet chestnut (*Castanea sativa*) |
| 12.30 | **LUNCH** | |
| 13.30 | Garbelotto, M | Disease Ecology of *Phythophthora ramorum* in sympatric transmissive and dead-end hosts |
| 13.45 | Burgess, T | Current and projected global distribution of *Phytophthora cinnamomi,* one of the world's worst plant pathogen |

## Wednesday 22nd March 2017

### 5. Genetics

| | | |
|---|---|---|
| 14.00 | Marcais, B | Genetic diversity and origins of the homoploid allopolyploid hybrid *Phytophthora ×alni.* |
| 14.15 | Rendondo, M | Traits associated with the establishment of *Phytophthora* in Scandinavia. |
| 14.30 | Brar, S | Population structure of an aerial *Phytophthora* species in a forest system |
| 14.45 | Henricot, B | Two distinct lineages in *Phytophthora austrocedri*, the cause of forest disease epidemics in Britain and Argentina. |
| 15.00 | Pánek, M | Evolutionary relationships within the *Phytophthora cactorum* species complex in Europe. |
| 15.15 | Engelbrecht, J | Identification and characterization of polymorphic microsatellite markers to study *Phytophthora cinnamomi* populations |
| 15.30 | **AFTERNOON TEA** | |
| 16.00 | Dunnell, K | *Phytophthora ramorum* and tree species susceptibility: Comparing virulence and sporulation of EU1 and NA1 isolates |
| 16.15 | Yuzon, J. | Host -induced genome alterations in the Sudden Oak Death pathogen *Phytophthora ramorum.* I. NA1 lineage on Coast live oak in California. II |
| 16.45 | Brasier, C | Host -induced genome alterations in the Sudden Oak Death pathogen *Phytophthora ramorum.* II EU1 lineage on *Chamaecyparis lawsoniana* in UK |
| 17.00 | Brasier, C | Biological differences between the evolutionary lineages within *Phytophthora ramorum, P. lateralis* and other Phytophthora species. Should they be formally taxonomically designated? |
| 17.15 | **SELF ORGANISED DINNER** | |

### 11. Surveys and new species

| | | |
|---|---|---|
| 19.00 | Jung, T | Diversity of *Phytophthora* species in Valdivian rainforests and their association with severe dieback. |
| 19.15 | Jung, T | Diversity of *Phytophthora* species in natural forests and streams and in rubber plantations in Vietnam. |
| 19.30 | Jung, T | *Nothophytophthora* prov. nom., a new sister genus of *Phytophthora* from natural and semi-natural ecosystems in Europe, Chile and Vietnam. |

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| | **Thursday 23rd March 2017** | |
| | **6. Pathways - urban/ horticulture** | |
| 8.15 | Drenth, A | Phytophthora diseases in Horticulture in Southeast Asia |
| 8.45 | Green, S | PHYTO-THREATS: Global threats from *Phytophthora* species: understanding drivers of emergence and opportunities for mitigation through nursery best practice |
| 9.00 | Hamelin R | Urban activities influence Phytophthora species diversity in BC, Canada. |
| 9.15 | Cerny K | Factors affecting *Phytophthora ×alni* distribution in Czech forests and its predictive modeling. |
| 9.30 | Paap, T | Botanical gardens: Sentinel plantings to detect new and emerging *Phytophthora* risks |
| 9.45 | O'Hanlon, R | Diversity and detections of *Phytophthora* species from trade and non-trade environments in Ireland. |
| 10.00 | Khdiar, M | Host range of *Phytophthora* species associated with declining trees |
| 10.15 | **MORNING TEA** | |
| 10.45 | Simamora, A | A forensic investigation into the sources of *Phytophthora boodjera* contamination in a Western Australian containerized production nursery. |
| 11.00 | Migliorini, D | Comparisons of *Phytophthora* incidence and diversity in cultivated and natural endemic species of Proteaceae in South Africa. |
| | **7. Isolation and ID techniques** | |
| 11.15 | Cooke, D. | Development and application of an amplicon metagenomics approach based on the ras-related Ypt1 gene for the detection of *Phytophthora* species |
| 11.30 | Cooke, D | Testing *in situ* water sampling and metabarcoding protocols to detect *Phytophthora* diversity for plant health testing and natural ecosystem surveillance |
| 11.45 | Oliva, J | Monitoring *Phytophthora* species in river systems in Sweden by high-throughput sequencing. |
| 12.00 | Khaliq, I | Best method for determining *Phytophthora* community. |
| 12.15 | Dunstan, W | Specific detection of *Phytophthora cinnamomi* DNA and mRNA in environmental samples using real time polymerase chain reaction assays. |
| 12.30 | **LUNCH** | |
| 13.30 | **FREE AFTERNOON AND EVENING; OPTIONS FOR ACTIVITIES** | |

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| | **Friday 24th March 2017** | |
| | **8. Pathogenicity** | |
| 8.15 | Sims, L | Evaluating eight plant families for *Phytophthora* species in California wildlands, early results from restoration nurseries |
| 8.30 | Quynh, D | First report of *Phytophthora cinnamomi* on *Cinnamomum cassia* in Vietnam. |
| 8.45 | Webber, J | Insights into the potential host range of *Phytophthora foliorum* |
| 9.00 | Milenkovic, I | Pathogenicity of *Phytophthora ×serendipita* to *Quercus petraea* and *Q. robur* in Serbia. |
| 9.15 | Serrano, M | *Phytophthora cinnamomi,* a highly variable pathogen with epidemiological consequences in California natural ecosystems |
| 9.30 | Gomez-Gallego | Red Needle Cast (*Phytophthora pluvialis*) studies on Douglas fir require Swiss Needle Cast suppression |
| 9.45 | Parke, J | Root rot of *Juniperus* and *Microbiota* by *Phytophthora lateralis* in Oregon horticultural nurseries |
| 10.00 | Arentz, F. | *Phytophthora cinnamomi* A1: an ancient resident of New Guinea and Australia of Gondwanan origin? |
| 10.15 | **MORNING TEA** | |
| | **9. Physiology** | |
| 10.45 | Corcobado, T | Effects of co-inoculations of *Alnus incana* with *Phytophthora alni* complex and *P. plurivora* on disease development and mortality |
| 11.00 | Dam, V | Biological characteristics of *Pythiaceae* species isolated from soil of Hevea brasiliensis plantations in the South of Vietnam |
| 11.15 | Gyeltshen, J | The decline in viability of *P. cinnamomi* survival structures under moist and dry soil conditions |
| 11.30 | Nguyen VT | Effect of silver oligochitosan against (AgNPs) on the growth and reproduction of *Phytophthora* species in vitro |
| 11.45 | Padamsee, M | Approaching the bioprotection of kauri *Agathis australis* from *Phytophthora agathidicida:* assessing the role of mycorrhizae and dark septate endophytes |
| 12.00 | Thu, PQ | Potential of endophytes as biological control agents of *Phytophthora* pathogens in rubber plantations in south-east Vietnam. |
| 12.15 | Widmer, T | The effect of *Phytophthora lateralis* soil populations on fine root densities of Port-Orford cedar (*Chamaecyparis lawsoniana*) |
| 12.30 | **LUNCH** | |

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|---|---|---|
| | **Friday 24th March 2017** | |
| | **10. Resistance** | |
| 13.30 | Bellgard, S | Testing kauri (*Agathis australis*) for resistance to *Phytophthora agathidicida.* |
| 13.45 | Chaendaekattu | *Phythopthora* abnormal leaf fall of *Hevea* and breeding for disease resistance |
| 14.00 | Garbelotto, M | A comparative transcriptomic analysis reveals mechanisms of resistance to *Phytophthora* in tanoaks |
| 14.15 | Williams, N | The hunt for a consistent phenotype for breeding for resistance to *Phytophthora pluvialis* in *Pinus radiata* |
| 14.30 | Hunter S | Testing the tolerance of *Phytophthora cinnamomi* from New Zealand avocado orchards to phosphite *in vitro* and *in planta.* |
| 14.45 | Scott, P | Use of chlorophyll florescence to infer root health and accelerate screening *Agathis australis* (Kauri) for resistance to *Phytophthora agathidicida* |
| 15.00 | **AFTERNOON TEA** | |
| 15.30 | **Group Workshop; A 50-100 year vision for managing *Phytophthora* in forests and natural ecosystems** | |
| 18.30 - LATE | **CONFERENCE DINNER** | |
| | **Saturday 25th March 2017** | |
| 8.30 | BUS DEPARTS SAPA FOR HANOI | |

# Session 1: Management and Control

## Predicting global and national *Phytophthora* diversity and biosecurity risk.

Peter Scott[1], Martin Bader[1], Treena Burgess[2], Giles Hardy[2] and Nari Williams[1]
[1]*Scion (New Zealand Forest Research Institute Ltd.), Rotorua 3046, New Zealand;* [2]*Centre for Phytophthora Science and Management, Murdoch University, Murdoch, Western Australia.*
peter.scott@scionresearch.com

The diversity and distribution of unknown *Phytophthora* species pose significant management and biosecurity challenges and advances in environmental DNA sampling are revealing significant diversity that has potential trade implications. New tools and approaches are required to model and predict *Phytophthora* diversity and contextualize the potential importance of rapidly identified *Phytophthora* species. Brasier (IUFRO, 2007) predicted there are between 200 and 600 *Phytophthora* species. We present a framework that leverages geographically and economically biased pathogen data against enviro-socio-economic metrics to model the global biogeography of the genus. This sets a context for *Phytophthora* research and a benchmark for biosecurity response. Time series models, using 12,120 reports from 135 countries, predict up to four times more described *Phytophthora* species than the 146 currently described. Constructing principal components analyses from gross national income, travel, imports, human population, vascular plant richness and land use allowed us to estimate national data deficits against key drivers of diversity, spread and knowledge bias. Two-thirds of trading nations have reported lower than predicted species numbers. Countries with greater reported diversity, therefore pose lower trade-related biosecurity risks as there is more opportunity to better manage known threats and pathways than those that remain unknown. Hierarchical clustering of invasiveness traits, including host and country range, align *Phytophthora* species as either cosmopolitan generalists or specialists historically tied to agriculture. Our framework facilitates improved biosecurity acumen across biological risks worldwide. We will also present an online web interface for examining known and predicted *Phytophthora* distribution, diversity and trade risk.

## Sudden Oak Death in Oregon Forests: Disease intensification and changes in disease management in the wake of a new clonal lineage.

Sarah Navarro[1], Alan Kanaskie[1], Ellen Michaels Goheen[2], Everett Hansen[3], Paul Reeser[3], Wendy Sutton[3], Nicholas Grunwald[4], Ron Rhatigan[2], Randall Wiese[1], and Ryan Porter[1]

[1]*Oregon Department of Forestry, Salem, OR, USA;* [2]*USDA Forest Service, Forest Health Protection, Central Point, OR;* [3]*Oregon State University, Department of Botany and Plant Pathology, Corvallis, OR, USA;* [4]*USDA ARS, Horticultural Crops Research Lab, Corvallis OR, USA.*
sarah.navarro@oregon.gov

Sudden oak death, caused by *P. ramorum*, kills tanoaks *(Notholithocarpus densiflorus)* in Curry County and has the potential to spread throughout its native range in Oregon. Since 2001, an interagency team has been attempting to eradicate and slow the spread of disease through a program of early detection, survey and monitoring, and destruction of infected and nearby host plants. In 2015, the EU1 clonal lineage of *P. ramorum* was detected on a single tanoak tree located approximately one mile north of a small private nursery, which is now closed for business. Genotype comparison of the tanoak and nursery isolates suggests the nursery as the probable source for the forest infestation. Until that time, all other forest isolates were the NA1 lineage. In 2016, two additional EU1 isolates were detected through stream monitoring in the same area, resulting in the detection of another EU1 forest infestation in Oregon. In pathogenicity tests, the EU1 lineage has demonstrated increased pathogen agressiveness on confier tree species, including Douglas-fir, a major timber species in Oregon. A sharp increase in disease between 2015 and 2016 forced the program to prioritize sites for full treatment due to available program funding. The highest priorities for treatment areas are located at or beyond the leading edge of the sudden oak death infestation, or near the quarantine boundary, or infestations of the EU1 lineage. Based on these new disease findings, we will discuss changes to sudden oak death management.

## Survival of *Phytophthora cinnamomi* in mine haul roads, road bunds and soil stockpiles.

William A. Dunstan[1], Giles Hardy[1], Treena I. Burgess[1], Vicki Stokes[2] and Andrew Grigg[2]
[1]*CPSM, Murdoch University, W. Australia, 6150;*[2]*Alcoa Australia, Huntly, W. Australia.*
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Alcoa Australia mine 23 Mt of bauxite p.a. at their Huntly mine in eucalypt forest in south-western Australia, in part infested with *P. cinnamomi (P.c.).* Post-mining, ca. 600 ha p.a. of mined forest is rehabilitated. The mine haul road network is constructed from *Pc* free materials, but may be infested during mining operations. Post mining, haul roads were maintained free of host plants and quarantined (fallowed) after mining operations. For rehabilitation of mined areas, a *Pc* free status for roads would greatly reduce complexity and cost of operations. If present in engineered structures, could *Pc* survive fallowing? We installed artificial inoculum in roads, road bunds and soil stockpiles, that were maintained plant free, and assessed survival of *Pc* over time. In a preliminary experiment in a haul road site there were no *Pc* recoveries after 24 months, and less than 1% recoveries from roads after 18 months in a larger replicated experiment. The mean for recoveries of *Pc* from road bunds after 12 months was 53%, but highly variable between sites (range 4-91%). Soil physical and chemical characteristics probably explain differences in recoveries from bunds between sites. In stockpiles, the rate of *Pc* recoveries was highly dependent on depth in the soil profile, where the mean rate of recovery of *Pc* at 10 cm was 8.5%, in contrast with 81% at 50 cm. In both roads and stockpiles, soil temperatures in summer frequently exceeded 46°C at 10 cm, likely to be lethal to *Pc.* At depth, soil temperatures were more favorable and soil moisture was likely to have been a significant factor in determining survival. Results show that *Pc*, if present in roads, is unlikely to survive the fallowing process and post-fallow roads could be considered safe to treat as pathogen free.

## Overview of phosphite treatment trials in forests with kauri dieback, and potential strategies for deployment.

Ian Horner, Ellena Hough and Mary Horner
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Phosphite treatment is being investigated to manage kauri dieback, a disease caused Phytophthora agathidicida which is having a significant impact in New Zealand kauri forests. Trials established on 162 infected trees (<40 cm trunk diameter) on four sites in 2012 showed a complete cessation of trunk lesion expansion in trees injected with a 7% or 20% phosphite. All above-ground lesions in treated trees healed. After four years very few lesions on treated trees showed any signs of *Phytophthora* activity or spread. In untreated trees, a majority of lesions remained active and expanding, some girdling the lower trunk and killing the tree. Phytotoxicity symptoms (leaf yellowing, browning or leaf/twig abscission) were seen in many phosphite-treated trees, particularly with the 20% concentration. In a small number of trees on two of the four sites, trunk cracking occured in line with the injection points and site or tree factors associated with this are being investigated Newly established trials are investigating treatment of trees up to 3 m diameter. In further trials, low phosphite concentrations (4%) and reduced injection frequency around the trunk (40 or 80-cm spacing, as opposed to 20-cm spacing in earlier trials) is being investigated. The potential deployment of phosphite treatment, either as a curative treatment on diseased trees, as a preventive treatment on threatened trees, or potentially as a barrier treatment to reduce the spread within infected forests will be discussed, together with the challenges of research into long-term barrier effectiveness in forests.

# The in vitro tolerance of some described *Phytophthora* species to phosphite in solid and liquid media.

Rajah Belhaj[1], Jen McComb[1], Treena Burgess[1], Leila Eshraghi[2] and Giles St. J. Hardy[1]
[1]*Centre for Phytophthora Science and Management, School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia, 6150* .[2]Biocenter Oulu, University of Oulu, Aapisie 5A,90220, Oulu, Finland.
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A number of new species of *Phytophthora* have been identified and little is known about tolerance compared with in planta analysis remains to be tested; this is currently in their host range, pathognicity, or response to phosphite. The tolerance to phosphite of 23 species was examined in vitro using solid or liquid Ribiero's medium.Tolerance was assessed by calculating the $EC_{50}$ of mycelial growth against a range of concentrations of phosphite: from 0-160 µg/ml (solid media) and 0-900 µg /ml (liquid media). Estimates of colony diameter on solid medium were unreliable as colonies were not always circular and differences in hyphal density were not reflected in the data. Assessment of mycelial growth in liquid medium was more reliable using the biomass dry weight after 7 or 14 days for fast or slow growing isolates respectively. If liquid medium is not buffered for pH, results will be anomalous. When pH of liquid medium is not buffered using 0.03M MES (2-(N-morpholino)ethanesulfonic acid) $EC_{50}$ values were lower than on solid medium, but in buffered medium there was a higher level of tolerance to phosphite (Table 1). The species analysed showed a range of $EC_{50}$ values to phosphite between 5->160 µg/ml (solid medium) and 30->900 µg /ml (liquid medium) but the ranking of species for tolerance was not the same on solid and liquid media (Table 2). The higher levels of tolerance observed were amongst the highest on record for *Phytophthora*. Example Species of most concern from a management point of view, are those that appear highly tolerant to phosphite in liquid medium: *P. sp.* cyperaceae*, P. inundata, P. sp.* condilina*, P. sp.* personii and *P.* sp. walnut. Species with the lowest $EC_{50}$ to phosphite were *P. constricta, P asparagi, P. lacustris, P. gregarta a*nd *P. multivora*. However, the value of measures of in vitro progress.

**Table 1.** $EC_{50}$ values for *Phytophthora* isolates after 7 days growth in solid or liquid Ribeiro's modified medium containing phosphite.

| | | Solid | Liquid | |
|---|---|---|---|---|
| Species | Isolate | | MES 0 | MES 0.03M |
| *P. cinnamomi* | MP94-48 | 25 | 6 | 250 |
| | MP128 | 95 | 40 | 180 |
| *P. multivora* | TP13-04 | 19 | 5 | 130 |
| | WAC13201 | 19 | 5 | 160 |
| *P. sp. kwonganina* | DDS3599 | 32 | 15 | 70 |
| | VHS23298 | 30 | 15 | 240 |

**Table 2.** Growth rate and $EC_{50}$ for inhibition of mycelial growth by phosphite for isolates of 23 *Phytophthora* species on solid and liquid Ribeiro's modified medium. Growth on solid medium was determined from colony diameter when growth reached the edge of control plates. Growth in liquid medium was determined from mycelial dry weight after 7 days for fast growing species, and 14 days for slow growing species.

| | | | Solid Media | | Liquid Media | |
|---|---|---|---|---|---|---|
| Clade | Species | Isolates | Growth Rate[1] | $EC_{50}$ solid medium | Growth Rate[2] | $EC_{50}$ liquid medium |
| 9 | *P. constricta* | CLJO695 | 6.82±0.03 | 65 | 12.48±0.83 | 50 |
| 9 | *P. constricta* | VHSC16130 | 6.84±0.02 | 65 | 13.81±2.37 | 30 |
| 7 | *P. cinnamomi* | MP 94-48 | 8.15±0.03 | 49 | 16.22±0.58 | 260 |
| 7 | *P. niederhauserii* | PAB13-29 | 7.74±0.18 | 44 | 10.43±0.36 | 270 |
| 4 | *P. boodjera* | VHS26806 | 5.02±0.07 | 135 | 6.45±0.17 | 300 |
| 4 | *P. boodjera* | VHSC27382 | 6.26±0.01 | > 160 | 5.40±0.12 | 450 |
| 4 | *P. arenaria* | ENA1 | 5.69±0.04 | > 160 | 6.02±0.22 | 250 |
| 4 | *P. arenaria* | ENA3 | 5.68±0.03 | > 160 | 6.67±0.20 | 225 |
| 2 | *P. elongata* | TP13-32 | 5.15±0.03 | 105 | 11.33±0.67 | 550 |
| 2 | *P. elongata* | TP13-36 | 5.16±0.02 | 125 | 12.14±0.08 | 550 |
| 2 | *P. multivora* | TP13-04 | 13.05±0.23 | 19 | 12.71±0.16 | 130 |
| 2 | *P. multivora* | WAC13201 | 13.73±0.06 | 19 | 12.09±0.83 | 160 |
| 6 | *P. asparagi* | VHS17175 | 4.86±0.01 | 140 | 5.74±0.44 | 30 |
| 6 | *P. lacustris* | HSA1959 | 3.96±0.09 | > 160 | 9..14±0.33 | 70 |
| 6 | *P. litoralis* | VHS17085 | 4.66±0.01 | 80 | 5.81±0.84 | 160 |
| 6 | *P. litoralis* | VHS20763 | 4.78±0.01 | 90 | 4.00±0.46 | >900 |
| 6 | *P. thermophila* | PN42.13 | 4.64±0.01 | 5 | 3.50±0.25 | 240 |
| 6 | *P. thermophila* | VHS13530 | 1.20±0.09 | > 160 | 2.71±0.25 | 450 |
| 6 | *P. fluvialis* | DH086 | 8.22±0.01 | 5 | 12.76±0.21 | 260 |
| 6 | *P. fluvialis* | DH213 | 11.04±0.09 | 5 | 13.85±0.58 | 80 |
| 6 | *P. moyootj* | VHS16108 | 3.34±0.22 | 160 | 5.77±0.14 | 250 |
| 6 | *P. moyootj* | VHS27218 | 5.66±0.05 | 143 | 6.82±0.46 | 80 |
| 6 | *P. gibbosa* | VHS22007 | 11.89±0.02 | 70 | 16.33±0.61 | 810 |
| 6 | *P. gibbosa* | VHS21998 | 11.91±0.01 | 50 | 13.57±0.45 | >900 |
| 6 | *P. gregata* | VHS21962 | 8.00±0.00 | 40 | 17.71±0.65 | 40 |
| 6 | *P. gregata* | VHS21992 | 11.77±0.01 | 60 | 17.48±0.62 | 40 |
| 6 | *P.* sp. walnut | P281 | 9.94±0.03 | > 160 | 3.10±0.09 | >900 |
| 6 | *P.* sp. kwonganina | DDS3599 | 11.93±0.07 | 32 | 6.10±0.25 | 70 |
| 6 | *P.* sp. kwonganina | VHS23298 | 11.62±0.02 | 30 | 13.43±0.08 | 240 |
| 6 | *P. pseudorosacearum* | VHS29592 | 10.08±0.06 | 150 | 7.33±0.95 | 180 |
| 6 | *P. pseudorosacearum* | HSA2530 | 10.15±0.02 | 60 | 8.14±0.52 | 200 |
| 6 | *P. pseudorosacearum* | VHS24266 | 8.00±0.04 | 125 | 7.09±0.27 | 300 |
| 6 | *P. rosacearum* | HSA1658 | 8.91±0.02 | 120 | 4.95±0.31 | 315 |
| 6 | *P. rosacearum* | HSA1650 | 8.93±0.03 | 120 | 6.52±0.56 | 250 |
| 6 | *P. rosacearum* | VHS25476 | 6.62±0.03 | 110 | 5.86±0.49 | 250 |
| 6 | P. sp. cyperaceae | VHS25675R1 | 13.73±0.04 | 130 | 5.62±0.58 | >900 |
| 6 | P. sp. cyperaceae | VHS25675R3 | 13.75±00 | 30 | 5.34±0.32 | >900 |
| 6 | *P. inundata* | P178 | 11.21±0.08 | >160 | 6.29±0.22 | >900 |
| 6 | *P. inundata* | VHS15512 | 11.29±0.04 | > 160 | 6.57±0.25 | >900 |
| 6 | *P.* sp. condilina | VHS25244 | 11.71±0.07 | 70 | 8.67±0.58 | >900 |
| 6 | *P.* sp. condilina | PAB11.04 | 11.45±0.16 | 80 | 9.86±1.28 | >900 |
| 6 | *P.* sp. personii | SA278 | 10.39±0.02 | 160 | 8.76±0.45 | >900 |
| 6 | *P.* sp. personii | SLPA133 | 11.44±0.04 | >160 | 6.57±0.21 | >900 |

[1] growth rate mm $day^{-1}$ on solid Ribeiro's modified medium
[2] growth rate mg $day^{-1}$ in Ribeiro's modified medium with 0.03M MES

## Developing a chemical control programme for management of red needle cast in planted Pinus radiata forests in New Zealand.

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Red needle cast (RNC) is a foliar disease of *Pinus radiata* caused by *Phytophthora pluvialis* that has the potential to cause significant (up to 38%) annual growth loss in badly infected plantations. Together with Scion, the New Zealand forest industry is actively investigating management solutions to reduce disease both in existing stands of *P. radiata,* through chemical control, as well as in future plantations, through breeding for resistance. Ultimately, an operational, cost-effective, chemical treatment that can be aerially applied to foliage is required to control outbreaks of RNC in existing stands. Over a period of five years both laboratory and field trials have investigated several active ingredients that have the potential to control RNC. Phosphite has been a key active ingredient included in the research programme, together with cuprous oxide, and promising results have been obtained with both. However, inconsistency of infection in the laboratory or field, variation in persistence of efficacy, product formulation and host variation in response to infection and chemical application have indicated that finding a management solution is more complex than initially anticipated. We will discuss research progress as well as the challenges of investigating efficacy and dose response of active ingredients in controlled versus field environments. The implications of the results for the development of robust and operational recommendations that can be used by the forest industry for management of this disease will also be discussed.

## From 'then to now'- *Phytophthora* science and management in Western Australia.

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*Phytophthora cinnamomi* is recognised as a *"Key threatening process to Australia's biodiversity"* by the Australian Government. It is having a major impact on flora and fauna across most of southern Australia and Tasmania. In the south-west of Western Australia over 41% of the nearly 7000 plant species are susceptible to the pathogen. Consequently, industries such as mining companies and natural resource land managers spend huge resources trying to limit the impact and spead of the pathogen. This talk will describe how management and control methods have evolved in Western Australia based on our understanding of the pathogen's biology and ecology over 25 years of research. This will include approaches to hygiene management, use of phosphite and eradication and containment methods.

## Kauri Dieback (*Phytophthora agathidicida*) Cryptic challenges of disease detection and long term management.

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Kauri (*Agathis australis*) is a conifer endemic to northern New Zealand. In recent years, kauri trees have been found to be suffering from a disease given the common name of 'kauri dieback' and caused by *Phytophthora agathidicida* (PA). The disease infects the feeder and structural roots, killing trees of all ages. Kauri dieback has been identified as a significant threat to kauri-dominated ecosystems of northern New Zealand (Beever et al. 2009). In 2009, the Kauri Dieback Programme was formed as a joint agency response to the disease. The Programme consists of the impacted regional councils (Northland, Auckland, Bay of Plenty and Waikato), central government (Ministry for Primary Industries, Department of Conservation [DOC]) and representatives of local Māori. The Kauri Dieback Programme has established a substantial programme of science, public engagement and surveillance to determine the extent of the problem and to mitigate against the transport of PA to other areas (see www.kauridieback.co.nz). This talk will detail the challenges, approaches and progress made by the Programme with emphasis on the cryptic expression of the disease symptomology and the tactics that have been applied to develop a surveillance programme that integrates technical innovation with field experience. Details of the key findings will be presented as a 'snap shot' of what we do and don't know, and how that knowledge translates into managing the spread of the disease as the Programme transitions from a reactive phase through to the long term recovery of kauri.

## Update on *Phytophthora ramorum* mitigation at the Bloedel Reserve in Western Washington.

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In March of 2015, *Phytophthora ramorum* was detected on a sample of *Pieris* exhibiting lower crown dieback and foliar symptoms from the Bloedel Reserve. This 150 acre botanical garden encompasses undeveloped forest, pastures, a marsh, woodland plantings, and intensely maintained gardens. A team comprised of personnel from WSDA, USDA APHIS and WSU Puyallup was formed and infected plants were detected in two initial sites, the Rhododendron Glen and the Camellia Trail areas in April and May of 2015. Subsequent delimitation surveys uncovered 4 additional positives in these managed landscape areas. No positive samples were detected during a fall survey of both the unmanaged native areas and the entire perimeter of the Reserve. Beginning in November of 2015, monthly surveys were conducted and an additional 6 positive plants were detected within the Glen area, and one additional plant was located in the Camellia Trail area. Destruction of infected plants and adjacent hosts within a minimum 2-meter zone around positive plants was done as they were located. Steam mitigation of the soil in the infested areas was undertaken by WSU Puyallup. In addition to these mitigation steps, a series of best management practices were implemented to limit the potential spread of waterborne inoculum on the trails. Access to eradication zones by visitors and workers was restricted and subjected to strict sanitation procedures. During early 2016, an IPM program was initiated that included additional removal of native host material, soil applications of *Trichoderma* and mulches, applications of protectant fungicides and stringent biosanitation procedures. As of December 2016, all of the monthly surveys conducted in 2016 have been negative. Surveys will continue at this site through 2017. This project represents an example of successful collaboration between the private sector, academia, and state and federal agencies to deal with the issue of invasive plant pathogens.

# Practical solutions to ecosystem changes from sudden oak death.

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As a genus, *Phytophthora* are remarkable organisms for many reasons. These microbes can live in a broad range of environments, form and utilize a range of survival and reproductive structures, and infect and/or sporulate on a broad range of plants – both living and dead. In conjunction with ready spread in industrial nursery practices, movement of soil, and via wind and rain, it quickly becomes apparent that controlling spread of *Phytophthora* often does not have a simple or obvious solution. The destructive capacity of these organisms, in conjunction with these other characteristics, means that they pose very challenging disease management problems once established at levels where eradication is unrealistic. Cynicism within the public, management, and policy-making communities is a significant barrier to developing responses to these diseases once established. Eradication often receives attention because it offers a tangible goal, provides a clear solution, and can be highly cost effective when applied during the early stages of establishment (Lovett *et al.* 2016). As an overall strategy, eradication relies on a strong surveillance program where threats are rapidly recognized. However, this approach can leave management systems vulnerable to "black swan" events: high impact, novel, and unpredictable disease or pest emergence (Ploetz *et al.* 2013). Understanding of *Phytophthora* pathogens is rapidly developing; this means that their potential impacts are more predictable and avoidable through detection and eradication. However, for many established *Phytophthora* removal is not realistic and both realized and potential ecological, economic, or cultural impacts are severe. This is clearly the case for *Phytophthora ramorum* and the disease sudden oak death in California where pathogen populations are so spatially extensive and local inoculum loads so great that pathogen removal and limiting regional spread is unrealistic (Cunniffe *et al.* 2016). The ecological impacts of this pathogen are substantial and include deleterious interactions with wildfire, alterations of ecosystem processes regulating nutrient and carbon cycling, and a severe but selective removal of overstory tree species (Cobb *et al.* 2012; Metz *et al.* 2013).

*Phytophthora ramorum* in California is by no means a unique case study in regard to the pathogen's extensive establishment in wildlands. For example, in much of the invasive range of *P. cinnamomi* and *P. lateralis*, eradication is no longer realistic. But, epidemiological differences among these three *Phytophthora* influence the feasibility and efficacy of various management approaches. *Phytophthora cinnamomi* is a soil bone pathogen where spread can be limited or arrested by sanitary protocols among other practices (Hardy *et al.* 2001); similarly, *P. lateralis* spread can be limited by restricting movement of soil-contaminated equipment (Jules *et al.* 2002). *Phytophthora ramorum* spread via rain events up to several km greatly and complicates slow the spread management. Inoculum reduction through local eradication or removal of at-risk hosts has been effective in Curry County Oregon through a combination of intensive monitoring and aggressive treatment of invaded stands (Hansen *et al.* 2008). This effort has been successful in limiting the acreage of forest invaded by *P. ramorum* and has likely slowed the spread of the pathogen into adjacent counties and at risk forests (Cunniffe *et al.* 2016). However, this epidemiological scenario and management strategy is obsolete in a growing region of central-coastal California, an area with almost a billion at risk trees and upwards of ~40 Tg of at risk stored forest carbon (Haas 2014). Recent estimates suggest 30-45 million host trees have been infected or killed by the pathogen in California and Oregon combined (Haas 2014). Clearly, eradication efforts can be cost effective and beneficial when applied in an appropriate invasion scenario such as at the early invasion stages or in the southwest Oregon example where there is an economic justification for landscape-level control. However, these conditions do not represent hundreds of thousands of hectares of disease-impacted forests where overstory tree cover has been greatly diminished, hazardous fuels have accumulated (fuel amounts, composition, and distribution), and valuable ecosystem processes have been compromised (Cobb *et al.* 2012; Metz *et al.* 2013).

This project confronts the need to develop ameliorative solutions to *Phytophthora* impacts by designing and applying a series of forest-level management experiments aimed at restoring ecosystem structure transformed by sudden oak death. My aim is to protect the few remaining overstory tanoak (*Notholithocarpus densiflorus*) trees in highly disease impacted stands while also increasing regeneration of disease-resilient trees such as redwood (*Sequoia sempervirens*) and Douglas fir (*Pseudotsuga menziesii*). While these species are susceptible to *P. ramorum* infections they support very low levels of sporulation and do not suffer lethal cankers meaning they are likely to reestablish in the overstory and provide services such as carbon sequestration despite established local pathogen populations. Tanoak readily develops basal sprouts following an above ground injury including cutting, fire, and stem mortality from *P. ramorum* infection. Tanoak resprouting combined with dead fuel accumulation at the forest floor can be substantial and increase fire-related mortality risk trees that would otherwise be resilient during wildfire (Metz *et al.* 2013). Tanoak resprouting is sufficiently vigorous and individual stems can survive long enough that natural regeneration of resilient species is unlikely on the scale of at least several decades (Cobb *et al.* 2012). Development of desired ecosystem structure is unlikely in the most disease-impacted forests without intervention. Reducing local inoculum and slowing landscape-

level spread have received much greater attention from managers and policy makers while restoration, resiliency, and host resistance have largely been overlooked. Development of resistant hosts and restoration of disease-impacted stands is relatively unexplored by the management and research communities suggesting useful techniques may exist to protect resources and reduce risks including fire and secondary pathogen spread. Solely focusing on treatments at the expanding edge of invasion implicitly ignores existing and rapidly increasing ecological and economic costs of the disease. Furthermore the lack of forest-level restoration experiments also means that managers have few objective case studies to rely upon for tailoring a response at a particular forest.

This project is new and results are preliminary; established treatments include actions aimed to increase ecosystem resiliency by decreasing host density prior to disease invasion, and reestablishing canopy trees in forests degraded by disease outbreak (Cobb et al. 2017). Forest and disease conditions required flexible approaches in the pursuit of common goals: restricting disease impacts and creating forests resilient to multiple factors including disease (but not exclusively disease). Forest with high-priority for restoration treatments were those where multiple ecosystem services influenced the need and immediacy of disease treatments such as high recreational use, water provisioning for municipalities, and fire-risk or structure protection issues. Any treatment which reduced host density and removed sporulseeation sources resulted in measurable disease-reduction benefits using a model designed for stand-level spread and intensification of sudden oak death. Each treatment resulted in some trade-offs such as greater loss of carbon for greater disease suppression, or greater resiliency against disease and increased water provisioning but at greater initial monetary investment. Tradeoffs among cost, protection of remaining tanoak, and/or other ecosystem resources are likely to be common for *P. ramorum* and *Phytophthora* pathogens. The optimal approaches or specific treatment types are likely to vary in accordance with specific *Phytophthora* epidemiology and a greater range of management case studies may yield general predictions that accelerate management to mitigate *Phytophthora* impacts.

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# Characterising the growth and disease expression of *Phytophthora agathidicida* within the soils of contrasting land-uses.

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New Zealand kauri (*Agathis australis*) is an iconic tree species of cultural significance, and an important forest ecosystem engineer and timber resource. A disease caused by *Phytophthora agathidicida*, an aggressive root and collar pathogen affects kauri trees of all ages. This soilborne pathogen infects roots and damages tissues which distribute water and nutrients within the tree. Surveillance studies between 2008 and 2017 have shown the widespread distribution of the disease in many of the regions where kauri is established illustrating the threat to the long-term survival of the species. However, significant areas of pristine forest remain. This study is attempting to characterize the abiotic factors (e.g. C:N, particle analysis) of soils from contrasting land uses (indigenous kauri forest, pasture land, commercial pine forest), to provide understanding of growth response and gene expression of *P. agathidicida* within them. We hypothesise that the growth response of the pathogen will vary between land uses and soil depths with implications for the potential spread of the pathogen across the landscape. An observational study of *P. agathidicida* in each soil sample has been conducted to obtain response curves over an 8 day period, in parallel to which, the gene expression of mycelial mats of the pathogen exposed to soil extracts from each land-system have been analysed to better understand the direct response of the pathogen to each soil. Preliminary results suggest that there is a difference in sporangium production with time and between soils and soil depths in association with observed variation in gene expression. This study has implications for local disease management, pathogen containment and biosecurity.

# Session 2: Community Engagement

## A case study for culturally relevant *Phytophthora* research: beyond engagement and working together to cross inform indigenous and modern science knowledge systems.

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Kauri (*Agathis australis*) is a culturally and ecological keystone tree species within the kauri forest ecosystem of northern New Zealand. This iconic species is currently heavily impacted by kauri dieback caused by the soilborne pathogen *Phytophthora agathidicida.* While contemporary science offers some new approaches to understanding forest health, indigenous narratives and culture harbour an extensive knowledge of the ecological linkages (*whakapapa*) and health indicators (*tohu*) of forest health. With objectives of identifying whether there is resistance to kauri dieback and applying modern genomic and biochemical analyses to identify markers for breeding and further understanding of the risk of kauri dieback across the regions (*rohe*) threatened with kauri dieback, the Healthy Trees, Healthy Future program has partnered with Mana Whenua to progress the research in a culturally informed, relevant and respectful manner. As representatives of the Maori communities (*mana whenua*) across the regions in which kauri grow naturally, these communities are directly impacted by the introduction of *P. agathidicida.* This talk will discuss the establishment of common understanding, language and objectives in the formation of this research partnership.

## Citizen science project to accelerate research on control tools for kauri dieback.

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A new project has just been started in the northern part of New Zealand to get community involvement in research to refine tools for kauri dieback control. The programme, named Kauri Rescue™, will initially focus on phosphite treatment, but will also facilitate investigation of other tools, including potential solutions from manawhenua (Maori) traditional knowledge. Under the programme, land-owners with *Phytophthora agathidicida*-infected kauri trees will be provided with tools to treat trees on their own properties, with the expectation that they collect prescribed data about the site, and at regular intervals monitor tree health, lesion development and responses to treatment. This data will be collated and fed into the pool of knowledge on treatment of diseased and threatened trees. It is anticipated that the programme will help determine appropriate phosphite dose rates for tree ranging in size from 10 cm to 3+ m trunk diameter, and help establish whether phytotoxicity symptoms are related to site parameters or factors such as timing of treatment or weather conditions during treatment. The project team comprises scientists, social scientists, Maori, and community groups. It is anticipated that community engagement in kauri dieback control work will encourage future participation in other biosecurity and conservation-related issues. The early roll-out of the programme and engagement with the community will be discussed.

## Managing sudden oak death on Federally-administered lands in Southwest Oregon, USA: past, present, and future.

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Since 2001, approximately 2,165 hectares of tanoak forests along the southwestern coast of Oregon have been treated to eradicate *Phytophthora ramorum* and slow the spread of sudden oak death. Over 525 of these hectares are on lands administered by the United States Department of the Interior, Bureau of Land Management (BLM CB), Coos Bay District and the United States Department of Agriculture, Forest Service, Rogue River-Siskiyou National Forest (USFS RRS). Treatments include using herbicides to reduce tanoak sprouting, cutting, piling and burning known infected tanoaks, and cutting, piling, and burning exposed tanoaks and other selected hosts in a buffer area around known infected trees. Affected sites have ranged from highly accessible and heavily used hiking trails to remote, relatively inaccessible and rugged terrain. Treatments have occurred in many different land allocations including Inventoried Roadless Area, Late Successional Reserve, and Wild and Scenic River Corridor. Over the years the BLM CB and USFS RRS have made great progress to streamline the procedural side of treating sudden oak death. The tools being used include multi-year contracts with designated contractors and programmatic consultation with federal regulatory agencies. Staff positions dedicated to sudden oak death management have been created and knowledgeable people are in those positions. But still, access, fire restrictions, timing of funding, and timing treatments to avoid disturbing federally-protected threatened and endangered species, pose challenges to managing sudden oak death as rapidly as possible.

## Responding to *Phytophthora* introductions in California restoration areas: The Phytophthoras in Native Habitats Work Group.

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Plantings of native and landscape flora into parks and wildlands are a significant pathway for introduction of numerous *Phytophthora* species in California More than 40 *Phytophthora* species were recorded from San Francisco Bay Area restoration plantings, two never previously been documented in the United States, *P. quercina* and *P. tentaculata*; additionally previously undescribed species have been identified. Preliminary surveys of purchased landscape plants in California, Oregon and Washington found approximately 25% of the plants, comprised of over 100 plant species, were infected with one or more *Phytophthora* species. In Washington, *P. uniformis* (aka *P. alni* ssp. *uniformis*) was recovered from red alder, *Alnus rubra;* its first detection in a US nursery. Phytophthoras and other plant pathogens are of particular concern in restoration areas because nursery plants are placed into habitats where rare vegetation grows, setting up a direct pathway for pathogen introduction and spread. Several water departments and land management agencies have taken a precautionary approach towards detections of infested plants outplanted in their restoration sites or on nursery stock being grown for their use. Managers suspended plantings, cancelled orders or invested millions in solarization and other treatments to clean-up contaminated sites. However, not planting also has negative effects since it slows or precludes attainment of restoration goals. To address these problems, the Phytophthoras in Native Habitats Work Group, www.calphytos.org, is bringing together all aspects of the problem to coordinate a comprehensive, unified program of management, monitoring, research, education and policy to minimize the spread of *Phytophthora* pathogens. Our strategy and progress on guidelines to prevent pathogen introductions will be presented.

# Sharing plant disease information for better biodiversity outcomes using online technology - "Dieback Information Delivery Management System" – DIDMS.

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*Phytophthora cinnamomi* is a plant pathogen spread via roots, soil and water and threatens more than 600 species of native flora within the internationally recognised biodiversity hotspot of South Western Australia. Project Dieback is an ongoing ten year project funded through the Western Australian state NRM Office and federal government that addresses the threat of *P. cinnamomi*. The occurrence, distribution and threat of *P. cinnamomi* do not discriminate between differing land tenure, managers or owners. Effective management of *P. cinnamomi* requires open and transparent exchange of information between stakeholders. The online *'Dieback Information Delivery and Management System'* (DIDMS) is a web-based GIS database that facilitates stakeholders in sharing *P. cinnamomi* information to assist with awareness raising, planning and management. DIDMS is developed using Gaia Resource's "Geographic Reporting Information Database" (GRID) platform. DIDMS enables registered users to create, store, modify and share data online using standardised templates, and map production tools (Figure 1).

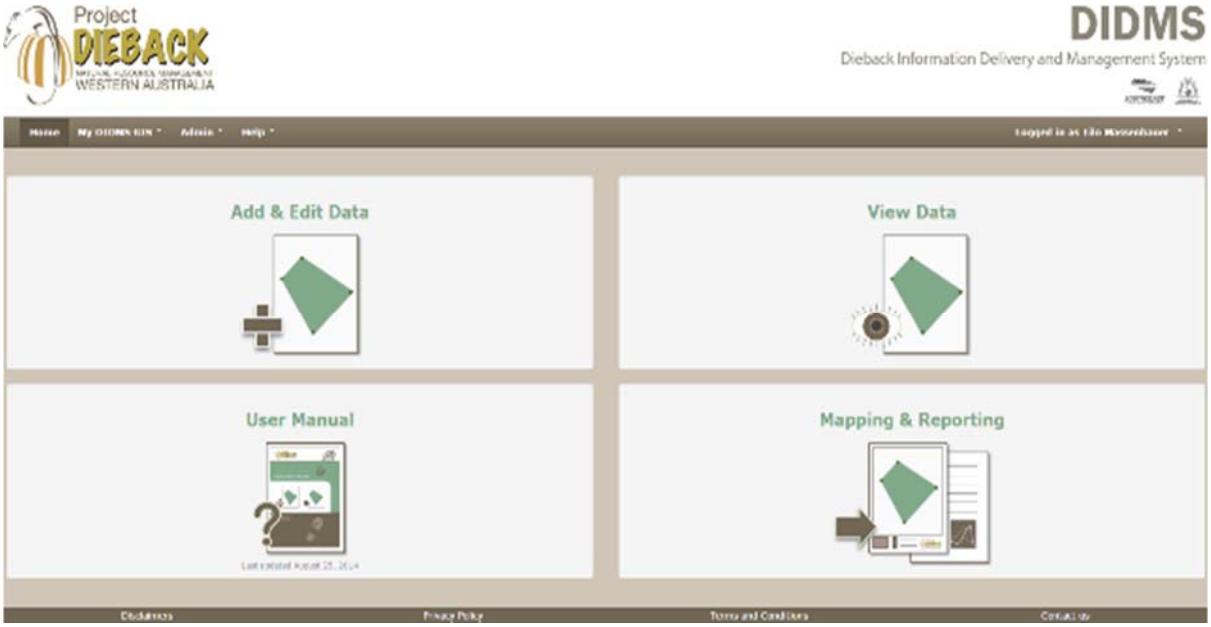


*Figure 1. DIDMS web page layout*

Data templates and information can be downloaded for further desktop GIS bulk data management and strategic planning. DIDMS caters for expert and non-expert data with disclaimers outlining how to use *P. cinnamomi* disease information. Some data within DIDMS is linked to a *'Dieback public map'* page as a quick and easy viewing platform for the general public. Registered DIDMS Users can view data as (Figure 2):

1. Activities – Where users add their own data to share with one another including GIS, photos and reports;
2. Baselayers – Data provided by the DIDMS administrator such as baselayer Plant Disease information, hydrology, climate, vegetation, Priority Protection Areas, Government data and mapbox imagery; and
3. User Layers – Where uses add their own private data that no one else can see or access such as GPS data, specific imagery, restricted threatened species data.

DIDMS contains important plant disease data as both Activities and baselayers. This information is stored in three formats, all of which used together assists users to plan, manage and communicate the threat of *Phytophthora*. The format types of plant disease data include:

1. Laboratory confirmed field samples for five different species of soil borne *Phytophthora* and a specie of Armillaria, which are stored as disease points.

2. Department of Parks and Wildlife Registered Phytophthora Dieback interpretation area mapping of six *P. cinnamomi* disease status categories, which are stored as polygons; and
3. South Coast NRM generated soil borne Phytophthora Hazard Dispersion GIS Modelling as a raster image.

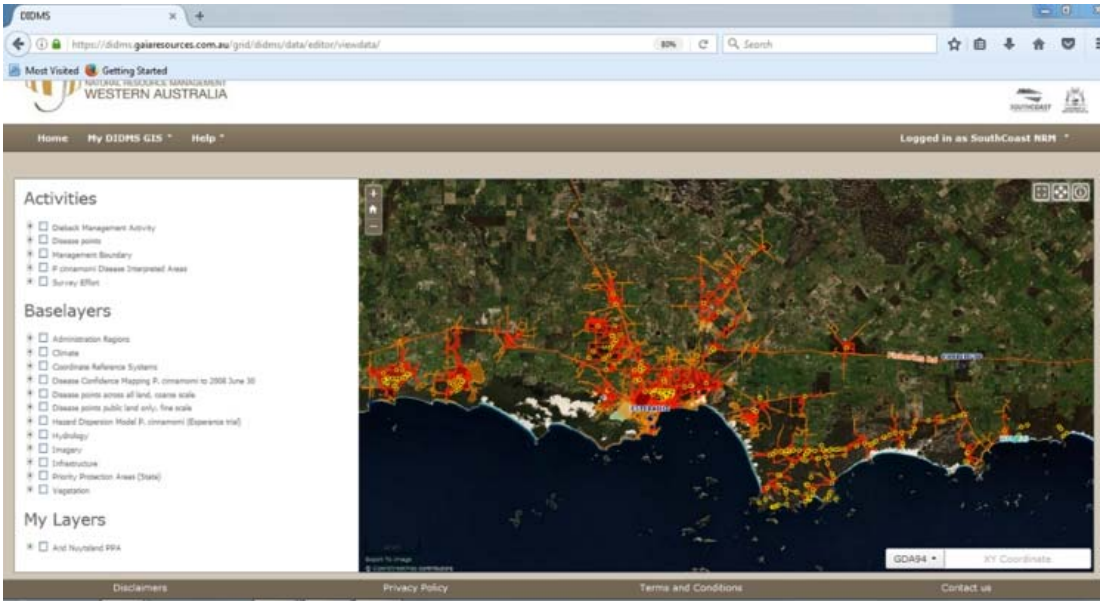


*Figure 2. Viewing data types in DIDMS showing plant disease points and Phytophthora Hazard Dispersion Modelling*

Once a DIDMS user has viewed data they can rapidly create a map within minutes to be downloaded as a PDF file and imported into a report (Figure 4). To assist in making the process of adding data, viewing data and creating maps within DIDMS easier there is a simple User Manual available to view online. South Coast NRM have also provided several 3 hour DIDMS Training workshops to key stakeholders to support adoption and ongoing efficacy of use. There are more than 240 registered users from government, private and not-for-profit organisations using DIDMS and all have access to free online training resources. DIDMS provides stakeholders a safe repository to share *P. cinnamomi* information for education and planning purposes. The information stored within DIDMS helps stakeholders implement targeted *P. cinnamomi* mitigation actions resulting in better biodiversity outcomes. For further information regarding DIDMS visit: http://www.dieback.net.au/


*Figure 3: Example of a simple DIDMS Map showing P. cinnamomi interpretation mapping disease status*

# An Investment Framework for Managing Phytophthora Dieback in south-west Western Australia.

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Phytophthora Dieback is a plant disease caused by *Phytophthora cinnamomi*, an introduced pathogen that affects up to 40% of native species in the south-west of Western Australia. In 2012, South Coast Natural Resource Management received funding from the Western Australian state NRM office to undertake *Project Dieback – 'Action and Opportunities for Protecting Biodiversity Assets'* in Western Australia. As part of Project Dieback, A **State Phytophthora Dieback Management and Investment Framework (Framework)** was developed in part to identify and rank the top 100 Priority Protection Areas (PPAs) in Western Australia that represent the most significant examples of ecosystems supporting plant species and communities vulnerable to *Phytophthor*a Dieback, in Western Australia that should be protected and conserved over the next 50 years (Figure 1).

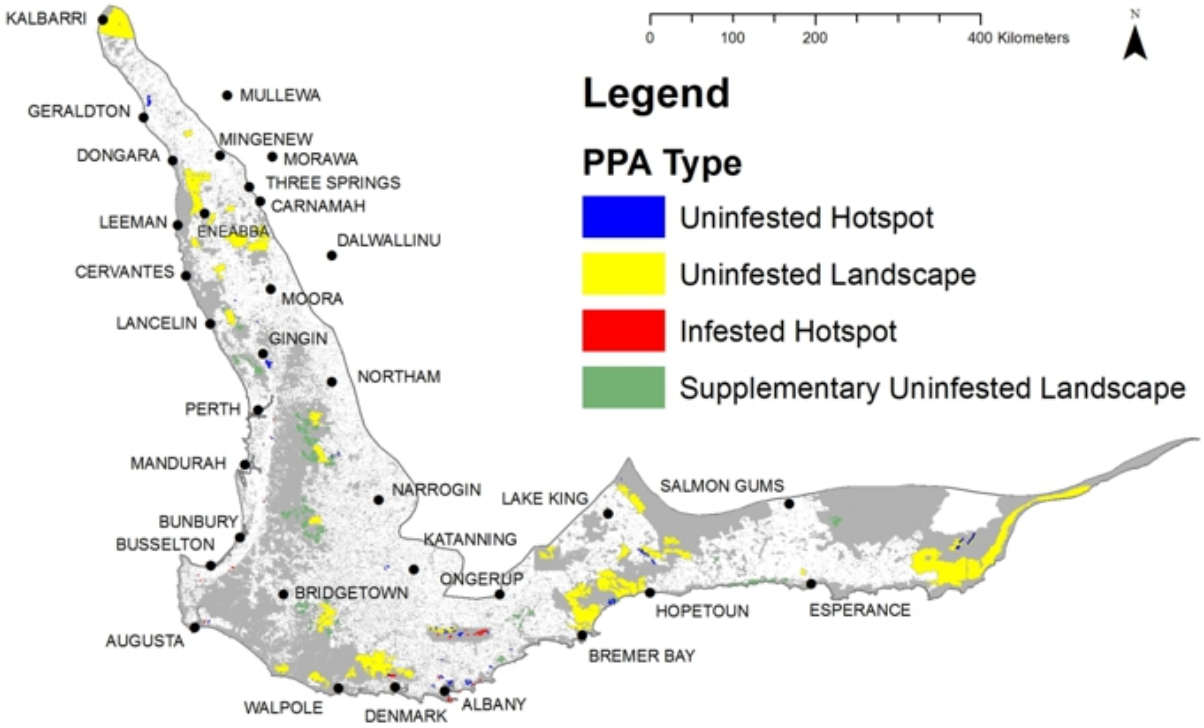


Figure 1. Representative biodiversity PPAs

The **Framework** provides a logical process and operational toolkit to plan collaborative, area-specific management actions and investment strategies required to protect PPAs against the adverse impacts of Phytophthora Dieback at a landscape scale (Figure 2). It provides a structure to:

1. Identify high value biodiversity representative *P. cinnamomi* susceptible areas considered a priority for management (the PPAs) using multiple criteria, GIS modelling, state wide datasets and expert weightings.
2. These PPAs are grouped into types based on the GIS analysis of weighted values, geographic concentration, susceptibility, known broad scale landscape infestation and down-stream risk, and local area stakeholder review. The PPA types are:
    a. Uninfested Hotspot – Uninfested areas with concentrations of *P. cinnamomi* susceptible high value flora species, communities and landscapes.
    b. Uninfested Landscape – Extensive uninfested *P. cinnamomi* susceptible high value areas with dispersed values included rare flora species, communities, and landscapes.
    c. Infested Hotspot – *P. cinnamomi* infested high value areas with a concentration of high value susceptible flora species and communities confined to a small area.

d. Supplementary Uninfested Landscapes – Lower scored Uninfested value areas identified through a local stakeholder review to supplement biodiversity representative gaps and *P. cinnamomi* disease status gaps for Uninfested Hotspots and Landscapes.

3. Identify values, objectives, threats, management strategies and actions as part of a standardised area planning process.
4. Assess the feasibility of management strategies and actions.
5. Develop risk reduction plans for identified PPAs using standardised template documentation, so as to compare like-with-like when allocating resources.
6. Implement priority projects.
7. Monitor and evaluate implementation and if necessary adapt management strategies, objectives and/or goals.

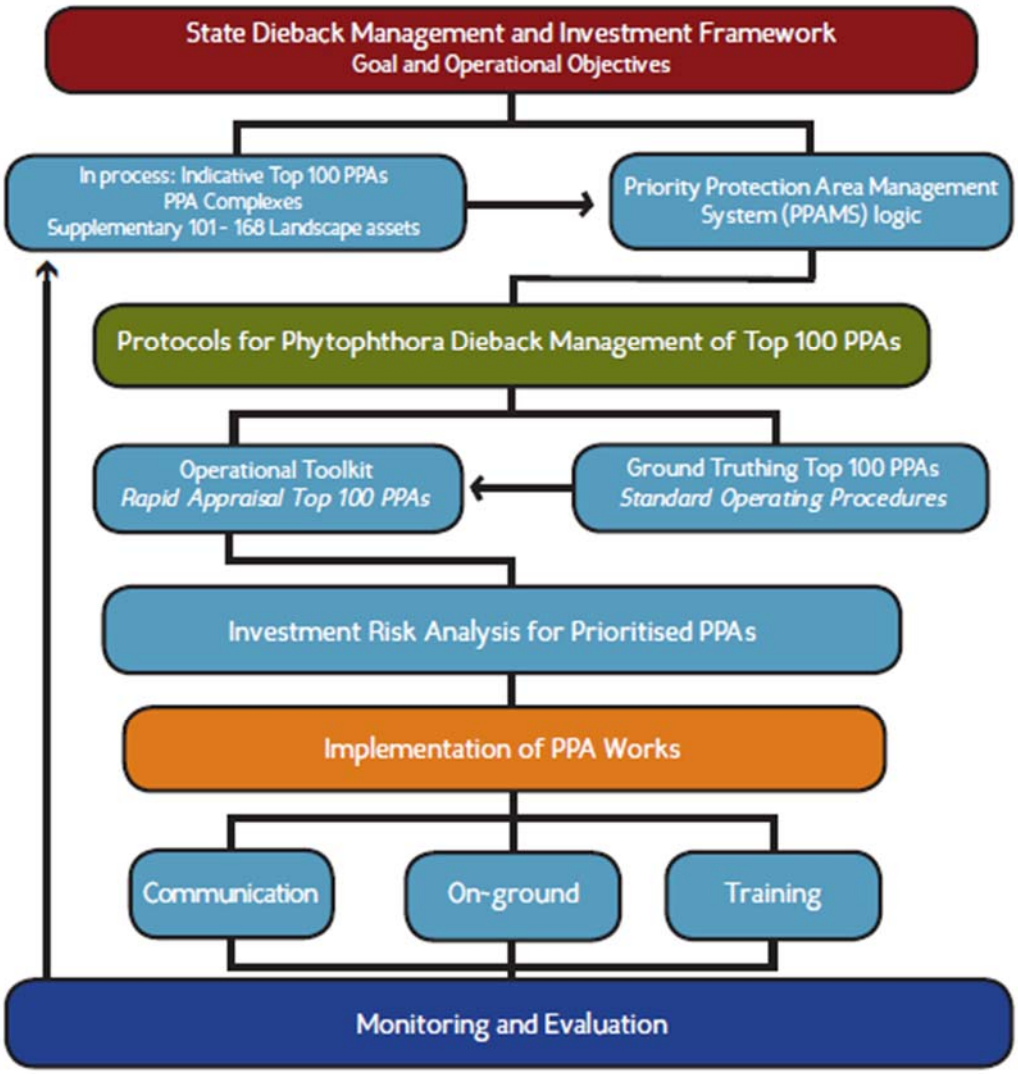


*Figure 2. Flow chart outlining the hierarchical steps of the **Framework**.*

The Framework provides direction for targeted investment that can be applied as a standardised tool for any parties wishing to prioritise *Phytophthora* Dieback management in their local area, large or small, complex or simple. Further information about the **Framework** is available at:

South Coast NRM (2014), State *Phytophthora* Dieback Management and Investment Framework VERSION 1/JULY 2014. http://www.dieback.net.au/about/state-dieback-management-and-investment-framework.html

## Cape Citizen Science: public engagement to survey *Phytophthora* in a developing country.

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Developing countries often play a major role in the dissemination of invasive forest pathogens. This is due to suboptimal quarantine systems and the subsequent establishment of pathogen populations increasing the likelihood of further spread via the "bridgehead effect". These countries generally have limited resources for early detection and monitoring, specifically with regards to training and human capacity. Citizen science projects may aid monitoring programs in these countries because they incorporate contributions from non-scientists, broadening sampling distributions at low costs, and they provide training to increase scientific inquiry and invasive species reports. *Cape Citizen Science* (http://citsci.co.za) is a project that involves the general public in the detection and sampling of plants affected by *Phytophthora* species in an important biodiversity hotspot of the world, the Western Cape Province of South Africa. Preliminary results indicate that non-scientists are invaluable for reporting disease incidence, isolating, and submitting *Phytophthora* isolates for larger ecological research programs. This clearly demonstrates that citizen science is a useful tool to increase monitoring capacity and to promote the early detection of invasive plant pathogens, especially in developing countries.

## "Unlocking a Nation of Curious Minds": a science participatory platform for the next generation of *Phytophthora* scientists.

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The "Curious Minds" program is an initiative of the central government of New Zealand, aiming to encourage and enable better engagement with science and technology across all sectors of New Zealand, starting at school-level. The objective is to partner schools with science-delivery organisations, to develop fun and interesting science projects to which, students are able to bring their unique, and fresh perspectives, to answer real-life questions. It also aims to demonstrate the relevance of science in the students' everyday lives. "Keeping Kauri Standing" is a national, multi-agency, bicultural response to manage and control Kauri dieback. Part of the approach to active, landscape-level, surveillance of this pathogen, is the baiting of streams to capture the diversity of *Phytophthora* in waterways, downstream of infested forests. The collaborative project, provided the students with opportunities to design a stream-based sampling strategy, using study sites which we have previously established in west-Auckland. The students invented a re-usable "bait cassette", designed with material engineers using CAD for Kids and extruded on a 3D-printer. The innovative cassette has a number of advantages over the conventional technology, and enabled the students to carry out the disinfestation, dissecting and plating of the leaf baits to *Phytophthora*-selective media. Students successfully recovered a diverse array of *Phytophthora*, *Pythium* and *Phytopythium* species, which they were able to sub-culture from their initial isolations. The vision is to extend the program within NZ to engage schools with acsces to kauri forests. There is also an opportunity to involve schools in Australia (e.g. Melbourne, Perth), South Africa and USA (e.g. Corvallis) to develop an "International Student Surveillance Network", through which students can share their experiences in forest and the values which they are trying to preserve.

## *Phytophthora* control in Western Australian backyards and bushland, where the solution isn't the only solution!

Glenn Tuffnell[1]
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Glenn and his team at Dieback Treatment Services have been using phosphite to control the root to root spread of *Phytophthora cinnamomi* in Western Australia since early 2003. Prior to this time Glenn was an employee in a Western Australian State Government Conservation and Land Management Agency before establishing a dedicated Phytophthora Mapping Service catering to a wide range of clients. Glenn's on ground Phytophthora management experience spans a quarter of a century with a current list of clients that range from individuals with one *Eucalyptus marginata* that they wish to protect to Local government organization's responsible for managing hundreds of hectares of natural ecosystem. This presentation will discuss the formation of this business from the perspective of the business owner, the challenges presented by community attitude and government systems and the key role that the scientific community play in formulating strategies that translate into on ground management.

## Green Card: A new training standard for biosecurity hygiene management.

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The Australian Environmental Protection Biodiversity Conservation Act 1999 is a key piece of environmental legislation which lists 'Disease in natural ecosystems caused by *Phytophthora cinnamomi*' as a key threatening process to Australia's biodiversity. As the legislation requires, a Threat Abatement Plan (TAP) was developed for this threat, outlining how the threat is to be addressed. A 2014 revision of this TAP prescribed the development of a national training standard for on-ground operators in areas threatened by *Phytophthora cinnamomi*. In Western Australia (WA), comparable training was already being administered internally within the Department of Parks and Wildlife. To meet the need for a national training standard however, the scope of the applicability and deliverability of the training needed to be broadened so that any person or organisation requiring the training could access it. With the cooperation of Parks and Wildlife, the Dieback Working Group adapted the training and developed a new training standard that could be offered to operators across tenure, tiers of Government and a wide range of industries. Green Card Training has now been run throughout the South West of WA for hundreds of people from a wide range of organisations and industry types. An increasing number of organisations are also adopting the training as a standard for on-ground operators on their tenure or within their organisation. The focus of Green Card Training on understanding *Phytophthora* diseases, their impact, trainee responsibilities for management and relevant biosecurity hygiene practices makes it an effective management tool for any organisation. I will present an overview of the training program and our progress towards creating a national training standard for Phytophthora Dieback hygiene management in Australia as well as plans for the future of the program.

# The challenges of *Phytophthora* study and management in the forest of Nepal (Review study).

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Forests are important resource for practically in Nepal. Apart from timber and wood products, forest supplies variety of valuable goods and services including implications for global climate regulations. In Nepal due to its altitudinal variation ranging from a 60 meters to the highest vegetation line, Nepal harbours critical forest ecosystems. However the factor affecting the establishment and growth of trees is unpredictable since we do not have field studies information on effects of disease and pathogens in forest of Nepal. Previous research found that the occurrence of a previously unknown *Phytophthora* in a remote forest in Nepal highlights the plant health risk associated with moving rooted plants and soil between different bio-geographical regions of the world and the need for rapid pathological screening of potential risk organisms. Therefore, the programme of actions to cope with forest pathogens is quite urgent in Nepal. However, understanding exactly how the Himalayan forest will be affected by *Phytophthora* is extremely challenging, and is further complicated by lack of knowledge, data and monitoring of forest diseases. Understand the effect of *Phytophthora* and eradication at a management level is necessary at diverse plant communities and varying soil types in Nepal.

# Session 3: Diversity

## A study of *Phytophthora* communities in the soils of two French Guiana forest sites, using three different identification methods.

Jean Legeay[1,2], Claude Husson[1,2], Marc Buée[1,2], Benoît Marcais[1,2] Chris Baraloto[3], Heidy Schimann[3] , Eliane Louisanna[3]
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jean.legeay@inra.fr

*Phytophthora* communities in neo-tropical soils and the factors structuring them are not well-known. The relatively new metabarcoding techniques could allow us to better describe this diversity compared to more traditional baiting methods. To answer this question, soil was sampled at two sites of French Guiana, a natural forest and a tree plantation made of monospecific plots. From these soils, three strategies were used to investigate *Phytophthora* diversity: metabarcoding of DNA directly extracted from soil; metabarcoding of leaves used as baits for the *Phytophthora*; and baiting followed by isolation on Petri dish. Surprisingly, with all three methods, a very low *Phytophthora* diversity was retrieved, counting only 5 species with an overwhelming dominance of *Phytophthora heveae*. No correlation to the host tree species or to habitat (soil or litter) could be established. Although the different methods yielded different results, the diversity obtained with each method were equal, which confirmed the low diversity of *Phytophthora* communities in the soils of this site.

## Distribution and causal agents of ink disease of *Castanea sativa* in Southern Switzerland.

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In southern Switzerland, European chestnut (*Castanea sativa*) is the dominant tree species up to about 900 m a.s.l., forming a continuous forest belt of 30,000 hectares. Besides having a high cultural value, local chestnut stands are an important landscape component and protect the mountain slopes from erosion and other natural hazards. In the last three decades, an increasing dieback of chestnut trees has been observed. The specific symptoms displayed by the declining trees (i.e. thinning of the crown, small chlorotic leaves, necroses at the root collar) suggested an emergence of ink disease. In this study, we determined the geographic distribution of this phenomenon and which *Phytophthora* species were involved. An inquiry conducted at the local forest service showed that chestnut dieback is concentrated in three main regions. A total of 19 declining chestnut stands were investigated for the presence of *Phytophthora* in the rhizosphere of 5 symptomatic trees and 10 asymptomatic trees (5 in a symptomatic and 5 in an asymptomatic sector of the stand). *Phytophthora* was isolated in 14 chestnut stands and its highest incidence was observed in the soil around symptomatic trees. ITS sequencing revealed a low *Phytophthora* species diversity, with 84% of the isolates belonging to *P. cinnamomi*, 11% to *P. plurivora*, and 2.5% each to *P. cactorum* and *P. cambivora*. The non-native *P. cinnamomi*, a well-known causal agent of ink disease, was the most frequent species in the rhizosphere of all three types of tree considered, and was particularly dominant in the geographic region with the mildest winter climate. This raises a major concern about a possible further spread of this invasive species in a scenario of climate change.

## Metabarcoding of *Phytophthora* communities in Scottish soils.

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*Phytophthora* species such as *P. ramorum*, *P. kernoviae*, *P. lateralis* and *P. austrocedri* have emerged in Britain over the last fifteen years to cause significant mortality on a range of woody hosts. The number of forest and woodland sites known to be infected with these pathogens is increasing, and therefore a better knowledge of the diversity of *Phytophthora* and the mechanisms of spread from site to site is of great importance in developing management and mitigation strategies for these diseases. The soil environment plays an integral role in the spread and establishment of *Phytophthora* pathogens, which can persist long term in soil in the form of resilient thick-walled spores. Waterlogged soils may also harbour free-swimming zoospores, the main mechanism by which *Phytophthora* infects plants. In this study, DNA was extracted from soils taken from around 10 trees in 15 sites located in Scotland. The selected sites represent various habitats including gardens, woodlands and forests. ITS1 amplicons were generated using modified *Phytophthora* primers and sequenced on an Illumina MiSeq platform. Results reveal a diversity of *Phytophthora* species with new locations for several quarantine species and new species to science and/or Scotland. This study demonstrates the extent to which different *Phytophthora* species are endemic in various soil environments across Scotland and the potential risks posed by spread of contaminated soil.

## *Phytophthora* affecting protected beech forests across Southern Sweden.

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Michelle.Cleary@slu.se

European beech (*Fagus sylvatica* L.) is an important forest tree species. Beech is susceptible to a range of Phytophthora species. Since 2010, concern has been growing around the increasing number of reports of damaged beech trees in protected forests and urban settings in southern Sweden. To reveal causal agents and assess damages, surveys were conducted in parks and protected beech forests in southern Sweden. Field sites were surveyed and symptomatic trees sampled to identify causal agents of damage. Classical isolation techniques using direct plating and baiting were used on tissue samples collected from the leading edges of bleeding cankers, and soils samples, respectively. Oomycete diversity was determined by sequencing ITS environmental DNA (eDNA) from stem tissues and soil (multiplex-sequenced using 454- and Illumina MiSeq), while isolates were identified via Sanger sequencing. Results revealed the presence of *P. plurivora, P. cactorum, P. syringae, P. cambivora*, and *P. gonapodyides.* It is the first time *P. gonapodyides* has been associated with stem damages on beech in Sweden. Implications for management of beech in protected forests and urban parks will be discussed.

## Multiple new cryptic *Phytophthora* species from Fagaceae forests in Europe.

Thomas Jung[1,2], Marília Horta Jung[1], Santa Olga Cacciola[3], Thomas Cech[4], Jozsef Bakonyi[5], Diana Seress[5], Saveria Mosca[6], Leonardo Schena[6], Salvatore Seddaiu[7], Antonella Pane[3], Gaetano Magnano di San Lio[6], Cristiana Maia[1], Alfredo Cravador[1], Antonio Franceschini[8] and Bruno Scanu[8]
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During surveys of *Phytophthora* diversity in natural and semi-natural Fagaceae forests in Austria, Italy and Portugal, four new cryptic species were isolated from rhizosphere soil samples. Multigene phylogeny based on nuclear ITS, ß-tubulin and HSP90 and mitochondrial *cox*1 and NADH1 gene sequences demonstrated that two species, *Phytophthora tyrrhenica* nom. prov. and *P. vulcanica* nom. prov., belong to phylogenetic Clade 7a with *P. uliginosa, P. europaea* and *P. flexuosa* being their closest relatives. The other two species, *P. castanetorum* nom. prov. and *P. tubulina* nom. prov., were related to *P. quercina* and the informally designated taxon *P.* sp. ohioensis from Clade 1. All four new species are homothallic, have low optimum and maximum temperatures for growth and very slow growth rates at their respective optimum temperatures. They differed from each other and from related species by a unique combination of morphological characters, cardinal temperatures and growth rates. Interestingly, three of the four new species always co-occurred with other *Phytophthora* species in the rhizosphere of declining trees. Pathogenicity tests, using a standardized soil infestation method, demonstrated that all new species were able to cause damage to the roots of seedlings of their respective hosts *Fagus sylvatica*, *Castanea sativa*, *Quercus ilex* and *Q. suber*. However, in comparison to the aggressiveness of their co-occurring *Phytophthora* species, *P. cambivora*, *P. cinnamomi* and *P. plurivora*, they should be considered as relatively weak pathogens. Further research is required to understand the ecological role of these cryptic species in the Fagaceae ecosystems.

## A third *Phytophthora* species causing wild olive decline in Spanish forests.

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*[1]Agronomy Department (Agroforest Pathology). ETSIAM, University of Córdoba. Córdoba, Spain. [2]Disease Diagnostic and Advisory Service. Forest Research. Alice Holt Lodge, Farnham, United Kingdom.*
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Since 2009, a severe decline caused by *Phytophthora cryptogea* and *P. megasperma* was observed in a protected wild-olive forest (*Olea europaea* var. *sylvestris*) of high ecological value (Dehesa de Abajo, Seville, Spain). In this natural forest, two new samplings of roots and soil were done on 25 symptomatic wild-olives in autumn 2014 and 2015. For each sample, feeder root segments were directly plated on NARPH; and olive leave baits were prepared for soil isolations. Apart from *P. cryptogea* A1 and *P. megasperma*, a third *Phytopththora* species were consistently isolated from roots in 2014 (24% of sampled trees) and 2015 (36% of sampled trees). These isolates were morphologically identified as belonging to the *P. citricola* complex and confirmed by analysis of their ITS regions. Their morphology fit well to *P. plurivora* or *P. multivora* and it will be confirmed according to β-tubulin and cytochrome oxidase sequences. Temperature growth relationships obtained in CA medium (5 to 35º C) showed no growth at 5º C nor at 30º C, with maximum growth registered at 19.9º C. Pathogenicity was confirmed on 1 year-old healthy wild olives inoculated by adding oospore suspensions (50 ml per plant, $2.2\times10^4$ oospores×ml$^{-1}$) to the rootballs. Pathogenicity on wild-olive was similar for the three *Phytophthora* species associated, but temperature requirements are quite different. It is hypothesized that they may show their active periods in different seasons depending on the climatic conditions.

# Diversity of *Phytophthora* species in forests, forest nurseries and riparian ecosystems of Portugal.

Marília Horta Jung[1,2], Alfredo Cravador[1], Cristiana Maia[1], Leonardo Schena[3], Saveria Mosca[3], Beatriz Mora Sala[4], Giuseppe Carella[5], Salvatore Moricca[5], József Bakonyi[5], Diana Seress[6], Bruno Scanu[7], Lidia Dionísio[8] and Thomas Jung[1,2,9]

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From 2014 to 2016, in the frame of the European BiodivERsA Project RESIPATH, a survey of *Phytophthora* diversity and diseases was performed in 68 forest stands, 12 forest nurseries, 38 river systems and 4 lagoon ecosystems across Portugal. In forests of *Quercus suber*, *Q. ilex*, *Q. robur*, *Q. faginea*, *Q. pyrenaica*, *Castanea sativa*, *Fagus sylvatica*, *Betula celtiberica* and other tree species *Phytophthora*-type decline and dieback symptoms were common. Bleeding stem cankers were frequently observed in *Q. suber* and *C. sativa*. Severe collar rot and dieback of *Alnus glutinosa* was observed along multiple rivers. In all nurseries typical *Phytophthora* symptoms and scattered or patchy mortality were common. A total of 2131 isolates were obtained from 61 forest sites, 37 rivers and all 12 nurseries using baiting assays and direct plating of necrotic tissues. Isolates were identified using both classical identification and sequence analysis of ITS and *cox1* and belonged to 24 known species, 1 informally designated taxon and 9 previously unknown taxa of *Phytophthora*. In addition an array of *Phytophthora* hybrids from Clades 6 and 9, *Nothophytophthora amphigynosa* nom. prov., *Halophytophthora avicenniae* and a new *Halophytophthora* species, 7 known species and one new taxon of *Phytopythium* and multiple *Pythium* species were isolated. The detection of 9 new *Phytophthora* taxa, the first records of *P. amnicola*, *P. boodjera*, *P. hydropathica*, *P. meadii*, *P. quercetorum* and *P. thermophila* in Europe and the finding of *P. ramorum* in a forest stream are of particular concern. Extensive host range testing of new species is needed to clarify their potential threat to European forests. Multigene phylogenetic analyses and morphological and physiological studies are underway for the official description of all new *Phytophthora* taxa. The ubiquitous *Phytophthora* infestations of forest nurseries pose a serious threat to reforestations and afforestations in Portugal.

# Section 4: Ecology

## Role of *Phytophthora* species in the lack of seedling recruitment syndrome in Mediterranean oak forests.

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Oak forests are far the most important ecosystems in the Mediterranean regions. However, their sustainability is under threat due to severe decline process, climate change and lack of natural regeneration. The oomycetes *Phytophthora* spp. have been associated with oak decline, with *P. cinnamomi* being the most widespread species. Besides killing adult trees, *Phytophthora* can in turn act as damping-off pathogen affecting the natural regeneration. This study aimed to explore the diversity of *Phytophthora* species occurring in Sardinian oak forests (Italy) and to investigate the variation in early survival of oak seedlings to *Phytophthora* infections. Soil and root samples underneath oak trees and from seedlings showing symptoms of *Phytophthora* infection were baited using fresh oak leaves. Several *Phytophthora* species were isolated and identified based on morphology and DNA sequences analyses. The susceptibility of germinating acorns to *Phytophthora* infections was tested by immerging growing taproot in a zoospores suspension. Acorns germination and seedlings survival rates were assessed in naturally and artificially infested soil. Although with different rates, *Phytophthora* assayed species were able to cause a significant reduction of roots development. In the field, the lack of seedling recruitment was assessed in randomly selected plots through *Phytophthora* infested and disease-free sites. First results showed that *Phytophthora* is impacting on oak seedling recruitment, with considerable post-emergent damping-off occurring on diseased sites. This observations have potential devastating long-term impact on oak forests that are succumbing to *Phytophthora* but with no recruitment. The ecological implications of these findings are discussed.

## Damping-off of native southwest Australian plant species by *Phytophthora* and *Pythium* species.

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*Phytophthora* species have the ability to kill seed and seedlings pre- and post-emergence (damping-off). Large quantities of seed are sown in southwest Australia to rehabilitate hyper-diverse shrubland removed during mining. A high proportion of the seed mix does not emerge and damping-off pathogens are hypothesised to contribute to this loss. Additionally, native damping-off oomycetes can maintain the diversity of tropical plant communities and may play a role in shaping Mediterranean shrubland. A glasshouse experiment has been established to determine if these oomycetes are damping-off pathogens, and if they could impact native and rehabilitated plant communities. The experiment will test the effect of five *Phytophthora* and a *Pythium* species, commonly isolated from hyper-diverse shrubland and rehabilitation in southwest Australia, on the germination of 30 native plant species. The statistical analysis will additionally attempt to determine if seed and plant traits make particular plant species more susceptible to damping-off. A preliminary glasshouse experiment indicated *Phytophthora arenaria*, believed to be native to southwest Australia, significantly reduced the emergence and survival of seedlings from four different plant species. The introduced *P. cinnamomi* was a prolific damping-off pathogen as it significantly reduced seedling emergence in seven of the fourteen plant species. If these results are confirmed, it will indicate *Phytophthora* species may be the cause of seed loss in rehabilitation, and native *Phytophthora* might play a role in shaping hyper-diverse shrubland plant communities, warranting further study.

## Native soil-borne pathogens equalize differences in competitive ability between plants of contrasting nutrient-acquisition strategies.

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Soil-borne pathogens are usually seen as detrimental agents of both agricultural crops and natural ecosystems. Their effects as invasive organisms has been widely studied but their role native ecosystems remains poorly understood. In tropical rainforests, soil-borne pathogens can contribute to the maintenance of high plant diversity by reducing differences in competitive ability between co-occurring plant species. However, evidence of this in other hyperdiverse ecosystems is lacking. In hyperdiverse shrublands in south-western Australia, non-mycorrhizal cluster-rooted Proteaceae are the dominant species but co-occur with many other plant species using other phosphorus-acquisition strategies, such as Myrtaceae that forms ectomycorrhizal (ECM) associations. In a glasshouse experiment, we grew Proteaceae and Myrtaceae species from hyperdiverse shrublands alone and in competition with each other, and in the presence or absence of native soil-borne pathogens (*Phytophthora* spp.). We hypothesized that native *Phytophthora* species are more detrimental to Proteaceae than co-occurring ECM plants, and that this reduces differences in competitiveness between these two plant families. When seedlings where grown alone, biomass of non-mycorrhizal plants was lower in the presence of *Phytophthora*, while ECM species were unaffected. When non-mycorrhizal and ECM species were planted together, ECM plants grew better with *Phytophthora* than in its absence, because *Phytophthora* reduced growth of non-mycorrhizal competitors. Growth of ECM plants was positively correlated with percent root colonization by ECM fungi, but this was only significant when ECM plants were grown in the presence of *Phytophthora*. We showed that native soil-borne pathogens equalized differences in competitive ability between seedlings of contrasting nutrient-acquisition strategies, potentially due to a trade-off between nutrient-acquisition efficiency and pathogen defense.

## Current status of *Phytophthora pluvialis* in western North America.

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*Phytophthora pluvialis* was formally described in 2013, although it was first recognized as a distinct species in 2002. *P. pluvialis* was recovered from baited streams, soil, canopy drip and rarely from necrotic tanoak tissues in the mixed tanoak (*Notholithocarpus densiflorus*)-Douglas-fir (*Pseudotsuga menziesii*) forest in southwestern Oregon, USA. In 2014 the pathogen was reported from New Zealand, associated with red needle cast of radiata pine (*Pinus radiata*) and Douglas-fir. Pathogenicity testing on Douglas-fir in Oregon was completed in 2014, and surveys were initiated in streams and in Douglas-fir plantations in California, Oregon, and Washington. *Phytophthora pluvialis* has now been identified in forest streams in northern California and western Oregon. It was recovered from rain traps and/or symptomatic Douglas-fir needles in western Oregon and southern Washington. In most years it appears to be a widespread but inconspicuous foliar pathogen in the Douglas-fir forest. Until the winter of 2014-2015 it was not associated with specific symptoms on Douglas-fir. In that season dramatic chlorosis and reddening of needles were observed on trees of all ages in many locations in the central coast range of Oregon. In many cases current year needles were cast from trees while they were still green. In 2016, however, dramatic symptoms were no longer observed and most trees recovered, although the pathogen was still identified from raintraps in stands known to be infected in previous years.

## Do *Phytophthora* species migrate from natural forest to plantations of non-native trees in South Africa?

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*Phytophthora* spp. are amongst the world's most devastating plant pathogens. In this study, we considered the diversity of *Phytophthora* species associated with *Eucalyptus grandis* and *Acacia mearnsii* grown as non-natives in plantations and compared these with species in adjacent natural forest in eastern South Africa. In so doing, we wished to determine whether *Phytophthora* species are migrating between these ecological niches. Both isolation by soil baiting and ITS-profiling using *Phytophthora*-specific primers were used. A total of 120 soil samples were collected from four sites, (Commondale, Melmoth, Vryheid and Howick). Using soil-baiting, we recovered 85 *Phytophthora* isolates representing 5 species: *P. alticola* (3), *P. cinnamomi* (33), *P. drecshleri* (4), *P. frigida* (36) and *P. multivora* (8). For ITS-profiling, environmental DNA (eDNA) was extracted from the soil samples and then, amplified using primers 18Phy2F/5.8-1R and ITS6/5.8-1R. Clustering of 123,308 reads resulted in 314 Molecular Operational Taxonomic Units (MOTUs) corresponding to a total of 33 *Phytophthora* species including seven undescribed taxa spanning across all 10 ITS clades. Natural forest at all sites had the highest species diversity. The *Phytophthora* diversity failed to cluster based on vegetation type. There was also a clear indication of species migration from natural forest to plantations. Most species in plantations were present in the natural forest sites and there were no exclusive species within *A. mearnsii* and *E. grandis* plantations.

## Shifting disease dynamic of *Phytophthora ramorum* causing localized epidemics on sweet chestnut (*Castanea sativa*).

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Since the first findings of *Phytophthora ramorum* in Britain in 2002, disease impacts have altered as the epidemic has progressed. Initially, ornamental plant nurseries then valuable heritage plants in gardens were affected and by the mid-2000s *P. ramorum* was established in woodlands where species such as European beech (*Fagus sylvatica*) and sweet chestnut (*Castanea sativa*) were susceptible hosts although relatively few trees (<100) succumbed. *Rhododendron*, particularly invasive *Rhododendron ponticum*, was the common host across all these environments and it proved essential to the epidemic because it sustained pathogen sporulation whereas most tree hosts did not. However, in 2009 the shift of *P. ramorum* to Japanese larch (*Larix kaempferi*) saw the start of widespread mortality as larch stems were girdled and *P. ramorum* sporulated prolifically on infected needles causing landscape scale losses across parts of western Britain. Recently, another change in the epidemic has occurred with observations pointing to ramorum disease cycling on sweet chestnut in some woodlands in the absence of any sporulation from larch or rhododendron. Although laboratory trials have shown that *P. ramorum* can sporulate on sweet chestnut leaves, there had been little evidence that this was significant in the wider environment. But in some remnant ancient semi-natural woodlands in south west England, more than 40% of chestnuts now have aerial infections caused by *P. ramorum*, and in some trees this is leading to major crown dieback. Evidence suggests this is due to the pathogen extending from multiple lesions on leaves, shoots and epicormics into scaffold branches and trunks. Even though infected, sporulating leaves are shed during autumn, infection may occur via leaf scars or be initiated the following year from inoculum persisting in buds. Current *P. ramorum* management concentrates on the clearance of infected larch and rhododendron, but if sweet chestnut is providing additional sporulation foci management protocols will need to be reconsidered.

## Disease Ecology of *Phythophthora ramorum* in sympatric transmissive and dead-end hosts.

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Although models incorporating uniform estimates of infection rate and susceptibility are powerful tools for predicting disease epidemics and designing interventions, the role of transmission heterogeneity at individual, species-level, and spatial scales is increasingly being recognized as an important driver of disease epidemics. For instance, particular individuals, host species, and/or sites may have an outsized effect on transmission, earning the designations "superspreading" hosts and "hotspots," respectively. This study examines bay (transmissive host for the Sudden Oak Death or SOD pathogen *Phytophthora ramorum*) infection at the individual and plot levels and its association with oak (dead-end host) and tanoak infection and mortality across seasons and years in order to understand the role of individual, spatial, environmental, and temporal heterogeneity in propagating the SOD epidemic. We constructed a network of sampling transects within a single watershed that include stands characterized by varying host composition, density, and site characteristics (e.g., slope, aspect), but a comparable history of exposure to the disease. The data obtained were then analyzed to describe outbreak cycles of the disease and to infer the overall ecology of SOD, its impact on hosts, and the nature of temporal disease dynamics in mixed evergreen forests. Results provide for the first time a robust overview of factors strongly correlated with disease incidence including disease spread, pathogen survival, host infection, and disease cycling. These results shed light on the role of transmission heterogeneity in the survival and spread of the epidemic, and suggest potential avenues for response.

## Current and projected global distribution of *Phytophthora cinnamomi*, one of the world's worst plant pathogens.

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Globally, *Phytophthora cinnamomi* is listed as one of the 100 worst invasive alien species and active management is required to reduce impact and prevent spread in both horticulture and natural ecosystems. There are regions thought to be suitable for the pathogen where no disease is observed. We developed a CLIMEX model for the global incorporating extensive empirical evidence on the presence and absence of the pathogen. The CLIMEX model captured areas of climatic suitability where *P. cinnamomi* occurs that is congruent with all available records. The model was validated by the collection of soil samples from asymptomatic vegetation in areas projected to be suitable by the model for which there were few records. DNA was extracted and the presence or absence of *P. cinnamomi* determined by high throughput sequencing (HTS). While not detected using traditional isolation methods, HTS detected *P. cinnamomi* at higher elevations in eastern Australia and central Tasmania as projected by the CLIMEX model. Further support for the CLIMEX model was obtained by using the large dataset from southwest Australia where the proportion of positive records in an area is related to the Ecoclimatic Index value for the same area. We provide for the first time a comprehensive global map of the current *P. cinnamomi* distribution, an improved CLIMEX model of the distribution, and a projection to 2080 of the distribution with predicted climate change. This information provides the basis for more detailed regional scale modelling and supports risk assessment for governments to manage this pathogen.

# Session 5: Genetics

## Genetic diversity and origins of the homoploid allopolyploid hybrid *Phytophthora* ×alni.

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Understanding the process that gives rise to hybrid pathogens is central to understand the evolution of emerging plant diseases. *Phytophthora ×alni*, a pathogen of alder, results from the homoploid hybridization of two related species, *Phytophthora uniformis* and *Phytophthora ×multiformis*. Describing genetic characteristics of *P. ×alni* should help understanding how reproductive mechanisms and historical processes shaped the population structure of this emerging hybrid pathogen. The population genetic structure of *P. ×alni* and the relationship with its parental species was investigated using twelve microsatellites and one mitochondrial DNA (mtDNA) marker on a European collection of 379 isolates. Populations of *P. ×alni* were dominated by one multilocus genotype (MLG). The frequency of this dominant MLG increased after the disease emergence together with a decline in diversity, suggesting that it was favoured by a genetic mechanism such as drift or selection. Combined microsatellite and mtDNA results confirmed that *P. ×alni* originated from multiple hybridization events that implicated different genotypes of the progenitors. Our detailed analyses point to a geographic structure that mirrors the one observed for *P. uniformis* in Europe. Our study provided more insights on the contribution of *P. uniformis,* an invasive species in Europe, to the emergence of *Phytophthora*-induced alder decline.

## Traits associated with the establishment of *Phytophthora* in Scandinavia.

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New introductions of *Phytophthora* species are being increasingly reported worldwide as a result of international nursery trade and human movement. However, not all introduced *Phytophthoras* are able to establish in ecosystems at the point of arrival and then further spread. In this project we studied the composition of the *Phytophthora* communities in eight nurseries, 16 rivers, and 14 forests in Sweden to identify functional traits associated with establishment. *Phytophthora* was isolated by baiting of river water, soil, and/or isolation from plant tissue, and identified based on the ITS region. In total 1670 isolates were obtained corresponding to 19 different species. We observed large differences in the composition of *Phytophthora* communities between the three environments. Nurseries and forest stands with low human influence showed the highest and lowest species richness, respectively. Three *Phytophthora* species were detected in all environments (*P. cactorum*, *P. plurivora*, and *P. cambivora*), while other species such as *P. ramorum* or *P. quercina* were only found in nurseries and forests, respectively. Current analysis of the differences between communities might shed light on the functional traits associated with the establishment of *Phytophthoras* in northern forests.

## Population structure of an aerial *Phytophthora* species in a forest system.

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*Phytophthora pluvialis* the pathogen responsible for red needle cast on *Pinus radiata* was first discovered in New Zealand in 2008 but had previously been recovered from Tanoak and Douglas-fir trees in Oregon (USA) in 2002. *P. pluvialis* was described as a new species in 2013 and classified in clade 3 based on the ribosomal ITS sequence. Little is known about the genetics of *P. pluvialis* in New Zealand and this project aimed to gain a better understanding of the genetic diversity, population structure and origin of this pathogen. A total of 368 *P. pluvialis* isolates from infected needles, bait buckets, streams and soil were included, 226 from the USA and 142 from New Zealand. Isolates were genotyped using 27 single nucleotide polymorphism (SNP) markers, which had been identified based on heterozygosity in the genome sequences of two *P. pluvialis* strains sourced from New Zealand and Oregon. The genotypic data were analyzed using an R package called Poppr. A total of 22 multilocus genotypes (MLG) were identified among the 142 New Zealand samples and 134 MLGs from the 226 samples from the USA, indicating the New Zealand population is clonal and less diverse than the USA population. A minimum spanning network suggested two distinct migration events of *P. pluvialis* into New Zealand. An analysis of molecular variance (AMOVA) and fixation index showed there was no clear population structure or significant variation between the two populations. This is evidence that *P. pluvialis* was introduced into New Zealand and there are two genetically distinct groups. Further work on virulence and gene expression will give insights on the difference between the two groups.

## Two distinct lineages in *Phytophthora austrocedri*, the cause of forest disease epidemics in Britain and Argentina.

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*Phytophthora austrocedri* was first described in 2007 from southern Argentina where it is associated with widespread mortality of the native Chilean cedar *Austrocedrus chilensis* (Cupressaceae). In 2011, it was reported in the United Kingdom associated with dieback and mortality of juniper, (also Cupressaceae). Following its detection, surveys in the UK revealed that *P. austrocedri* is widely distributed and contributing to the severe decline of *Juniperus communis*, an ecologically important native conifer species. Being homothallic with amphigynous antheridia, semi-papillate and non-caducous sporangia with coralloid hyphae, isolates from the UK resemble those from Argentina but can be discriminated by their higher percentage of viable oogonia and significantly slower growth rates. In the phylogenetic analysis, isolates of *P. austrocedri* from both origins form distinct clades within Clade 8d with their closest relatives being *P. obscura* and *P. syringae*. Based on morphological characteristics, growth-temperature relationships, sequences of eight DNA gene regions and pathogenicity assays, isolates originating from the UK are distinct from those isolated in Argentina and are proposed to be considered as two different lineages of the same species. Both lineages are able to infect *A. chilensis* and *J. communis* but they are more aggressive on their natural hosts. The full extent of their host range is yet to be determined. Work is under way to elucidate the relatedness of both lineages through genome sequencing.

## Evolutionary relationships within the *Phytophthora cactorum* species complex in Europe.

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The *Phytophthora cactorum* species complex in Europe is composed of *P. cactorum, P. hedraiandra,* and a hybrid species *P.* ×*serendipita*. Evolutionary analyses using the amplified fragment length polymorphism (AFLP) method were carried out on 133 isolates from 19 countries. The AFLP data were complemented by sequence analysis of three genes (ITS region of ribosomal RNA gene, phenolic acid decarboxylase – Pheca I, and Cytochrome oxidase – Cox I), morphometric analysis and cardinal temperature data. The high proportion of clonal genotypes, low gene flow among groups, which was defined by the structure analysis, and low Nei's gene diversity confirms the homothallic life cycle of the groups. On the other hand, the ITS, Cox I and Pheca I sequence data support occasional hybridization between species. The structure K=5 grouping revealed two groups of hybrid origin (C2 and F). While the C2 group resembles *P.* ×*serendipita*, the F group includes Finnish isolates characterized by high oogonial abortion rates and slow growth. The morphological characters routinely used in identification of *Phytophthora* species are not useful for delimitation of species from the *P. cactorum* complex. Therefore, we discuss the status of *P. hedraiandra* as a separate species. The epitypification of *P. cactorum* is proposed.

## Identification and characterization of polymorphic microsatellite markers to study *Phytophthora cinnamomi* populations.

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*Phytophthora cinnamomi* is the causal agent of root rot, canker and dieback of thousands of plant species around the globe. This oomycete not only causes severe economic losses but threatens natural ecosystems. In South Africa *P. cinnamomi* affects eucalyptus, avocado, macadamia and indigenous fynbos. Despite being one of the most important plant pathogens with a global distribution, little information is available regarding origin, invasion history and population biology of this species. This is partly due to the limited number of molecular markers available for studying *P.cinnamomi.* The aim of this study was to develop a set of microsatellite (SSR) markers for *P. cinnamomi.* Using available genome sequences for three isolates of *P. cinnamomi*, seventeen polymorphic microsatellite markers were developed. The application of these markers on *P. cinnamomi* populations from avocado production areas in South Africa revealed that they were highly polymorphic, with the number of alleles per locus ranging from 2 to 12 (mean 2.813) and observed heterozygosity ranging from 0.621 to 0.665 (mean = 0.649). These markers were carefully designed and optimised so that they can be combined in one or two multiplex PCR genotyping assays. The markers developed in this study represent a valuable resource for studying the population biology and movement of *P. cinnamomi* and will aid in the understanding of the origin and invasion history of this important species.

## *Phytophthora ramorum* and tree species susceptibility: Comparing virulence and sporulation of EU1 and NA1 isolates.

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*Phytophthora ramorum* has four clonal lineages: NA1, NA2, EU1, and EU2. Until recently, forest infestations in Oregon and California have all been the NA1 clonal lineage whereas EU1, NA1, and NA2 clonal lineages have only been found in western US nurseries. In Europe, by contrast, only the EU1 and the newly described EU2 lineages are found in forests and in nurseries. In February 2015, *P. ramorum* was isolated from a dying *Notholithocarpus densiflorus* forest tree in Curry County, Oregon. Microsatellite genotyping showed that these isolates belonged to the EU1 lineage and were nearly identical to EU1 isolates collected in 2012 from a nearby horticultural nursery. In 2016, a new cluster of EU1 infected trees was confirmed about 1 km from the previous EU1 sites. Isolates were obtained from symptomatic tanoak and understory saplings of *Abies grandis*. In some past pathogenicity tests the EU1 lineage exhibited higher pathogenic aggressiveness and faster growth rates than the NA1 lineage, suggesting potential for a more severe forest epidemic. In order to evaluate the relative threat of the new EU1 lineage compared to the established NA1 lineage to Oregon forests two log inoculation experiments and a sporulation assay were conducted. The specific objectives were to: (i) Compare the aggressiveness of three NA1 and three EU1 isolates in common forest trees found in Southern Oregon; (ii) determine if there are any qualitative differences in virulence between the two lineages; and (iii) compare sporulation of NA1 and EU1 isolates on several host species.

## Host-induced genome alterations in the Sudden Oak Death pathogen *Phytophthora ramorum.* I. NA1 lineage on Coast live oak in California. II EU1 lineage on *Chamaecyparis lawsoniana* in UK.

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Rapid phenotypic diversification in clonal invasive populations is often observed, although the underlying genetic mechanisms remain elusive. Lineages of the Sudden Oak Death pathogen *Phytophthora ramorum* are exclusively clonal, yet isolates of NA1 lineage from oak (*Quercus* spp.) frequently exhibit host-dependent, unstable colony phenotypes called non-wild type (*nwt*). This phenotypic variation is seen despite population genetic and host-specificity studies negating any host-driven population subdivision. We also found comparable *nwt* phenotypes in EU1 isolates from Lawson cypress (*Chamaecyparis lawsoniana*) in the UK: isolates from the middle of a large ~4m long lesion on a mature tree were normal wild type (*wt*), those from the extremities were variable *nwt* types. Previously only *wt* isolates were known in the EU1 lineage, including those from larch (*Larix*). We hypothesize that the environment in the bark of oak and Lawson cypress is responsible for the unusual phenotypic diversification in *P. ramorum*. A series of passage experiments tested this hypothesis. When *P. ramorum* NA1 isolates from foliar host California bay (*Umbellularia californica*) were inoculated and re-isolated from mature oaks, both *wt* and *nwt* phenotypes were obtained. In contrast, no such phenotypic changes were observed when the same isolates were passed through the foliar host California bay. High-throughput sequencing-based analyses identified major genomic alterations in NA1 *nwt* isolates from oaks included partial aneuploidy and copy-neutral loss of heterozygosity. Chromosomal breakpoints were found to be located at or near transposons, linking transposon de-repression caused by the chemical environment of oaks to structural genomic changes. Similarly, two EU1 *nwt* isolates and one *wt* isolate from *C. lawsoniana* exhibited large chromosomal regions with copy number variations and loss of heterozygosity, consistent with the isolates being copy number heterokaryons. Such genomic alterations were not identified in NA1 isolates from California Bay.

# Biological differences between the evolutionary lineages within *Phytophthora ramorum*, *P. lateralis* and other *Phytophthora* species. Should they be formally taxonomically designated?

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The four known evolutionary lineages of *Phytophthora ramorum* (informally designated NA1, NA2, EU1 and EU2) are probably anciently divergent and recently introduced into Europe and North America from different, unknown geographic locations. Recombinants between them are genetically unstable and probably unfit to survive, indicating reproductive isolation. They also differ in growth rates and aggressiveness. EU1 is faster growing and also more pathogenic on bark of *Quercus rubra* than NA1.Our recent studies show that all four lineages can be readily discriminated on colony and growth behaviour alone in gene x environment stress tests. Also that EU2 lineage is more aggressive than EU1 on larch stems but EU1 may produce more sporangia on larch needles. Other tree Phytophthoras also exhibit multiple lineages. We have revealed four lineages within *P. lateralis* (TWK, TWJ, PNW and UK) exhibiting distinctive colony types, sporangial sizes and shapes, chlamydospore sizes and differing aggressiveness on *C. lawsoniana.* Overall these lineage differences may be greater than in *P. ramorum. P. cinnamomi* has A1 and A2 lineages that are strongly reproductively isolated, exhibiting zygotic and post zygotic abortion when crossed. Within *P ramorum* or *P lateralis* the lineages tend to share some characters such as sporangial type and host range that are broadly consistent with their being conspecific and sharing a common ancestor. Other differences between them, morphological, behavioral and genetic, suggest they were previously adapted, via selection, drift and reproductive isolation, to somewhat different biogeographic environments. They might be considered equivalent to sibling or cryptic species or their phenotypic differences sufficiently large to warrant formal taxonomic recognition. In *P. lateralis* the differences between the lineages are probably as large as those between some described *Phytophthora* species. Whether such lineages should be formally recognized as subspecies or even as species will be discussed.

# Session 6: Dispersal Pathways/ Urban Horticulture

## Phytophthora diseases in Horticulture in Southeast Asia.

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Tropical crops such as cocoa, durian, papaya, pineapple, custard apple, coconut, pepper, citrus, avocado, macadamia and tomatoes are susceptible to one or more species of *Phytophthora*. The wet tropics provides an ideal environment for Phytophthora diseases due to high levels of humidity, seasonal heavy rainfall, and an abundance of susceptible host plant species (all year round in the case of perennial crops). Many *Phytophthora* species cause multiple diseases on the same host from seedling dieback, root rot, stem canker, flower blight, leaf blight and fruit rot. Many of the diverse tropical food and fibre crops are susceptible to one or more different *Phytophthora* species eg. *P. palmivora* and *P. capsici* attack a wide range of cultivated tropical species. These factors combined often mean that there is no break in the disease cycle, sporangia are produced continuously and conditions for infection are often present. Despite the challenges many options for management of Phytophthora diseases have been developed which can be successfully applied across different crops. I will give an overview of some of the major *Phytophthora* diseases which cause problems in horticulture in Southeast Asia and draw some links between common *Phytophthora* problems and their management experienced in forestry and horticulture.

## PHYTO-THREATS: Global threats from *Phytophthora* species: understanding drivers of emergence and opportunities for mitigation through nursery best practice.

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Over the past 13 years, seven new species of *Phytophthora* have been detected in the UK, all of which infect tree species. In most cases, trade in imported planting material has been either confirmed or strongly implicated as the most likely route of introduction. The PHYTO-THREATS project is an interdisciplinary collaboration of seven institutions aimed at addressing risks to UK forest and related ecosystems from *Phytophthora.* This three year project, from April 2016, will examine the distribution and diversity of Phytophthoras in UK plant nursery systems and provide the evidence base for the development of a set of enhanced nursery 'best practice' accreditation criteria to mitigate risk of further *Phytophthora* introduction and spread. This evidence base will also be informed by a greater understanding of the emerging *Phytophthora* threats from both global and evolutionary perspectives. The objectives are (i) examine the distribution, diversity and community interactions of *Phytophthora* in UK plant nursery systems, (ii) provide the evidence base for a voluntary nursery 'best practice' accreditation scheme to mitigate further spread, (iii) identify and rank global *Phytophthora* risks to the UK and (iv) gain a greater understanding of the evolutionary pathways of Phytophthoras. We will present a precis of the first year of the project including methods to secure stakeholder participation and approaches to nursery sampling. A database of biological traits and distribution of all known *Phytophthora* species has been created for modelling global environmental niches and the risk of new arrivals to the UK from source regions through trade networks. We will discuss how best to exchange and share these data with the international community of *Phytophthora* researchers.

## Urban activities influence *Phytophthora* species diversity in BC, Canada.

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We hypothesize that the nursery and agricultural trades affect the diversity of Phytophthora species. To test this, we characterized and compared *Phytophthora* diversity in natural and urban environments. We collected soil samples from sites in urban and natural stands or at the interface of urban/natural areas around Vancouver, BC and south Vancouver Island, in 2012 and 2013. DNA was extracted from 130 soil samples and DNA metabarcoding of the internal transcribed spacer one (ITS1) was conducted. In 2011 five urban waterways located around agricultural or residential areas were baited with mesh bags containing Rhododendron leaves. *Phytophthora* species were isolated and barcoded using the internal transcribed spacer (ITS1 and ITS2). DNA metabarcoding analyses identified 25 putative *Phytophthora* species, 8 of which are potentially new species. *Phytophthora syringae* and a hybrid between *P. polonica* and an unknown species were the most widespread species. Urban sites had the highest species diversity, ranging from 3 to 12 species per site, whereas natural sites had between 4 and 8 species per site. In total, 23, 14 and 11 species were found in urban, urban/natural interface and natural locations, respectively. Most of the unknown species were in urban or urban/natural interface sites. Several species in urban sites were present in low frequency and could represent introductions via urban activities. Seventeen *Phytophthora* species were found by stream baiting; the most widespread were *P. gonopodyides* and *P. lacustris*. Eight species were common to both DNA metabarcoding and baiting experiments. This study suggests that urban activities influence *Phytophthora* diversity and composition.

## Factors affecting *Phytophthora ×alni* distribution in Czech forests and its predictive modeling.

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*Phytophthora ×alni* is an invasive organism significantly damaging the forest and riparian vegetation of alder trees in Central and Western Europe. The analysis of environmental factors affecting its distribution and the modelling of its potential occurrence are important tools for its management. In this study, we analyzed the distribution and extent of phytophthora root and collar rot in 854 alder forest stands over the area of the Czech Republic with use of thorough statistical analysis of data obtained from field surveys as well as available geodatabases. The alder disease was identified in 53% stands. Its extent was significantly influenced by many environmental and silvicultural characteristics: for instance by ecological series, presence of a watercourse in the stand, temperature, altitude, proportion of alder in the stand and its area, age and standing timber stock (volume of alder biomass). Models developed for particular ecological series explained 33–51 % of data variability. Watercourses were detected as the main way of pathogen spread. Its spread through alder planting is less important in the area. The predictive model of *P. ×alni* potential distribution was made for the database of all alder stands in the Czech Republic containing ca120 000 individual polygons. The map based on the final model shows the potential risk of occurrence of *P. alni* in forests on a five-point scale ranging from very low risk for alder stands to very high levels of damage. Nearly 49 % of the total area of alder stands (255 476 hectares) was predicted as very high risk for the pathogen spread with only 9 054 hectares (4 %) mapped as very low risk. *P. ×alni* poses an important continuing risk for alder cultivation in the area. A management strategy of the disease based on outcomes of the study is proposed.

## Botanical gardens: Sentinel plantings to detect new and emerging *Phytophthora* risks.

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Global forests are increasingly under threat from invasive pests and pathogens. In an era of rapidly growing global trade, this threat is set to continue. For example, alien *Phytophthora* species have caused devastating disease epidemics on most continents of the world. Importantly, many of the most damaging forest pathogens were unknown to science prior to their arrival in a new environment. *Phytophthora ramorum's* devastation of native forests of the western United States provides an apt example. Botanical gardens play an important role as custodians of outstanding plant collections, and can often act as 'traps" for new pest and pathogen introductions. Against this backdrop, the International Plant Sentinel Network (IPSN) has been established to provide a platform to coordinate information exchange and support for sentinel plant research in botanic gardens and arboreta. The IPSN aims to facilitate the identification and to provide warnings of new and emerging pest and pathogen risks. We present a novel, systematic and comprehensive survey undertaken in botanical gardens of South Africa to obtain baseline information on *Phytophthora* species currently present in the gardens. The work was undertaken in close collaboration with garden staff, utilising their existing knowledge and familiarity with collections. In return, the project has provided training sessions to increase staff awareness and understanding of *Phytophthora* related plant health issues. Intensive soil sampling and baiting carried out with the staff of three botanical gardens in the Western Cape province resulted in the isolation of nine *Phytophthora* species: *P. amnicola, P. asparagi, P. cinnamomi, P. lacustris, P. hydropathica, P. nicotianae, P. multivora, P. psuedocryptogea* and two Clade 9 species. Survey results were able to directly inform management options for diseases caused by these pathogens. The work highlights the benefits of the IPSN framework in building capacity in botanical gardens to support survey efforts and effective biosecurity practice.

## Diversity and detections of *Phytophthora* species from trade and non-trade environments in Ireland.

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Statutory monitoring for *Phytophthora* species focuses on the species regulated in the EU and recommended for regulation by the European and Mediterranean Plant Protection Organization (Plant Health Directive 2000/29 EC and the EPPO A2 list). This research provides details for the *Phytophthora* species detected from trade and non-trade environments in Ireland between 2013-2015. The results of statutory surveys for the regulated *species Phytophthora ramorum, Phytophthora kernoviae* and *Phytophthora lateralis* from 2003–2015 are also presented. Testing of more than 11,000 samples was carried out using morphological and/or DNA identification using specifically designed *Phytophthora* conserved primers. This led to the detection of 19 species and 3 unpublished taxa of *Phytophthora*, including 8 new records for Ireland. Eight species were found in both trade and non-trade locations, and three undescribed species were also detected. *Phytophthora ramorum* was found on the most hosts (30 hosts), followed by *Phytophthora syringae* (6 hosts) and *Phytophthora kernoviae* (3 hosts). Rhododendron was the host on which *Phytophthora* species was most frequently detected (12 *Phytophthora* species). The role of the plant trade in spreading invasive *Phytophthora* species is discussed.

## Host range of *Phytophthora* species associated with declining trees.

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*Phytophthora species have been isolated from dying and declining tress in the urban or peri-urban environment. They include those commonly found in natural ecosystems, but also others s better known for associations with agricultural crops. For many of these species, little is known about their host range.* We examined the host range of 19 *Phytophthora* species including newly described ones associated with declining urban trees from Perth Western Australia; *P. amnicola, P. arenaria, P. boodjera, P. capensis, P. capsici, P. cinnamomi, P. citrophthora, P. combivora, P. crassamura, P. drechsleri, P. frigida, P. multivora, P. nicotianae, P. niederhauserii, P. palmivora, P. pseudocryptogea, P. taxon* walnut, *P. thermophila* and *P. 'versiformis.* A preliminary pathogenicity screen used excised branches from 16 trees species; *Agonis flexuosa, Banksia sessilis, Callistemon* sp., *Corymbia calophylla, Eucalyptus gomphocephala, Eucalyptus marginata, Ficus macrocarpa, Fraxinus raywoodii , Hakea laurina, Magnolia grandiflora, Melalecua* sp., *Metrosideros excelsa, Olea sp., Plantanus sp., Pyrus ussuriensis, Viburnum sp*. Excised branches (28 to 32 cm long 3.5 to 4.5 mm in diameter) were under-bark inoculated with one of nineteen *Phytophthora* species. They were incubated in the dark at 25°C and lesions measured 8 days after inoculation. Lesions developed in *Eucalyptus marginata* and *Corymbia calophylla* with all *Phytophthora* species. *Hakea laurina* was resistant to 16 of the 19 *Phytophthora* species. *Phytophthora pseudocryptogea* was pathogenic to all 16 tree species while *P. 'versiformis'* formed lesions in only two tree species. *P. pseudocryptogea*, *P. cinnamomi* and *P. multivora* were the most pathogenic species causing the largest lesions in most of these trees species. It is likely these pathogens have a significant negative impact on the health of the urban forest worldwide.

## A forensic investigation into the sources of *Phytophthora boodjera* contamination in a Western Australian containerized production nursery.

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The recently emerged plant pathogen *P. boodjera* is responsible for damping-off and mortality of *Eucalyptus* seedlings in Western Australian nurseries. It emerged in 2011, in a nursery producing mostly eucalypt seedlings for restoration purposes in agricultural land. We undertook a systematic sampling strategy to test and eliminate the possible sources of on-site contamination in the nursery in order to understand the epidemiology of *P. boodjera* and develop methods for its control. The nursery was sampled over a period of three years: at the start of seeding, throughout the growing season, and when the nursery was fallow after the seedlings had been dispatched. Eucalypts and other plant genera were sampled at all stages of production. Additionally potting media, trays, water from tanks, dams and ponds, outlet water, rhizosphere soil from windbreak hedgerow trees and soils from paddocks immediately adjacent to the nursery were sampled. Despite the nursery following best practice guidelines as prescribed by Nursery Industry Accreditation Scheme Australia, such as using chlorine solution or dry heat to sterilise used containers, the pathogen persisted on-site during the fallow period. Oospores of *P. boodjera* profusely formed in infected roots of eucalypt seedlings, are resistant to dry heat treatment of up to 65°C and were the inoculum source surviving on the trays between seasons, and reinfected young seedlings in subsequent years. *P. boodjera* was recovered from the lawn and the roots of eucalypt hedgerow plantings adjacent to the nursery benches, but the primary source of re-infection was from re-used seedling trays from the previous season. Inoculation trials indicated that *Eucalyptus* species were the main host of *P. boodjera* and excess water was not required for the infection process. The problem was completely and readily solved by steam sterilization of trays containing moistened potting substrate.

# *Comparisons of Phytophthora* incidence and diversity in cultivated and natural endemic species of Proteaceae in South Africa.

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The Cape Floristic Region (CFR) of South Africa includes at least 332 species of Proteaceae and thus the second largest (after Australia) diversity of this family. The genera *Protea* (on which the Family is based) and *Leucadendron* are also restricted to Africa with most species occurring in the CFR. Many species are also cultivated for the international cut-flower market and are thus traded globally. *Phytophthora* root rot is one of the most important constraints to the propagation of the Proteaceae in South Africa and *P. cinnamomi* has invaded the natural CFR ecosystem where it kills large numbers of susceptible species. *Phytophthora nicotianae* has also been isolated from dying plants but there is a general lack of knowledge regarding the diversity of *Phytophthora* species in the area. A study has consequently been designed to describe the diffusion of *Phytophthora* species along the South African range, where ornamental varieties of this family are cultivated. Four transect of five soil samples (ten from cultivated plots inside a Protea farm and ten from the surrounding environment) were made in different places. Each sample was generated by bulking roots and soil from five plants. *Phytophthora* spp. were isolated by baiting and identified base on DNA sequences. We expect to find a diversity of species and differences in incidence related to the different geo-climatic location of sampling areas. Comparisons between the occurrence of *Phytophthora*. spp. occurring in nature and in adjacent cultivated Proteaceae should also reveal patterns of movement of these pathogens and inform management options for them.

# Session 7: Isolation and ID techniques

## Development and application of an amplicon metagenomics approach based on the ras-related Ypt1 gene for the detection of *Phytophthora* species.

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A metagenomic approach to detect *Phytophthora* species in agricultural and natural ecosystems was developed using the *ras*-related protein Ypt1 gene as an alternative genetic barcode. Two pairs of degenerate nested primers were designed by aligning sequences of 111 different *Phytophthora* species retrieved from GenBank, genome sequences or from unpublished USDA APHIS data. Outer and inner primers pairs amplified target regions of approximately 600 bp and 500 bp, respectively. Both primer pairs proved to be appropriate to amplify the target region from a comprehensive collection of isolates representative of the whole genetic diversity within the genus *Phytophthora*. For both primer pairs the detection limit of a single round of PCR amplifications was 100 pg of DNA increasing to 1 pg in nested PCR. The method was validated using a large number of soil samples and corresponding baiting leaves, collected in different natural ecosystems in Sicily. All samples were also analyzed using a previously reported metagenomics method targeting the ITS1 region of the rDNA. For soil samples, a nested approach was necessary for positive amplifications while a limited number of baiting leaves produced amplicons of the expected size also after a single round of PCR. Overall, amplicons were obtained from approximately 65% of the investigated samples. All positive amplicons were sequenced using Illumina MiSeq platform and data were processed and analyzed using the bioinformatics pipeline QIIME v. 1.8. Preliminary results indicate that the Ypt1 gene is an appropriate target to detect a low quantity of *Phytophthora* inoculum in natural samples providing that a nested approach is utilized. The higher diversity of this gene region as compared to the ITS1 region improves discrimination among closely related species allowing more in-depth investigations of *Phytophthora* diversity.

## Testing *in situ* water sampling and metabarcoding protocols to detect *Phytophthora* diversity for plant health testing and natural ecosystem surveillance.

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Water-borne propagules are a key dissemination pathway for many species of *Phytophthora* and their capture and isolation via baiting has proved useful for detection and surveillance. Molecular detection of the pathogen in water offers an alternative, but transporting water samples to the laboratory is impractical and most detection assays are species-specific. Plant health protocols are generally based on known listed species but awareness is increasing of their failure to detect novel undescribed pathogenic taxa. We have thus combined an *in situ* water filtration sampling method and a generic *Phytophthora* PCR test based on the rDNA ITS1 region. The amplified DNA barcodes are analysed using high-throughput sequencing technology allowing an unparalleled depth of sampling. We used this technology to examine *Phytophthora* diversity in natural ecosystems and to test planting material and irrigation water in nurseries. It proved valuable but the downstream computation biology platform must be robust and based on a robust database of reference sequences that copes with 'fuzziness' and overlap around species boundaries. As an objective measure of the benefits of the technology for plant health legislation and ecosystem surveillance we are running replicate field and reference samples to evaluate reproducibility of the method, potential bias within sampling, PCR and sequencing steps and cross-validation of traditional and metabarcoding.

## Monitoring *Phytophthora* species in river systems in Sweden by high throughput sequencing.

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River systems concentrate water and *Phytophthora* inoculum from their catchment areas. River filtrates can be used for detecting new outbreaks and for monitoring established *Phytophthora* populations. In this research, we developed a novel high throughput sequencing (HTS) protocol to assess the diversity of *Phytophthora* species in rivers and we compared the results with those obtained by baiting *in situ*. DNA from river filtrates was extracted from 96 randomly placed plots along 16 river systems in Sweden during two consecutive years. We amplified the entire ITS region by using specific primers for *Phytophthora*, and we sequenced the products by single molecule real time (SMRT) sequencing. Preliminary results show a larger number of species obtained by HTS than by baiting (39 vs 12 species). Seven of the 29 species not detected by baiting in the rivers had been isolated in Sweden by either soil baiting or directly from cankers, indicating that HTS may be able to capture species present in the surrounding areas with perhaps too low zoospore abundance to be detected by baiting. No previous records are available for the remaining 22 species indicating that new species may have been discovered with this technique. The most abundant species were *P. lacustris* and *P. gonapodyides*, but also other tree pathogenic species such as *P. pseudosyringae*, *P. cactorum* or *P. plurivora* were abundantly represented in the assemblages obtained by either HTS or baiting. The assemblage obtained by SMRT sequencing is comparable with the one obtained with other protocols using Illumina or Iontorrent sequencing platforms, though the longer reads obtained by SMRT sequencing may allow a finer species identification.

## Best method for determining *Phytophthora* community.

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The genus *Phytophthora* causes significant losses to agricultural and horticultural production throughout the world. Due to their wide variety of ecological roles, broad distribution and economic impact, proper isolation and identification of *Phytophthora* is of great importance. In this study five different sites were sampled and seven methods were used to establish the *Phytophthora* community. Three methods of traditional isolation were conducted (1) soil baiting where leaves are floated on flooded soil and infected baits plated onto selective media (2) filtering of the bait water followed by direction isolation on selective media from the filters and (3) placement of field roots into an incision in a Granny Smith apple and then placing lesioned material onto selective media. These were compared to four sources of eDNA used for metabarcoding using *Phytophthora* specific primers (1) sieved soil (2) roots from field (3) filters from baiting water and (4) roots from bait plants grown in the glasshouse in soil collected from the sites. Five *Phytophthora* species were recovered by traditional baiting using bait leaves (method 1) and six species were recovered from baited filtering water (method 2). However, no *Phytophthora* species were recovered from Granny Smith apples (method 3). eDNA extracted from field roots detected the highest number of *Phytophthora* species (25). These were followed by isolation from roots from bait plants grown in the glasshouse (20), direct DNA isolation off filters (14) and DNA extraction from field soil (12). Therefore, roots were determined to be the best substrate for detecting *Phytophthora* communities.

## Specific detection of *Phytophthora cinnamomi* DNA and mRNA in environmental samples using real time polymerase chain reaction assays.

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A species-specific primer and probe for *Phytophthora cinnamomi* were designed based on a mitochondrial locus encoding subunit 2 of cytochrome c oxidase (COII). Eight PCR primers, including three forward and five reverse, were designed and tested in all possible combinations. Annealing temperatures were optimized for each primer pair set to maximize both specificity and sensitivity. Each set was tested against *P. cinnamomi* and related species, *P. parvispora* and *P. niederhauseri*. A sub-set of four sets of primers were selected and tested with a species-specific *P. cinnamomi* probe labelled with FAM and ZEN dyes to evaluate their specificity for *P. cinnamomi*. The specificity of the qPCR primer was confirmed with the genomic DNA of 29 *Phytophthora* isolates, including 17 isolates from 11 species from clade 7, along with isolates of a representative species from each of the remaining 9 clades. The assay showed no cross-reaction with other *Phytophthora* species, (except for *P. parvispora* which showed late amplification at high DNA concentrations), and was able to detect as little as 1.5 fg of *P. cinnamomi* DNA. The efficiency of the real time PCR protocol was evaluated with environmental samples (soil, plant roots) artificially colonised with *P. cinnamomi*. *P. cinnamomi* was detected in all samples with a sensitivity of 15 picograms. DNA from dead organisms may persist in soils very much longer than RNA, and a positive result from a DNA extraction is not proof of the presence of a living organism. A protocol was developed for the extraction of RNA from environmental samples, and following cDNA synthesis, the same assay was then used successfully to confirm the presence of living propagules.

# Session 8: Pathogenicity

## Evaluating eight plant families for *Phytophthora* species in California wildlands, early results from restoration nurseries.

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We started a project in 2016 with the primary goal to improve our understanding of the ecology and epidemiology of the *Phytophthora* species assemblages in California wildlands. Areas evaluated include wildland restoration nurseries, restoration sites, and remnant sites. The plant species examined are woody perennial California native plant species from eight families: Sapindaceae, Rosaceae, Rhamnaceae, Phrymaceae, Platanaceae, Salicaceae, Ericaceae, or Fagaceae. These families all contain known susceptible plant species. From this, we will develop a management approach to reduce the chance of introduction and establishment of *Phytophthora* species pathogens based on our improved understanding. We will present our nursery findings for the *Phytophthora* species isolated, conditions, and possible pathways of spread; and approach to sampling wildlands. Preliminary results include isolation of *Phytophthora* species in nurseries from plants in the five families tested thus far and the *Phytophthora* species recovered fall into ITS clades 1, 2, 6, 7, and 8. Poor nursery conditions observed include reusing without treatment dirty containers and potting mixture, growing plants on the ground with flooding, allowing weeds to proliferate in containers with growing plants, having drive-thru areas on the same surface as 'new' potting material, cycles of over-under-watering, and irrigation in disrepair.

## First report of Phytophthora cinnamomi on Cinnamomum cassia in Vietnam.

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*Phytophthora cinnamomi* is a pathogen that has a wide host range world wide, and results in considerable damage to natural vegetation, tree plantations and and crops. In Vietnam the pathogen has been reported to cause heart rot of pineapple in 2001, and of great concern is its isolation from *Acacia mangium* in 2013 in Yen Son, Tuyen Quang province, where it caused severe decline of the *A. mangium* plantations. The pathogen was believed to be derived from natural forests as It also caused disease on chestnut plants in Hoang Lien National Park and Van Ban Nature Reserve in 2015. It is now known to cause root rot leading to mortality of cinnamon plantations all over the country. In Yen Bai province, the mortality rate of cinnamon plantations was about 10%. The disease is difficult to detect at early stage because the pathogen first attacks the smaller rootlets. Soil and small rootlets were taken and used for trapping and the isolated oomycetes were identified using morphological characteristic of oogonia, sporangia and chlamydospores and as well as ITS1/ITS4 sequence. A pathogenicity trial of 15 *P. cinnamomi* isolates was assessed by adding mycelium to the root containers of year old cinnamon seedlings. and scoring disease symptoms 15 days. The number of trees with visible symptoms, wilting and death, were recorded. Isolate YBC1 was highly virulent killing all seedlings. Management options for disease control in plantations are discussed.

## Insights into the potential host range of *Phytophthora foliorum.*

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During a survey for *Phytophthora ramorum* undertaken in north west Scotland in early 2016, shoots of the invasive shrub *Rhododendron ponticum* were found to be infected by *P. foliorum.* Prior to this finding, the recently described *P. foliorum* had only been reported from foliage of hybrid azaleas in nurseries in California and Tennessee and from azalea plants in an ornamental nursery in Spain. No other hosts are known and much of the behaviour of *P. foliorum* remains enigmatic. However, the species is classified in *Phytophthora* Clade 8c, with closest relatives *P. ramorum* and *P. lateralis, both of which are highly damaging tree pathogens. To explore the potential threat that P. foliorum could pose to trees, experiments were undertaken to compare the bark colonising capability of this pathogen with P. ramorum* and *P. lateralis. Initial comparisons focussed on R. ponticum, Larix decidua (European larch) and L. kaempferi (Japanese larch) as all three are significant hosts of P. ramorum in the UK. Later experiments included broadleaf tree species Fagus sylvatica (European beech) and Quercus robur (pendunculate oak) as well as the main host of P. lateralis, Chamycaeparis lawsoniana (Lawson cypress). Preliminary findings suggest that as well as being a significant pathogen of R. ponticum, P. foliorum has the potential to colonise the bark of larch although its growth is influenced by season.*

## Pathogenicity of Phytophthora ×serendipita to Quercus petraea and Q. robur in Serbia.

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During studies of broadleaved tree declines in Serbia, *Phytophthora ×serendipita* was isolated from declining *Quercus petraea*, *Q. frainetto* and *Pyrus pyraster* trees and identified based on morphological and physiological characters, and ITS sequence analyses. In order to test the pathogenicity of the obtained isolates, roots of each twelve 6-months-old seedlings of the important oaks *Q. petraea* and *Q. robur* were inoculated with one selected isolate of *P. ×serendipita*, using a standardised soil infestation test. In addition, isolates of *P. ×cambivora*, *P. plurivora*, *P. polonica* and *P. quercina* were included as comparison. The control group received sterile substrate. After eight months, roots were examined for the presence of necroses and scanned using the WinRhizo® software. With the exception of *P. polonica* which was only weakly aggressive, all *Phytophthora* species caused significant fine root losses compared to control plants. In *Q. petraea*, the most aggressive species were *P. quercina* and *P. ×serendipita* with the average fine root length (FRL) of inoculated seedlings being ca. 3 times shorter than in the control seedlings, followed by *P. plurivora* and *P. ×cambivora*. In *Q. robur*, the most aggressive species were *P. ×cambivora* with 3.8 times shorter FRL, *P. quercina* with 2.8 times shorter FRL, and *P. ×serendipita* with 2.4 times shorter FRL. All differences to the controls were statistically significant. All *Phytophthora* species including *P. ×serendipita* were re-isolated by plating pieces from root lesions and necrotic fine roots onto PARPNH agar. These results demonstrate the ability of *P. ×serendipita* to colonize fine roots of oak species and cause significant damages. This aggressive hybrid species was previously not recorded on woody hosts and its role in forest tree declines remains unclear. Therefore, additional field surveys and host range testing are required, as well as a clarification of potential pathways of introduction to Serbia.

## *Phytophthora cinnamomi,* a highly variable pathogen with epidemiological consequences in California natural ecosystems.

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Multiple introductions in California of the exotic pathogen *Phytophthora cinnamomi* are severely threatening the biodiversity of its native ecosystems. Virulence of 10 different genotypes representatives of the worldwide genetic variability within *P. cinnamomi* species was studied both on roots and stems and on four important natural Californian hosts for this pathogen. The disease expression depended on the individual genotypes which initiated the infection, although the high susceptibility exhibited by Pacific madrone and whiteleaf manzanita may mask possible variation in virulence. Some genotypes better adapted as root than stem pathogens have enhanced their abilities to cause symptoms on aerial vs. underground portions of plants, producing a different disease expression. Likewise, bay laurel acts mainly as a root host, although with meager aerial symptom progression, whereas Pacific madrone and manzanita are general hosts. In general, the highest level of disease was caused by four isolates, two of them of a genetic lineage recently identified in farms and wild lands in California (Clade B), but not worldwide spread. In addition, the highest virulence on roots of Douglas firs was produced by one the genotypes isolated in Papua New Guinea. So, the differences in the genotypes' affinity to both hosts and parts within hosts should be taken account by regulations to appropriately protect wild resources and plants production facilities.

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## Red Needle Cast (*Phytophthora pluvialis*) studies on Douglas fir require Swiss Needle Cast suppression.

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*Phytophthora pluvialis* is associated with early defoliation and shoot dieback in Douglas fir in both Oregon and New Zealand plantations. In 2013, *P. pluvialis* was described as a new species recovered from mixed tanoak-Douglas-fir forest in Oregon. Concurrently, it was recognized as the main causal agent of the Red Needle Cast disease in New Zealand radiata pine plantations. However, little is known about the infection cycle of *P. pluvialis* in Douglas fir and its impact on Douglas fir physiology. Studying the *P. pluvialis* infection in Douglas fir poses a challenge due to the widespread presence of the endophyte *Phaeocryptopus gaeumannii* in Douglas fir needles. This fungus can cause the Swiss Needle Cast disease (SNC), under proper conditions including needle wetness. In our study, we devised a strategy to successfully suppress SNC development when inoculating *P. pluvialis* in Douglas fir needles. As both *P. pluvialis* and *P. gaeumannii* are triggered by high humidity levels in the needle environment, it is difficult to achieve exclusive expression of *P. pluvialis*. To minimize the amount of *P. gaeumannii* inoculum in Douglas fir foliage, we sheltered Douglas fir saplings from rain or overhead irrigation during spring time, when both *P. gaeumannii* sporulation and new flushing take place. This inoculation routine lead to new foliage with low *P. gaeumannii* abundance that was available for more targeted, exclusive *P. pluvialis* inoculation and research. This approach has enabled the development of a *P. pluvialis* inoculation bioassays on Douglas fir and is enabling further investigation of the epidemiology of this important disease.

## Root rot of *Juniperus* and *Microbiota* by *Phytophthora lateralis* in Oregon horticultural nurseries.

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*Phytophthora lateralis* causes root rot of *Chamaecyparis lawsoniana* (Port-Orford cedar; POC) and *Taxus brevifolia* in the U.S. Pacific Northwest, and in landscape plantings of *Chamaecyparis* in the U.S. and Europe. *Thuja occidentalis* in an ornamental nursery has also been listed as a host. In 2015, following observations of mortality in two horticultural nurseries in Oregon, a *Phytophthora* was isolated from the roots of two conifer species: *Microbiota decussata* and *Juniperus communis*. Sequencing of the ITS, COX1, and COX2 regions identified the isolates as *P. lateralis,* Pacific Northwest clonal lineage. We conducted Koch´s Postulates on potted *Juniperus*, *Microbiota,* and POC plants with one *Juniperus* isolate, one *Microbiota* isolate, and a POC isolate used in POC resistance screening trials. Following application of zoospores to the base of plants, we observed foliage chlorosis, root and stem discoloration, and mortality on all three hosts. Mortality of *Juniperus* and *Microbiota* was greater when inoculated with nursery isolates than with the POC isolate. A branch-dip experiment with four nursery isolates and one POC isolate on susceptible and resistant POC clones indicated differences in virulence between isolates. While lesion lengths generally reflected differences in POC susceptibility, some nursery isolates produced significantly larger lesions than the POC isolate across all levels of resistance. Because disease occurred in nurseries despite frequent application of the Oomycete-specific fungicide Subdue Maxx, we tested all isolates *in vitro* for resistance to the active ingredient mefenoxam at 0.1 to 100 ppm. No evidence for resistance was detected. This work completes Koch's Postulates and extends the host range of *P. lateralis* to members of two additional genera, *Juniperus* and *Microbiota*. The nursery isolates belong to the same clonal lineage as current known U.S. populations, but should be monitored due to their greater virulence, potential for development of resistance to mefenoxam, and possible spread to forests.

## *Phytophthora cinnamomi* A1: An ancient resident of New Guinea and Australia of Gondwanan origin?

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This paper re-examines the hypothesis, first proposed by Shepherd (1975), that *Phytophthora cinnamomi* is an ancient organism in Australia and New Guinea*. It further evaluates data that suggest the A1 mating type is Gondwanan in origin and may have been present in New Guinea for up to 10 million years. It is postulated that there has been a mating type change in *P. cinnamomi* from A1 to A2 in relatively recent times as a result of genetic transformation of the A1 mating type.
*New Guinea refers to the geographic entity whereas Papua New Guinea refers to the political entity encompassing the eastern half of the New Guinea mainland and islands including the Bismarck Archipelago.

Arentz, F. (2017) *Phytophthora cinnamomi* A1: an ancient resident of New Guinea and Australia of Gondwanan origin? Forest Pathology.*2017;00:e12342. https://doi.org/10.1111/efp.12342*

Shepherd, C.J. (1975) *Phytophthora cinnamomi*- an ancient immigrant to Australia. Search. 6:484-490.

# Session 9: Physiology

## Effects of co-inoculations of *Alnus incana* with *Phytophthora alni* complex and *P. plurivora* on disease development and mortality.

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The *Phytophthora alni* complex exhibits strong host specificity, damaging all European alder species. These pathogens overlap also in their distribution ranges, which may lead to contact and possible interactions between them. *P. plurivora* is widespread in Europe and has a broad host-range, which includes alder species. Under the same niches, co-infections of these *Phytophthora* spp. are expected to affect their pathogenic behavior and the disease development. The present study aims to assess the effects of co-inoculations of *P. xalni* (Pa), *P. xmultiformis* (Pm), *P. uniformis* (Pu) and *P. plurivora* (Pl) on *Alnus incana*, which is a representative European alder species, but not commonly used in inoculation trials. Two different inoculation treatments were performed on 320 three-year-old plants; soil infestation (70 plants infested with two pathogens) and soil-stem inoculation (250 plants infested with one pathogen and stem-wound inoculated with another pathogen). Mortality was higher in the soil-stem inoculation compared to the soil-infestation four months after inoculation. The presence of Pa or Pm in the stem and/or soil caused higher mortality than the other isolate combinations. When the same *Phytophthora* species was inoculated in the soil and the stem, survival curves between Pa, Pm and Pu were similar, while they differed significantly from Pl. In the soil infestation trial the two isolates combination groups (Pl/Pm, Pa/Pm), which caused mortality within 131 days after inoculation, showed no differences in their survival curves. In the soil-stem group, Pa and Pm produced higher numbers of collar and stem lesions compared to Pl, while Pu caused more damage in the stem than in the collar. Results also showed that the length of stem lesions differed significantly between Pl and the other species (either in presence of *Phytophthora* or control in the soil), and between Pa and the other species from *P. alni* complex. Contrary to this, no differences were observed in the length of the collar lesions in plants under the soil-stem inoculation, either in presence of *Phytophthora* or control in the stem. Regarding the soil infestation trial collar lesions and mortality were only observed when Pm was present in combination with Pa and Pl.

## Biological characteristics of Pythiaceae species isolated from soil of *Hevea brasiliensis* plantations in the South of Vietnam.

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In a leaf baiting assay oomycete isolates were obtained from 20 soil samples collected from rubber plantations in 8 areas in the South of Vietnam. Using ITS sequence analysis and classical identification methods, the isolates were assigned to 11 species from three Pythiaceae genera including *Phytophtora* (6 species), *Phytopythium* (4 species) and *Pythium* (1 species). Besides the known rubber pathogens *Phytophthora heveae*, *Phytophthora palmivora*, and *Phytophthora nicotianae* three new *Phytophthora* species were identified which were provisionally named as *Phytophthora* sp. insolita-like, *Phytophthora* sp. macrochlamydospora-like and *Phytophthora* sp. Four *Phytopythium* species were identified as *Phytopythium cucurbitacearum*, *Phytopythium vexans*, *Phytopythium chamaehyphon* and *Phytopythium* sp. Pathogenicity of these species, assessed by inoculating unwounded leaves of *Hevea brasiliensis* with mycelial plugs, ranged from non-pathogenic to highly aggressive. Five strains, *P. heveae* VN739, *Phytopythium* sp. VN604 and VN596, *P. nicotianae* VN722 and *Pythium* sp. VN602, showed very strong pathogenicity. Morphological and biological characteristics species with high aggressiveness to rubber leaves were investigated. In general, most of the species showed good growth at 25-30°C and in a pH range of 5-8. Growth characteristics of mycelia and formation of oogonia and clamydospores differed between the species and also depended on the medium used.

# The decline in viability of *P. cinnamomi* survival structures under moist and dry soil conditions.

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*Phytophthora cinnamomi* is a pathogen of great concern in Western Australia as it causes die-back disease in the jarrah trees (*Eucalyptus marginata*) and affects 3000 other plant species. The pathogen is spread through the landscape by movement of infested soil and water. Extensive mining operations in the infested areas can spread the disease to non-infested areas. We undertook the study to determine how long *P. cinnamomi* propagules survive in the heavily disturbed and exposed mine-site soils (the stockpiles) and consider the scope of using certain exogenous treatments such as smoke water, fish emulsion and living host plant to stimulate germination, and chemicals such asridomil and furalaxyl to kill or inhibit the growth and development of *P. cinnamomi* oospores. In particular, the aim of the study was to find out how moist and dry soil conditions affected the persistence of oospores as free soil propagules and to find out the scope of using exogenous treatments in managing *P. cinnamomi* in the mining environment.

The study was carried out under glasshouse conditions using soils collected from the stockpiles. Spores were produced *in vitro* and processed by filtering onto polycarbonate membranes and packaging in nylon-mesh sachets for placement in soil pots. The spore sachets were recovered after 3, 6, 12, 24, and 48 weeks and treated with tetrazolium bromide stain to determine spore viability. RNA assay was used to compare the precision of the two methods. The results showed a significant overall decrease in viability with time under both moist and dry conditions irrespective of treatments. At the end of 48 weeks, over 96% loss of viability was observed in all treatments, and in 60% of the treatments, 100% viability loss was observed. Furthermore, the rate of viability loss was significantly different amongst treatments under the moist conditions with viability decline occurring in the order of (first to last), smoke water, fish emulsion, ridomil, furalaxyl, control and living plant host. The RNA assay was partially in agreement with the results of the vital stain test as it confirmed viability of oospores for up to the 12$^{th}$ week under moist condition but failed to detect any viable oospores beyond the third week under the dry conditions. As indicated by the study, if oospores lose their viability in the soil within a period of one year under natural conditions, we see potential benefits of reducing inoculum load by fallowing the soil in the mining stockpiles before using them to restore and rehabilitate the mined sites.

# Effect of silver oligochitosan against (AgNPs) on the growth and reproduction of Phytophthora species in vitro.

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The genus *Phytophthora* with over one hundred species includes plant pathogens that cause the devastating diseases of industrial trees, fruit, spices crops and various horticultural herbs. Phytophthora diseases cause annual damage of billions of dollars in temperate and tropical regions. Nanotechnology provides new agrochemical agents and has the strong activity against microbial pathogens, and it promises to reduce pesticide use. Moreover, antifungal activity of oligochitosan has been observed against several fungal pathogens. Silver oligochitosan was prepared from 3,4 dihydroxyphenyl acetic acid - conjungated oligochitosan and silver core- shell nanoclusters by Institute of Applied Materials Science, Vietnam Academy of Science and Technology. Antifungal activity of silver oligochitosan against three *Phytophthora* species was investigated in vitro. Silver oligochotosan in inhibiting mycelia growth, sporangium production, zoospore release and zoospore germination was observed (Tables 1-4). Silver oligochitosan exhibited strong inhibition on the mycelium growth and sporangium production, zoospore release and zoospore germination of *P. capsici, P. nicotianae* and *P. colocasiae* at 9ppm. The current study suggests that use of silver oligochitosan has potential for control of *Phytophthora* diseases in crops.

**Table 1. Effect of silver oligochitosan on mycelial radial growth of *Phytophthora* species on PDA medium**

| Silver oligochitosan concentration | Diameter of colony (mm)[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 3 | | | Day 4 | | | Day 5 | | | Day 6 | | |
| (ppm) | *P.cap* | *P.nic* | *P. col* | *P.cap* | *P.nic* | *P. col* | *P.cap* | *P.nic* | *P. col* | *P.cap* | *P.nic* | *P. col* |
| 0 | 41.6[a] | 41.3[a] | 28.3[a] | 56.0[a] | 53.0[a] | 34.3[a] | 67.3[a] | 71.6[a] | 43.0[a] | 79.3[a] | 77.0[a] | 52.6[a] |
| 3 | 28.3[b] | 22.0[b] | 13.0[b] | 36.0[b] | 29.3[b] | 16.3[b] | 47.0[b] | 44.6[b] | 23.6[b] | 53.0[b] | 53.0[b] | 26.6[b] |
| 6 | 23.6[c] | 17.6[b] | 13.3[b] | 28.6[c] | 24.0[c] | 16.0[b] | 34.6[c] | 29.0[c] | 22.3[b] | 44.0[c] | 38.6[c] | 24.3[b] |
| 9 | 10.6[d] | 11.6[c] | 8.6[b] | 12.0[d] | 14.0[d] | 11.0[b] | 14.6[d] | 15.3[d] | 16.6[c] | 16.3[d] | 21.0[d] | 20.0[c] |

Colony diameter was measured on days 3-6 after incubation in the dark at 25°C and analysed by using Tukey's Multiple Comparison Test (*P*=0.05).
*P. cap: Phytophthora capsici*, *P. nic: P. nicotianae*, *P. col: colocasiae*

**Table 2. Effect of silver oligochitosan on sporangium production of *Phytophthora* species**

| Silver oligochitosan concentration (ppm) | *P. capsici* | | *P. nicotianae* | | *P. colocasiae* | |
|---|---|---|---|---|---|---|
| | NS[a] (sporangium/mL) | EH[b] (%) | NS (sporangium/mL) | EH (%) | NS (sporangium/mL) | EH (%) |
| 0 | $19.0x10^{4a}$ ± 14548.7 | - | $13.0x10^{4a}$ ± 13369.0 | - | $16.0x10^{4a}$ ± 511.8 | 0 |
| 3 | $8.7x10^{4b}$ ± 6359.5 | 54.0 | $4.4x10^{4b}$ ± 4333.3 | 66.3 | $10.6x10^{4b}$ ± 683.4 | 33.3 |
| 6 | $3.1x10^{4c}$ ± 1643.0 | 83.3 | $2.0x10^{4c}$ ± 2433.2 | 84.3 | $5.5x10^{4c}$ ± 4445.4 | 65.6 |
| 9 | $1.7x10^{4c}$ ± 2836.8 | 90.9 | $1.1x10^{4c}$ ± 1578.6 | 91.4 | $2.8x10^{4d}$ ± 3290.1 | 82.1 |

[a] NS: Numbers of sporangia were counted at day 5th after incubation in the dark at 25°C and analysed by using Tukey's Multiple Comparison Test (*P*=0.05). [b]Effect of inhibition (EH)

**Table 3. Effect of silver oligochitosan on zoospore production of *Phytophthora* species**

| Silver oligochitosan concentration (ppm) | *P. capsici* | | *P. nicotianae* | | *P. colocasiae* | |
|---|---|---|---|---|---|---|
| | NZ (spore/mL) | EH (%) | NZ (spore/mL) | EH (%) | NZ (spore/mL) | EH (%) |
| 0 | $12.0x10^{4a}$ ± 5925.4 | - | $25.1x10^{4a}$ ± 17211.5 | - | $11.8x10^{4a}$ ± 6148.9 | - |
| 3 | $7.4x10^{4b}$ ± 4634.2 | 38.5 | $13.8x10^{4b}$ ± 14331.6 | 44.9 | $8.1x10^{4b}$ ± 4404.1 | 31.1 |
| 6 | $2.6x10^{4c}$ ± 2681.5 | 78.4 | $10.6x10^{4b}$ ± 9730.4 | 57.5 | $5.0x10^{4c}$ ± 3273.2 | 57.6 |
| 9 | $1.8x10^{4c}$ ± 3408.6 | 85.0 | $2.3x10^{4c}$ ± 3217.0 | 90.6 | $2.4x10^{4d}$ ± 3132.0 | 79.7 |

NZ: Numbers of zoospores were counted on day 5th after incubation in the dark at 25°C and analysed by using Tukey's Multiple Comparison Test (*P*=0.05). EH: Effect of inhibition

**Table 4. Effect of silver oligochitosan on the germtube length of *Phytophthora* species**

| Silver oligochitosan concentration (ppm) | *P. capsici* | | *P. nicotianae* | | *P. colocasiae* | |
|---|---|---|---|---|---|---|
| | GL (µm) | EH (%) | GL (µm) | EH (%) | GL (µm) | EH (%) |
| 0 | $11.2^{a}$ ± 1.0 | - | $18.73^{a}$ ± 0.8 | - | $16.6^{a}$ ± 1.1 | - |
| 3 | $8.3^{b}$ ± 0.6 | 25.9 | $5.97^{b}$ ± 0.4 | 10.8 | $14.8^{a}$ ± 0.6 | 68.1 |
| 6 | $6.9^{b}$ ± 0.6 | 38.4 | $3.19^{c}$ ± 0.2 | 43.4 | $9.4^{b}$ ± 0.3 | 83.0 |
| 9 | $0.0^{c}$ ± 0.0 | 100.0 | $0.0^{d}$ ± 0.0 | 100.0 | $0.0^{c}$ ± 0.0 | 100.0 |

GL: Germtube length measured 1 hour after incubation of cystospores in the dark at 25°C and analysed by using Tukey's Multiple Comparison Test (*P*=0.05). EH: Effect of inhibition

## Approaching the bioprotection of kauri *Agathis australis* from *Phytophthora agathidicida:* assessing the role of mycorrhizae and dark septate endophytes.

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***Phytophthora agathidicida*** is a newly-described, exotic pathogen causing kauri dieback in the north island of New Zealand. A multi-agency response was initiated in 2009, and the search continues for ways to manage the short- and long-term impacts of this disease. Although there is on-going work to elucidate the mechanisms of disease transmission and host resistance, there is little information on the Fungi associated with *Agathis australis* and what, if any, roles they may play in disease resistance. We used light, scanning and transmission electron microscopy to characterise colonisation, and 454-sequencing to identify the arbuscular mycorrhizal fungi (AMF) associated with kauri roots and nodules. We interpreted the results in terms of the edaphic characteristics of the kauri-influenced ecosystem. Microscopic structures typical of AMF were found to be common in root nodules, but less noticeable in fine roots. Sequence data suggest that most of the molecular operational taxonomic units belong to globally-distributed *Glomus* species with little representation from other AMF lineages; however, there is also evidence for AMF species specific to kauri. Other than AMF, kauri roots are also colonised by dark septate endophytes (DSE). We isolated and identified cultures of these DSEs. In addition, we assessed the potential of these DSEs to inhibit *P. agathidicida* by performing *in vitro* inhibition assays. The results from plate inhibition assays suggest that helotialean ascomycetes such as *Pezicula* and *Leptodotidium* can have inhibitory effects on *P. agathidicida*. Our results are discussed in terms of the potential for the synergistic integration of these diverse groups of fungal microbes against *P. agathidicida.*

## Potential of endophytes as biological control agents of *Phytophthora* pathogens in rubber plantations in south-east Vietnam.

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Vietnam has become the world's leading exporter of natural rubber latex. The current area is about 800,000 ha in total in which about 46% is in the south-east region including Binh Phuoc, Dong Nai, Tay Ninh and Vung Tau provinces. In 2012 alone, Vietnam exported more than 1 million tons, valued at more than 2 billion USD. However, *Phytophthora* leaf fall and root rot diseases are main constrains to rubber growers. Recently, *Phytophthora heveae* VN739, *P. nicotianae* VN722, *Phytopythium* sp. VN604 and VN596, and *Pythium* sp. VN602 were isolated from the soil of rubber plantations and showed very strong pathogenicity in detached leaf tests. These pathogens are causing large scale economic losses. Control of disease with chemicals is difficult, costly and polluting to the environment. Endophytes reside in most healthy plants. Some endophytes are beneficial to host plants, providing growth promotion and disease prevention. Thirty five strains of bacterial endophytes (20 strains) and fungal endophytes (15 strains) isolated from rubber twigs and leaves collected from healthy trees. Isolated endophytes showed diversity in species and in antifungal activities to the above pathogens. Eight endophytic strains (NC1,NC3, NC5, N10, BC1, BC2, BC10 and BC7) demonstrated strong antagonism to the pathogens. These endophytes have a potential for use in biological control of *Phytophthora* leaf fall and root rot diseases in the region.

# The effect of *Phytophthora lateralis* soil populations on fine root densities of Port-Orford cedar (*Chamaecyparis lawsoniana*).

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*Phytophthora lateralis* is an exotic pathogen first reported in the U.S. in the 1920s on Port-Orford cedar (*Chamaecyparis lawsoniana*). The pathogen has caused extensive damage to Port-Orford cedar forests in North America following its likely introduction by the international plant trade pathway. Although *P. lateralis* has been isolated from aerial lesions on Port-Orford cedar, the most severe damage comes from collar and root rot. Motile zoospores released from sporangia are attracted to and infect fine roots leading to infection of larger roots. *Phytophthora lateralis* produces chlamydospores where upon favorable conditions will germinate to produce sporangia. Currently, it is unknown what role *P. lateralis* chlamydospores play in initial infection and cause of collar and root rot of Port-Orford cedar seedling. Pasteurized potting mix was infested with chlamydospores (10 chlamydospores per $cm^3$ potting mix) of two different *P. lateralis* isolates (US and Canadian) or an equal mixture of the isolates. Non-infested potting mix was included as a negative control. Susceptible and tolerant Port-Orford cedar seedlings were transplanted in 500 $cm^3$ pots containing the different treatments (n=5). Subdue GR was applied at the recommended rate after transplanting in one set of pots that contained potting mix infested with a mixture of the isolates. After 7 weeks, the plants were removed and rinsed free of potting mix. The fresh root and shoot were weighed. The roots of each plant were surface-sterilized, rinsed in sterile water and plated on a *Phytophthora*-selective medium. The potting mix for each of the five pots for each individual treatment was combined, thoroughly mixed, and plated on *Phytophthora*-selective medium to try and recover *P. lateralis*.

Results showed no difference in the root or shoot weight for any of the treatments. The susceptible Port-Orford cedar seedlings had a higher average root and shoot weight than the resistant seedlings but they were not always significant. *Phytophthora lateralis* was not recovered from the potting mix or the roots of any of the treatments.

Based upon these results, more basic questions need to be addressed. For example, what soil conditions are needed for chlamydospores to germinate and infect Port-Orford cedar roots? What is the survival of *P. lateralis* chlamydospores in a potting mix or soil medium? These questions will be investigated in further studies.

# Session 10: Resistance

## Testing kauri (Agathis australis) for resistance to Phytophthora agathidicida.

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***Phytophthora agathidicida*** is a newly-described, exotic pathogen causing kauri dieback in the north island of New Zealand. The decline was first noted on Great Barrier Island (Aotea) in 1972, but it was only in 2006 that the disorder was recognised on the mainland. A multi-agency response was initiated in 2009, and in 2014, research began to assess the presence of resistance within the remnant kauri forest-stands under the Scion-led "Healthy Trees Healthy Future Program". A number of non-destructive, low-impact experimental approaches have been used, as it is critical to the process that progeny can be obtained from parent-trees that display differential disease responses. To achieve this, our experiments currently use shoots, which were pruned from target trees and collected before they touched the ground. The shoots were divided into 15 one-year-old shoot piece replicates. Fifteen individual leaves were randomly selected from these shoot pieces; 10 leaves were inoculated with an isolate of *P. agathidicida* grown on V8 juice agar, and five were inoculated with a V8 juice agar plug as the negative controls. After 10 days, the leaves were photographed for Winfolia analysis and lesion length was scored, and post-hoc recoveries were taken from set distances beyond the point of inoculation. Ten shoot pieces were inoculated mid-way along the stem with a millet seed infected with *P. agathidicida* and five were inoculated with autoclaved millet seeds as the negative controls. After 28 days, the shoots were dissected and post-hoc evaluations of the test material included; i) plating to *Phytophthora*-selective media, ii) preserving material for cytological investigations (via fluorescent in-situ hybridization), and iii) cryostoring tissues for down-stream, "omics" – analyses. The results from the leaf and shoot assays were compared and correlated to determine if the levels of differential disease resistance observed from these ex-situ assays concurred.

# *Phytophthora* abnormal leaf fall of *Hevea* and breeding for disease resistance.

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*Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. (family, Euphorbiaceae; diploid, 2n=36), popularly called Para rubber tree, is a forest tree species native to the tropical rain forests of Central and South America. Para rubber tree is monoecious and insect-pollinated and possesses high outcrossing tendency. The tree produces latex which contains rubber particles (isoprene) and is used for making more than 50,000 end products (mostly tyres) from rubber bands to aviation tyres. Several thousands of hectares of Para rubber tree are being raised predominantly in South East Asian countries including India, China, Vietnam, Thailand, Indonesia and Malaysia. The plantations are also increasingly raised in new areas like Liberia. However, commercial rubber cultivation is under constant attack by native as well as exotic fungal pathogens. Climate change, which is clearly felt in many rubber growing regions, could possibly alter the host-pathogen interactions which can lead to emergence of disease epidemics in hitherto unknown regions posing serious challenges to productivity because *Hevea* breeding mainly focused on rubber productivity (Jayasinghe, 1999; Narayanan and Mydin, 2012; Mydin, 2014).

Severe economic losses have been reported in rubber plantations due to attack of pathogenic fungal diseases caused by *Phytophthora*, *Corticium*, *Corynespora* and *Oidium*. Among above diseases, abnormal leaf fall (ALF) caused by *Phytophthora* is a devastating disease capable of causing upto 40% loss in crop production. Every year, thousands of tons of fungicides are used for prophylactic spraying in plantations for prevention of major disease outbreaks and ensure good yield. Besides huge costs, long-term applications of chemical fungicides pose environmental and socio-economic constraints.

Many clones of *Hevea* exhibit variable levels of susceptibility to the fungal pathogen. Earlier selection and breeding for resistance to *Phytophthora sp*. in Brazil led to identification of resistant clones. Baptists (1961) reviewed the progress in selection and breeding of *Hevea* clones with resistance to *Microcyclus ulei* (=*Dothidella ulei*; causative agent of South American Leaf Blight) and *Phytophthora sp.* in Brazil and developed a list of those resistant clones which have been imported into Malaya and Ceylon since 1953-54.The list included selections from *H. brasiliensis* and *H. pauciflora* but most of the resistant material had been derived from *H. benthamiana* 'F4542', a selection of upper Rio Negro origin. Inter-specific hybridizations (*H. brasiliensis* x *H. pauciflora*, *H. camargoona* x FX 4098) were also attempted but a major breakthrough was not achieved.

Twenty six hybrid clones generated through a hybridization programme conducted during 1990 using Wickham clones and wild germplasm accessions were assessed to find clonal resistance with reference to abnormal leaf fall disease caused by *Phytophthora*. Results indicated high level of resistance in progenies belonging to the family 'RRII 105 x RO 142' as reflected by very high percentage of retention of healthier and uninfected leaves (59%) (Fig. 1). The study indicated that Amazonian accession RO 142 (RO - Rondonia, Brazil) used in the hybridization as well as its hybrid progenies possibly harbor genes for resistance to *Phytophthora* infection and hence useful for future resistance breeding. The above high-yielding hybrids with appreciable levels of disease resistance have already been advanced to final stages of evaluation in field trials laid out in experimental station and diverse environments through participatory plant breeding approach. Results from the above trials are awaited to evaluate and validate field level resistance of the clones under different environmental conditions.

Clone Fx 516 from Brazil was found to be highly resistant to ALF by *Phytophthora* and was used for hybridization with high-yielding modern clones to recover high-yielding recombinants with enhanced levels of resistance. Hybrids successfully produced with above cross combinations have been planted in nursery trials and are being evaluated for yield as well as disease resistance. In addition, open-pollinated progenies were collected from clone Fx 516 and nursery evaluation trials have been raised to rapidly recover progenies with high-yield and resistance to ALF disease. Among indigenous clones, RRII 5 and RRII 33, the clones developed by RRII, were more resistant to diseases including ALF, and hence, these clones and germplasm accessions can also be used in breeding for disease resistance in *Hevea*.

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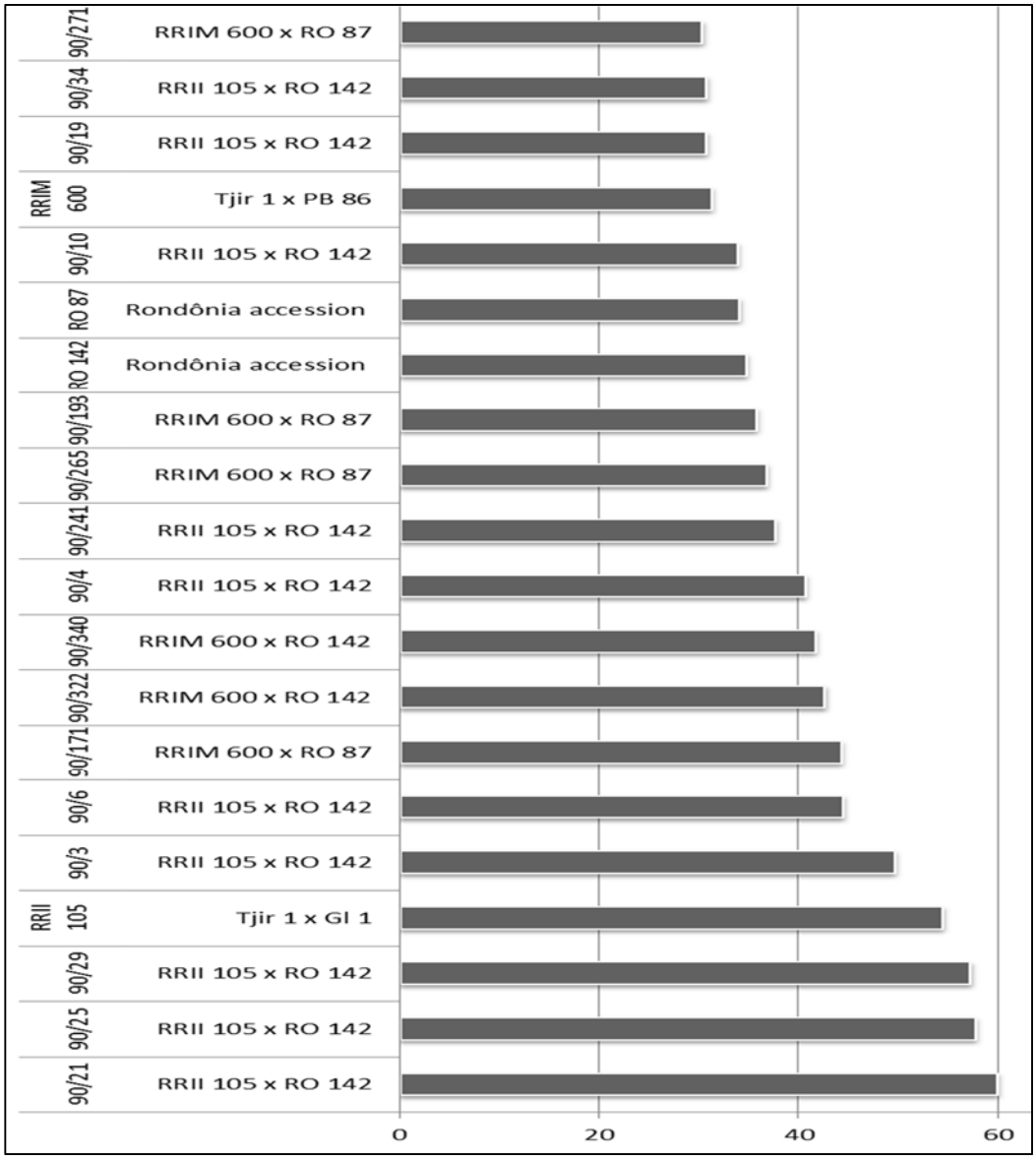


*Figure. 1. Percentage (X axis) of uninfected, healthy leaves in parental clones and their hybrids in response to Phytophthora leaf disease in a field trial (RRII – Rubber Research Institute of India, India; RRIM, Rubber Research Institute of Malaysia; RO – Rondonia, Brazil; RRII 105 and RRIM 600 are high-yielding parental clones; RO 87 and RO 142 are Rondonian germplasm accessions from Brazil).*

## A comparative transcriptomic analysis reveals mechanisms of resistance to *Phytophthora* in tanoaks.

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We have previously identified significant levels of tolerance to Sudden Oak Death, a tree disease caused by the oomycete *Phytophthora ramorum*, among populations of tanoaks (*Notholithocarpus densiflorus*) in Ca. In this study, we selected four families of half sibs grown in a common garden at U.C. Berkeley (California, USA) to study the genetic mechanisms underlying disease tolerance. Comparative analyses of the entire transcriptome were performed for approximately 100 individuals that were either a)-naturally tolerant, b)- naturally susceptible, c)- disease tolerant after treatment with phosphites, and, d)- disease susceptible after phosphite treatment. Results identified thousands of genes that were differentially expressed: a total of 80 genes (including metabolic controls) were selected for validation using Nanostring probes on approximately 100 plants, different from the ones fully sequenced. Final results showed that natural and phosphite-induced resistance are part of completely separate processes, with just a few genes involved in chemically induced disease resistance. Ten distinct genes were selected as markers for either constitutive disease tolerance or responsiveness to phosphite treatment. Genetic markers are being developed based on these ten loci to use for an associative transcriptomic analysis, through which naturally resistant and phosphite-responsive individuals may be identified in wild populations. Dual RNAseq data, showed that gene expression of the pathogen is minimal in most disease tolerant phenotypes, but also identified some resistant individuals in which the pathogen RNA was expressed at levels comparable with susceptible individuals. The implications of dual RNAseq results will be discussed.

## The hunt for a consistent phenotype for breeding for resistance to *Phytophthora pluvialis* in *Pinus radiata.*

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Diseases caused by *Phytophthora* species that infect the foliage of *Pinus radiata* are relatively new and are impacting New Zealand's production forestry. Both *Phytophthora. kernoviae* and *P. pluvialis* are present in *Pinus radiata* plantations. The former is the likely cause of physiological needle blight and *P. pluvialis* is known to be the cause of red needle cast on *Pinus radiata*. Variations in the growth and sporulation in relation to lesion development have been observed between susceptible and resistant genotypes of *Pinus radiata* following infection by both species. However, identifying consistent phenotypes for breeding and selection has proven problematic. In taking a systems biology approach, a range of phenotypic traits, genetic and biochemical markers are being assessed to inform breeding and selection programs. Analyses using Nuclear Magnetic Resonance, LC-MS and RNAseq in parallel to detailed histology are each providing insight into the host-pathogen interactions during infection across host genotypes. These results will be presented and discussed.

## Testing the tolerance of *Phytophthora cinnamomi* from New Zealand avocado orchards to phosphite *in vitro* and *in planta.*

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***Phytophthora cinnamomi*** is responsible for phytophthora root rot of avocado which is the most serious disease of avocado internationally. Disease control relies on application of phosphite trunk injections which suppress *P. cinnamomi* growth while also stimulating plant defence responses. *Phytophthora* species may become resistant to phosphite, with potentially resistance being observed in some isolates of *P. cinnamomi* from South African avocado orchards. In 2016 10 isolates of *P. cinnamomi* that were isolated from the rhizosphere of various host within New Zealand, were found to be variable in their sensitivity to phosphite with one isolate showing significantly more tolerance. New isolates of *P. cinnamomi* isolated from avocado orchards in the Bay of Plenty, Northland and Waikato regions of New Zealand will be tested for their *in vitro* and *in planta* tolerance to phosphite. Methods will include poison plate and liquid media assays, excised avocado branch assays and lupin assays. The aim is to determine if tolerance seen *in vitro* correlates with tolerance *in planta*. This work will be further expanded upon by examining the phylogenetic relationships of the isolates across the orchards.

## Use of chlorophyll florescence to infer root health and accelerate screening *Agathis australis* (Kauri) for resistance to *Phytophthora agathidicida.*

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***Phytophthora agathidicida*** is responsible for a significant decline in *Agathis australis* (kauri) forest, and is responsible for the death of some of the few remaining trees that predate human settlement. Significant research is required to efficiently screen the remaining diversity of kauri genotypes for disease resistance. Eco-physiological tools provide great insight into the non-visual impacts of infection on plant physiology. Assessments of photosynthetic efficiency have been widely used to infer root health associated with water use efficiency and root toxicity; however, they have rarely been applied to infer root health associated with soil borne pathogens. Here we demonstrate the application of non-invasive time series measurements of kauri chlorophyll florescence signatures to measure root damage and the relative susceptibility of seedlings to root infection and damage caused by *P. agathidicida*. This screening provides a greater understanding of the epidemiology of this poorly understood disease.

# Session 11: Surveys and New Species

## Diversity of *Phytophthora* species in Valdivian rainforests and their association with severe dieback.

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The Valdivian rainforest, one of the global hotspots of biodiversity, is considered a tropical rainforest in a non-tropical climate originating as a tertiary relic from the supercontinent Gondwana. In November 2014 a survey of *Phytophthora* diversity was performed in 13 natural forest stands and 20 forest streams and rivers located in two protected areas near Valdivia and in a temperate mountain forest in the Concepción area, and in each one planted stand of the introduced tree species *Castanea sativa* and *Fagus sylvatica*. Using baiting assays, eight described species and two previously unknown taxa of *Phytophthora* were isolated from 86% of the 50 rhizosphere soil samples from 7 of the 8 tree species sampled in 12 forest stands, and from 20 streams: *P.* x*cambivora, P. chlamydospora, P. cinnamomi* A2*, P. gonapodyides,* three lineages of *P. kernoviae* two of them most likely constituting a new species, *P. lacustris*, *P. madida* nom. prov. from Clade 8c, *P. plurivora*, *P. pseudosyringae*, and *P. valdiviana* nom. prov. from Clade 2b. *Phytophthora kernoviae* was also isolated from necrotic leaves of *Drimys winteri*. In addition, a diverse array of Clade 6 hybrids, *P. chlamydospora* x *P.* x*stagnum*, *P. thermophila* x *P. amnicola*, *P. thermophila* x *P. chlamydospora* and *P.* sp. xthermophila-like, were recovered from various streams. Moreover, *Nothophytophthora caduca* nom. prov., *Nothophytophthora chlamydospora* nom. prov. and *Nothophytophthora valdiviana* nom. prov., belonging to a new sister genus of *Phytophthora*, informally designated as *Nothophytophthora* nom. prov., were isolated from several forest streams. *Phytophthora cinnamomi* was associated with severe large-scale dieback of Valdivian rainforest trees, in particular *Saxegothea conspicua*, *D. winteri*, *Luma apiculata* and *Nothofagus dombeyi*. First pathogenicity trials demonstrated high aggressiveness of *P. cinnamomi* to several native tree species. Most of the other *Phytophthora* species were not associated with obvious disease symptoms.

# Diversity of *Phytophthora* species in natural forests and streams and in rubber plantations in Vietnam.

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In spring 2016 a survey of *Phytophthora* diversity was performed in 23 natural forest stands and 10 forest streams and rivers in temperate montane and tropical lowland regions and in 14 rubber plantations across Vietnam using baiting assays. Seventeen described species, 3 designated taxa and 17 previously unknown taxa of *Phytophthora* were isolated from 62% of 76 rhizosphere soil samples from 44 of 59 woody species in 19 forest stands, from 37% of soil samples in 5 plantations and from 10 streams: *P.* attenuata and 4 new taxa from the *P. attenuata* complex*, P. chlamydospora, P. cinnamomi* A1 and A2, *P. capensis*, *P. castaneae*, *P. citricola*, *P. drechsleri*, *P. gregata*, *P.* x*heterohybrida* (A1 and homothallic), *P. heveae*, *P. meadii*, *P. nicotianae*, *P. palmivora*, *P. parvispora*, *P. pseudocryptogea*, *P. ramorum, P. virginiana*, *P.* sp. citricola-like 1, *P.* sp. citricola-like 2, *P.* sp. kelmania, *P.* sp. insolita-like 1, *P.* sp. insolita-like 2, *P.* sp. Kunnunara, *P.* sp. macrochlamydospora-like, *P.* sp. multivesiculata-like, *P.* sp. Peru 4, *P.* sp. Peru 4-like, *P.* sp. sylvatica-like, *P.* sp. xGrenada 3-like, *P.* sp. xKunnunara-like 2-4, *P.* sp. xPeru4-like, *P.* sp. xsylvatica-like, and two other Clade 9 hybrid taxa. In addition, *Nothophytophthora vietnamensis* nom. prov. and a diverse array of known and new taxa of *Phytopythium*, *Pythium* and *Elongisporangium* were recovered including 15 genotypes of *Phytopythium vexans*. The finding of *P. ramorum* (A1 and A2 mating type) in six forest streams supports a Southeast Asian origin. The *P. ramorum* isolates differed only by one basepair in *cox1* from the NA2 lineage but show some behavioural differences and may be a new lineage. In Hoang Lien National Park above 1900 m altitude *P. cinnamomi* A2 was associated with severe large-scale dieback of many tree species mostly belonging to the Fagaceae and Lauraceae whereas in Ba-Vi National Park at lower altitudes *P. cinnamomi* A2 did not cause disease symptoms.

# *Nothophytophthora* prov. nom., a new sister genus of *Phytophthora* from natural and semi-natural ecosystems in Europe, Chile and Vietnam.

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During various surveys of *Phytophthora* diversity in Europe, Chile and Vietnam slow growing isolates were obtained from soil samples and small streams in natural and planted forest stands which morphologically resembled *Phytophthora*. Preliminary phylogenetic analyses of ITS, *cox1* and LSU sequences showed that they constitute six new species belonging to a new sister genus of *Phytophthora*, informally designated as *Nothophytophthora* nom. prov. Further gene regions are currently being sequenced as basis for a multigene phylogeny. All five species produce nonpapillate sporangia releasing zoospores with particularly long flagellae. The type species from forest streams in Portugal, *N. amphigynosa* nom. prov., has persistent sporangia and produces oogonia with mostly amphigynous antheridia in single culture. *Nothophytophthora intricata* nom. prov. from a riparian *Aesculus* stand in Germany is homothallic with paragynous antheridia and persistent sporangia. In contrast, the sporangia of the three species from Valdivian temperate rainforests in Chile, *N. caduca* nom. prov., *N. chlamydospora* nom. prov. and *N. valdiviana* nom. prov., are selfsterile and partially caducous with short pedicels. *Nothophytophthora vietnamensis* nom. prov. was isolated from a montane riparian forest in Vietnam. All isolates have partially caducous sporangia and are homothallic with both globose and elongated oogonia and paragynous antheridia. Including *Nothophytophthora* in phylogenetic and molecular clock analyses will give new insights into the evolutionary history of *Phytophthora*. Comparative morphological studies of both genera will allow clues about the morphology and ecology of their common ancestor.

# Posters

## The host range of selected new *Phytophthora* species on native Australian species in the glasshouse.

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The pathogenicity of some recently described species of *Phytophthora* together with *P. cinnamomi* as a control were tested against seven Western Australian plant species in the glasshouse. Host species were *Casuarina obesa*, *Banksia littoralis*, *B. occidentalis*, *B. grandis*, *Lambetia inermis*, *Corymbia calophylla*, and *Eucalyptus marginata*. 22 *Phytophthora* species were grown on vermiculite and used as soil inoculum when the plant hosts were approximately three months old. Pathogenicity was assessed after 6 weeks and plants were scored for death, reduction of shoot growth compared with control plants, and percentage of root damage. *Phytophthora* species were reisolated from roots for each host species but the degree of damage was varied. As expected from previous work *P. cinnamomi and P. niederhauseri,* killed *L. inermis, B. grandis*, *B. occidentalis*, and *C. obesa*, and also resulted in reduction of shoot growth and root damage in the other species except *Corymbia calophylla.* Based on root and shoot damage of the highest number of species, the most pathogenic species were *P. cinnamomi* and *P. niederhauseri, P. boodjera*, *P. rosacearum, P. multivora, P. thermophila, P. inundata, P. arenaria*, *P. gibbosa.* The plant species susceptible to the highest number of *Phytophthora* species were *Eucalyptus marginata, Banksia littoralis*, *B. occidentalis*, *B. grandis. Corymbia calophylla* was tolerant to most species. *Phytophthora* was isolated from asymptomatic plants.

# The host range of selected new *Phytophthora* species on native Australian species in the glasshouse

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A number of species of *Phytophthora* have been described from Western Australia and elsewhere. The host range and pathogenicity of these species is not known.

Investigation of the pathogenicity of the new *Phytophthora* species on a number of ecologically important woody species from Western Australia was undertaken. Plants were grown in the glasshouse and when 3 months old the soil was inoculated with each *Phytophthora* species. Shoot height and root health was assessed after 6 weeks.

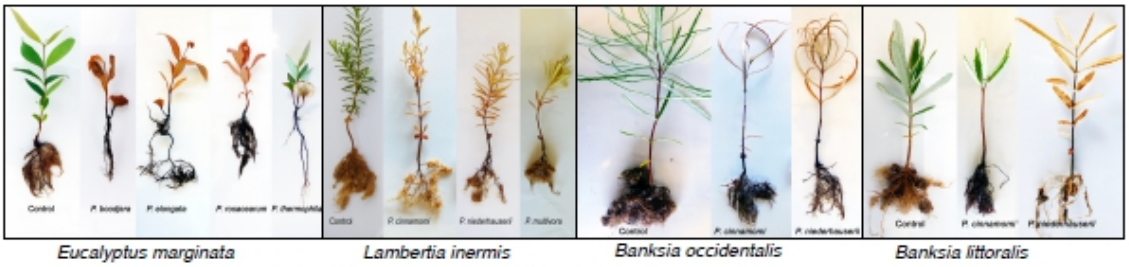
**Figure: 1:** Plants at the time of inoculation

## RESULTS

**Table1: Effect of *Phytophthora* species on shoot growth and root health of woody species from WA**

| Clade | 9 | 7 | 7 | 4 | 4 | 2 | 2 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Plant species | *P. constricta* | *P. cinnamomi* | *P. niederhauserii* | *P. boodjera* | *P. arenaria* | *P. elongata* | *P. multivora* | *P. asparagi* | *P. lacustris* | *P. litoralis* | *P. thermophila* | *P. flurialis* | *P. meyorae* | *P. gibbosa* | *P. gregata* | *P. sp. wangulina* | *P. pseudorosacearum* | *P. rosacearum* | *P. sp. cyperaceae* | *P. inundata* | *P. sp. condilina* | *P. sp. personii* | | |
| | CLJ0695 | VHSC16130 | MP 94-48 | PAB13-29 | VHS26806 | VHSC27382 | ENA1 | ENA3 | TP13-32 | TP13-36 | TP13-04 | WAC13101 | VHS17175 | HSA15939 | VHS13085 | VHS20763 | PN42-13 | VHS13530 | DH086 | DH213 | VHS16108 | VHS27218 | VHS32007 | VHS21998 | VHS21962 | VHS21992 | DBS3599 | VHS23298 | VHS29592 | HSA2530 | VHS24266 | HSA1658 | HSA1650 | VHS25476 | VHS2567381C | VHS2567383C | VHS15512 | VHS25244 | PAB11.04 | SA778 | SLPA133 |
| *Eucalyptus marginata* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Banksia occidentalis* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Banksia littoralis* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Lambertia inermis* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Casuarina obesa* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Corymbia calophylla* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Banksia grandis** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

( ) a statistically significant reduction in shoot height.
( ) a statistically significant increase in root damage
( ) dead plants
* shoot height could not be measured for *B. grandis*



*Eucalyptus marginata* *Lambertia inermis* *Banksia occidentalis* *Banksia littoralis*

**Figure 2:** A comparison of control and infected plants

## CONCLUSIONS

- Most of the new *Phytophthora* species were not highly pathogenic on the plant species tested.
- *P. niederhauserii* isolate PAB13-29 was as pathogenic as *P. cinnamomi*.
- The most pathogenic species were *P. boodjera*, *P. elongata*, *P. rosacearum*, *P. thermophila*, *P. gibbosa*, *P. multivora*, and *P. litoralis* on *Eucalyptus marginata*, *Banksia occidentalis*, and *B. littoralis*.
- The most pathogenic species were from clades 2, 4 and 7.

## *Castanea sativa* and global change: Searching for native trees tolerant to both water stress and *Phytophthora cinnamomi*.

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In Spain, ink disease caused by *Phytophthora cinnamomi* is the primary threat to Sweet chestnut (*Castanea sativa* Mill.). Commercial hybrids obtained with *C. crenata* and *C. mollisima* germplasm are extensively planted in infested areas to replace native forests and traditional fruit varieties, but do not always tolerate the current conditions of drought and high temperatures. Within an ongoing project funded by the Spanish Ministry of Economy and Competitiveness (AGL-2014-53822-C2-1-R) we evaluated 1) the tolerance of native chestnut: 360 chestnut trees from coppice, wild or fruit orchards from four Spanish provenances. Trees and seedlings from 60 of them were genotyped by both neutral SSRs and functional EST-SSRs markers: 2) the influence of water stress, waterlogging and grafting in the susceptibility of *C. sativa* to *P. cinnamomi.* Several experiments in the greenhouse with clonal material will be conducted, and the hormonal and biochemical profiling and the gene expression (by RT-qPCR) of ramets will be assessed; 3) native trees tolerant to both *P. cinnamomi* and water stress, suitable for being planted in dry areas in central and southern Spain. Based on EST-SSRs genotyping, mother trees will be selected in the field and their offspring will be inoculated. The results achieved will be shown and discussed.

## *Phytophthora* diseases in Nghe An province.

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Nghe An province in north-central Vietnamnormally has a wet season from June to September leading to waterlogging and flooding. Typhoons also affect the area. These wet conditions favour *Phytophthora* diseases leading to serious yield losses in crops including citrus, pineapple, and papaya. Two serious *Phytophthora* diseases were identified recently. In early September 2007, crops of *Telosma cordata*, a high-value cash crop, were severely affected by a root and stem rot disease referred to as "quick death" by local farmers. There was 100% loss of plants in the majority of crops by late October. The onset of the disease followed prolonged wet weather and flooding. The pathogen was identified as *Phytophthora palmivora* and is thought to have been introduced into the area on infected papaya seedlings from southern Vietnam. In August 2012 a severe stem rot (stem canker) disease was observed in a purple-fruit variety of passionfruit, *Passiflora edulis*, in a mountainous region of the province. The stem rot progressed rapidly along the stem affecting branches and fruit, leading to chlorosis, wilting and death of the distal part of the stem. The disease spread within and between small-holder plantings causing 100% loss of some crops. It is assumed that the pathogen was introduced on infected seedlings. Waterlogging and poor drainage are major factors favoring these *Phytophthora* diseases. Integrated disease management strategies include improved drainage, disease-free seedlings, and strategic application of fungicide.

## *Phytophthora cinnamomi* in the Central Highlands of Vietnam.

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In Vietnam, *P. palmivora*, *P. capsici*, *P. botryosa* and *P. nicotianae* are well known pathogens which have a significant impact on the production of high value crops including durian, chili, rubber, pineapple and longan. Over the last decade, the cultivation of avocado and macadamia has become popular and is rapidly expanding in the Central Highlands of Vietnam. Both of these host plant species are susceptible to *P. cinnamomi* but no work has been done to establish the presence and impact of *P. cinnamomi* on avocado and macadamia. We have observed bleeding stem canker, trunk rots, root rots and die back problems in the Central Highlands. Our initial isolation and morphological identifications revealed that these symptoms were caused by a *P. cinnamomi*. Our findings have already led to changes in practices of producing plants in nurseries and layout, design and drainage in new orchards. Based on these initial findings and the rapidly expanding avocado and macadamia industry, we plan to study the occurrence and diversity of *P. cinnamomi* throughout Vietnam. At the same time, we aim to design and implement management procedures to reduce the impact of *P. cinnamomi* on avocado and macadamia orchards in Vietnam.

# *Phytophthora cinnamomi* in the Central Highlands of Vietnam

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## Introduction

In Vietnam, *P. palmivora*, *P. capsici*, *P. botryosa*, *P. nicotianae* and *P. cinnamomi* are well known pathogens which have a significant impact on the production of high value crops including durian, chili, rubber, pineapple and longan. Over the last decade, the cultivation of avocado (*Persea americana*) and macadamia (*Macadamia integrifolia* and *M. tetraphylla*) has become popular and is rapidly expanding in the Central Highlands of Vietnam. Both of these host plant species are susceptible to *P. cinnamomi* but little work has been done to establish the presence and impact of *P. cinnamomi* on avocado and macadamia. We have observed the bleeding stem canker, trunk rots, root rots and die back problems in the Central Highlands. Our initial isolation and morphological identifications revealed that these symptoms were caused by a *P. cinnamomi*. Our findings have already led to changes in practices of producing plants in nurseries and layout, design and drainage in new orchards. Based on these initial findings and rapidly expanding avocado and macadamia industry, we aim to study *P. cinnamomi* diversity and reduce its impact.

## Objectives

- Study the occurrence and diversity of *P. cinnamomi* throughout Vietnam
- Design and implement management procedures to reduce the impact of *P. cinnamomi* on avocado and macadamia orchards in Vietnam

## Methodology

### 1. Study *P. cinnamomi* diversity

Collecting samples (soils, trunks, leaves, roots) throughout Vietnam

CARPP selective media

Isolation

Purification

Identification
- ✓ Taxonomic keys of Stamps *et al.* (1990) and Erwin & Ribeiro (1996)
- ✓ Koch's postulates
- ✓ DNA analysis

Diversification
- Microsatellites and SNPs of trnG-rns, rns-nad6, nad9, secY, cox2, rps10, nad3 or nad3-cox1

### 2. *P. cinnamomi* management procedures

**i. CULTURAL PRACTICES**

- Pathogen free seedlings
- Well-drained, well-aerated soil
- Irrigation right: dripping system
- Ridging/mounding, mulching,
- Balanced nutrients: chicken manures, bio-fertiliser
- Exploiting root growth stimulator: *Azotospilllium* spp., *Pseudomonas* sp., *Burkholderia* sp.

**ii. RESISTANCE/TOLERANCE**

**iii. BIOLOGICAL CONTROL**

- *Enterobacter cloacae*

**iv. CHEMICAL CONTROL**

- Phosphonates (Agri-Fos)
- Metalaxyl (Ridomil Gold)

## Acknowledgements

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## Changes in the population structure of *Phytophthora ramorum* associated with the host jump to larch in Britain.

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The first findings of the EU1 lineage of *Phytophthora ramorum* in Great Britain date from 2002 with many of the early outbreaks centred on nurseries and managed gardens and the affected hosts mainly broadleaf ornamental shrubs and trees. However, 2009 saw a major change as conifer plantations of larch (*Larix*) became affected by ramorum disease and started to show widespread infection and mortality. EU1 isolates collected from 2002-2012 over the course of the epidemic in Britain were investigated using eight polymorphic microsatellite markers with the aim of understanding possible drivers for the host jump to larch. Analysis of 347 isolates revealed 51 multi-locus genotypes (MLGs) which partitioned into two distinct population clusters. One cluster comprised MLGs unique to Britain and apparently not present elsewhere in Europe; the other cluster consisted of MLGs already known from Europe. Isolates obtained pre-2009 belonged predominantly to the unique British cluster with only a few typical of the European cluster. From 2009 onwards this pattern changed markedly, as European MLGs, especially EU1MLG1, became common as the disease epidemic on larch spread. Analysis suggests that the dominance of certain European MLGs may have been an important driver in the emergence of the *P. ramorum* epidemic on larch and they are closely associated with the distribution of larch along the west coast of Britain. In contrast, MLGs of the unique British population tend to be concentrated in south west England. These two distinct population clusters in Britain also suggest that there have been at least two separate introductions of *P. ramorum* EU1 lineage into the UK, each of which has subsequently diversified into the span of MLGs revealed by this study.

## Changing distribution of *Phytophthora ramorum* lineages in the UK.

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Until recently the population structure of *Phytophthora ramorum* was known to consist of three, largely clonal evolutionary lineages. The EU1 lineage has been reported from most European countries and very recently from Oregon in the USA, whereas in North America it is the NA1 and NA2 that dominate. Then in 2011 a fourth evolutionary lineage of *P. ramorum* was discovered in the UK which so far has not been recorded anywhere else. The consequences of EU2 arrival in the UK are under investigation but it has been found infecting a wide range of hosts including Japanese larch (*Larix kaempferi*). There is also emerging evidence that the EU2 is more aggressive when colonizing larch bark than the EU1 suggesting it could pose an even greater threat to forestry than the already widespread EU1. In 2012, isolates of *P. ramorum* collected from across the UK since 2002 were tested for lineage, plus more than 100 samples collected during 2013-14. The combined data set confirmed that the EU2 was restricted to Northern Ireland and a small area of south west Scotland. Continuing sampling and lineage testing of plants infected by *P. ramorum* in south west Scotland now show that the area occupied by the EU2 lineage is gradually extending and in some locations the EU1 and EU2 are close to overlapping. However, so far the EU2 remains absent from the other countries of the UK - England and Wales. This study shows through a series of maps the changing distribution of the EU2 five years after the initial finding.

# *Phytophthora* collection effort and potential hybrids identified from forests and rivers in France.

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**Introduction**

Introduction of alien species and potential hybridization with indigenous species is one of the principal threats to plants and forest ecosystems. In this context, decipher the mechanisms of hybridization of invading organisms and pinpoint the hot-spots of hybridization remains a major challenge. The *Phytophthora* genus constitutes a good model to study the emergence and evolution of hybrid species. As an example, the recent analysis of ploidy level and genetic diversity of the hybrid pathogen *P.* ×*alni* allows us to understand the emergence of alder disease (Aguayo et al. 2016, Husson et al. 2015). This pathogen which causes serious damages in riparian ecosystems in Europe was actually emerged as a result of the hybridization between an invasive pathogen, P. *uniformis* and a cryptic species, *P.* ×*multiformis*.

In order to achieve this objective, the first step is to collect *Phytophthora* isolates from different ecosystems to determine the environmental factors that increase the probability of hybridization. Then, analyze the chromatograms of the sequence of the highly conserved rDNA ITS region constitutes a preliminary method for detecting potential hybrids. Despite the multi-copy nature of ITS region, presence of double peaks in nucleotide positions could indeed indicate signals of a recent hybridization. We therefore collected isolates of *Phytophthora* spp. in forest soil and river water using baiting and isolation on Petri dishes and then amplified ITS region in order to screen hybrid species.

**Materials and Methods**

In August 2014, soil samples were collected in Northeastern France in 5 plots located in Northeastern France at the vicinity of 4 trees belonging to *Fraxinus excelsior*, *Quercus* sp., *Alnus glutinosa* or *Carpinus betulus* (Figure 1). In total, 20 soil samples were analyzed separately. Baiting using Rhododendron cv. Cunningham's white, *Prunus laurocerasus* and *Chamaecyparis lawsoniana* leaves was performed with 200 ml of soil sample and 500 mL of distilled water and analyzed after 3 days incubation. Moreover, baiting was directly performed in river water with Rhododendron leaves placed in a net floating on the water surface as a raft. The baits were installed during three days in June 2015 in 7 plots where Willow, Alder, Oak or Ash trees were present in riverbank (Figure 1). Necrotic spots on leaves (from river water or soil baiting) were cut and placed in Petri dishes containing *Phytophthora*-specific agar medium based on V8 juice amended with 10 mg/l rifampicin, 10 mg/l pimaricin, 200 mg/l ampicillin, 15 mg/l benomyl and 50 mg/ml hymexazol. Each isolate was then sub-cultured on V8 agar medium. DNA was extracted from mycelium using a commercial kit and the ITS region was amplified with ITS6-4 primers. Finally, Sanger sequencing of the amplicons was performed for species identification based on Genbank databases and for investigating double peaks in nucleotide positions.

**Results and Discussion**

Firstly, 112, 38 and 14 necrotic spots of Rhododendron, *P. lauracerasus* and C. *lawsoniana* leaf baits, respectively, were analyzed. 96% of spots from Rhododendron leaves were caused by *Phytophthora* species, compared to 63% of spots from bay laurel leaves. No *Phytophthora* were isolated from *Chamaecyparis* leaves. Our study confirmed that Rhododendron leaves appears to be an efficient bait for collecting *Phytophthora* isolates in temperate forests.

The sizes of ITS amplicons were from 760 to 840 bp. In total, 132 and 150 *Phytophthora* isolates were collected from forest and river stands, respectively. While 6 species were identified from forest soil samples (*P. plurivora*, *P. pseudosyringae*, *P. syringae*, *P.* sp. *hungarica*, *P. bilorbang*, *P. megasperma*), only two were detected in river water (*P. lacustris* and *P. plurivora*). The species *P. plurivora* was the main species in forest soil (50% of the isolates baited) and was found at the vicinity of the 4 hosts studied. *Phytophthora lacustris* isolates *were* overwhelmingly domimant in river (96%).

All of the 150 *Phytophthora* isolates from rivers exhibited at least 1 double peak in ITS sequences and 65% exhibited 3 or more double peaks. On the contrary, 80% of isolates collected in forest soil exhibited no double peaks in nucleotide position and 20% had a máximum of 2 double peaks. Our results show therefore that rivers and streams appear to be a hot-spot of hybridization in Europe compared to forest soil.

The isolates identified as *P. lacustris* in this study are probably good candidates for identifying potential hybrids. This taxon was only detected in river water and 68% of isolates had 3 or more double peaks in ITS region. To a lesser extent, isolates belonging to two other taxa, *P. pseudosyringae* and *P. plurivora*, could also be interesting taxa as 50% and 28%, respectively, exhibited 1 or 2 double peaks.

In forest, 16 out of the 36 isolates (44%) detected at the foot of alder trees were potentially hybrids although the species *P. ×alni* was never isolated. Thus, alder trees could be an interesting host to detect *Phytophthora* hybrids. On the contrary, only 2 *Phytophthora* isolates (and no hybrid) were isolated from soil samples collected close to *C. betulus*. In conclusion, this study tests a preliminary technique for detecting potential hybrids based on the analysis of chromatogram of ITS sequences and on the presence of double peaks in nucleotide positions and highlights potential hot-spots of *Phytophthora* hybrids in river water. Further studies are needed to validate the results and to clearly identify hybrid species in the genus *Phytophthora* using technologies as flow cytometry, genotyping-by-sequencing or sequencing of single copy genes.

**Acknownledgments** This work was supported by grants from the BiodivERsA project RESIPATH 2014-2017. The UMR1136 is supported by a grant overseen by the French National Research Agency (ANR) as part of the "Investissements d'Avenir" program (ANR-11-LABX-0002-01, Lab of Excellence ARBRE)

Aguayo J, Halkett F, Husson C, et al. 2016. Genetic diversity and origins of the homoploid allopolyploid hybrid *Phytophthora ×alni*. Applied and Environmental Microbiology. doi:10.1128/AEM.02221-16
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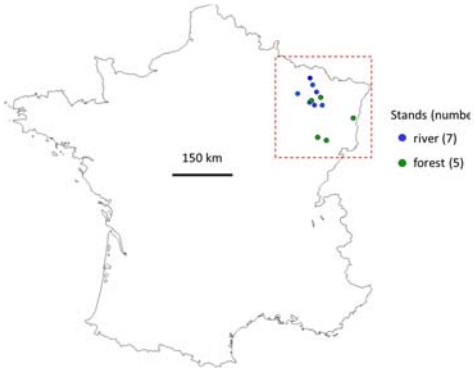
*Figure 1: location of the stands investigated in forest and river in Northeastern France*




## Can *Phytophthora cinnamomi* adapt to cold environments?

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The ability of *Phytophthora cinnamomi* to grow, sporulate and release zoospores at different temperatures 4, 7.5, 10, 12.5, 15, 20, 25, 30, 32.5, 35 and 37.5 °C was determined. Initially, 30 *P. cinnamomi* isolates were grown on V8 agar to determine their growth rates at different temperatures. From this 10 isolates were selected based on their different cardinal temperatures and growth curves. All the 10 isolates were grown on V8 agar for 5-7 days at 25 $^{0}$C. Four agar plugs were cut in triplicates from each isolate and transferred to sterile empty Petri dishes. Each Petri dish was flooded with V8 broth until the broth was just under the surface of the agar plugs. All the plates were immediately transferred to incubators set at the above-mentioned temperatures. Isolates kept at higher temperatures were left for 3-4 days and those at lower temperatures (7.5, 10 and 12.5°C) were left for two weeks to allow sufficient mycelium growth. The cultures were then rinsed three times with deionised water (at the correct temperature) and flooded with 10% soil extract after the final wash. Finally, cultures were incubated under light for 18 hours to encourage sporangia production. Numbers of sporangia were counted in six fields of view at 10x magnification, at each temperature. Growth on agar and numbers of sporangia formed and zoospores release were significantly greater at 25 and 30°C. Growth on agar and the ability of *P. cinnamomi* to sporulate were in harmony at lower temperatures 4 and 7.5°C, as no growth and sporulation was observed. Similarly, no growth on agar and sporulation was observed at 32.5, 35 and 37.5 °C. This study suggests that *P. cinnamomi* can produce sporangia only at those temperatures at which it can grow. Further experiments are underway to determine the effect of host on sporangia production and sporulation at low temperatures.

# Can *Phytophthora cinnamomi* adapt to cold environments?

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## Background

*Phytophthora cinnamomi* has been found in highly diverse and fragile sub-alpine ecosystems previously considered pathogen and disease free. This suggests that it could be adapting to colder environments. *What are the potential scenarios to explain this observation?* (Fig. 1)

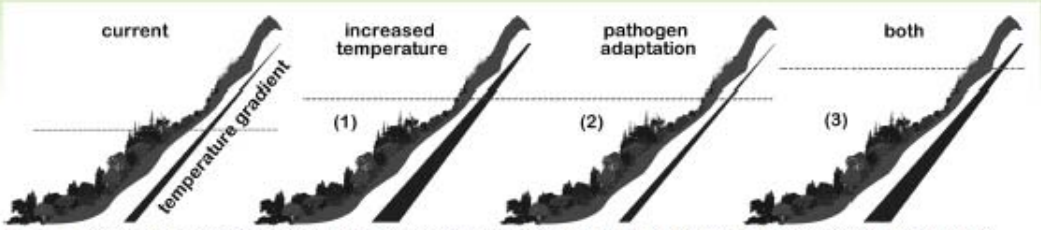


Figure 1. There are three overarching hypotheses to explain these observations. (1) The climate at higher elevations is no longer unfavourable for survival of *Phytophthora cinnamomi*, (2) *Phytophthora* cinnamomi has adapted to higher elevations and can now survive under colder conditions, or (3) a combination of both

## Materials and Methods

This study is examining the second of these scenarios. Can *P. cinnamomi* respond rapidly to selection pressure and adapt to new and colder environments?

Firstly, *in vitro* experiments were conducted to determine the temperature profile of 30 *P. cinnamomi* isolates ranged from 4 - 37.5 ° C. From these, 9 isolates were selected to determine their ability to produce sporangia and release zoospores across the same temperature range. Sporangia were produced in non-sterile soil extract at each temperature and zoospore release was observed. The number of intact and empty sporangia were counted in six fields of view at 10X magnification.

Secondly, an on-going experiment is attempting to 'train' four isolates to grow and sporulate at lower temperatures *in planta*. Briefly, *Oxylobium ellipticum* seedlings were germinated in a sterile river sand under glasshouse condition and inoculated with four *P.* cinnamomi isolates after three months. The plants were transferred to a growth cabinet set at 9 ° C and harvested after 96 days. To date only one isolate has been recovered from dying seedlings.

## Results

For all 30 isolates, the optimum temperature for growth was 25 to 30 ° C, and all isolates failed to grow at 4 and 37.5° C was found to be lethal. No sporangia production or zoospore release were observed at 4, 7.5, 32.5 and 35° C. Sporangia production was infrequent at 10 and 12 ° C and zoospore release was limited at less than 20° C . The temperature range for producing sporangia and releasing zoospores was more narrow than the range over which it can grow (Fig. 2). Additionally, the rate of sporangia production and zoospore release was much slower at 10 and 12.5° C than at higher temperatures, as they were observed after 10 days compared to 2 days at optimum.

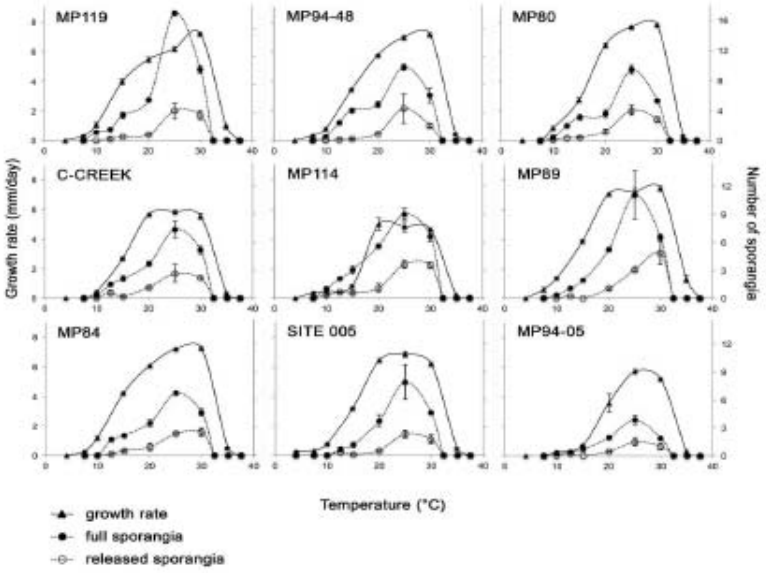


Figure 2. Mean radial growth rates, number of sporangia produced and number of empty sporangia (indicating zoospore release) for nine *P. cinnamomi* isolates at different temperatures.

Training *in planta* at 9 ° C in the growth cabinet resulted in symptoms appearance and plant deaths (Fig. 3). The recovered isolate C-CREEK-1 was able to increase its growth rate at 7.5 ° C on V8A compared to the original isolate.

Sporangia and zoospores release will now be tested. The experiment will be repeated numerous times to determine its adaptive capability to grow, sporulate and cause disease at increasingly lower temperatures.

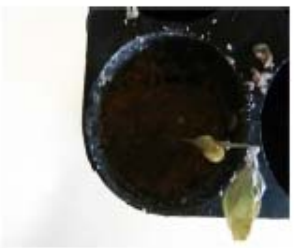

Figure 3. *Oxylobium ellipticum* seedling killed by *P. cinnamomi* isolate C- CREEK growing in the growth cabinet at 9 ° C .

## Conclusions

Preliminary results indicate that an isolate originally from sub-alpine vegetation in Tasmania (C-CREEK), was able to adapt to lower temperature (thus exhibiting adaptive plasticity). This indicates *P. cinnamomi* can respond rapidly to selection pressure and adapt to new environments and can cause disease in such environments. Further experiments are underway to confirm this observation.

## Association of *Phytophthora* with declining vegetation in an urban environment.

Mohammed Y. Khdiar [1, 2], Paul Barber [1, 3], Giles St. J. Hardy [1] and Treena Burgess [1]
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***Phytophthora*** species are important plant pathogens causing disease and mortality of a large number of trees and shrubs in forest ecosystems worldwide. 270 root samples were collected from declining trees in 87 parks and nature reserves in Joondalup, Perth Western Australia. Samples were collected from an extensive variety of declining exotic and native Australian trees and shrubs including *Acacia, Allocasuarina, Banksia, Corymbia, Eucalyptus, Grevillea, Hibbertia, Metrosideros* and *Xanthorrhoea.* DNA was extracted roots and a *metabarcoding approach was followed using Phytophthora–specific primers.* Out of the 270 samples collected, 175 contained at least one *Phytophthora* species. Overall, forty-seven *Phytophthora* species were detected. *Phytophthora multivora* was isolated the most frequently from 77% samples and *Phytophthora cinnamomi* was detected from 44% samples. In contrast, five species *P. asparagi, P. fallax, P. gonapodyides P. sp. pecan* and *P.* sp. walnut were present from only one sample. The results reinforced the primary role of *Phytophthora* species in the declining health of urban trees.

# Host range of *Phytophthora* species associated with declining urban trees

Mohammed Y. Khdiar, Treena I. Burgess, Paul A. Barber and Giles E. St. J. Hardy

Murdoch University

## Introduction

Members of the genus *Phytophthora* are important pathogens causing forest declines worldwide. The genus *Phytophthora* contains approximately 200-600 species including 150 formally described species. *Phytophthora* cause many significant plant diseases to agricultural, horticultural and forest ecosystems around the world. The aim of this study was to develop a rapid bioassay to screen the potential host range and pathogenicity of 19 Phytophthora species including many newly described species on a range of plant species commonly planted in the urban landscape in Western Australia.

## Materials and Methods

An inoculation experiment with nineteen *Phytophthora* species was conducted on excised branches (28 to 32 cm long 3.5 to 4.5 mm in diameter) of sixteen tree species. The plant branches were underbark inoculated with one of twenty-one isolates of the 19 *Phytophthora* species. There were 10 replicate stems of each tree species for each *Phytophthora* isolate. These were incubated in the dark at 25°C and lesions were measured 8 days after inoculation

## Results

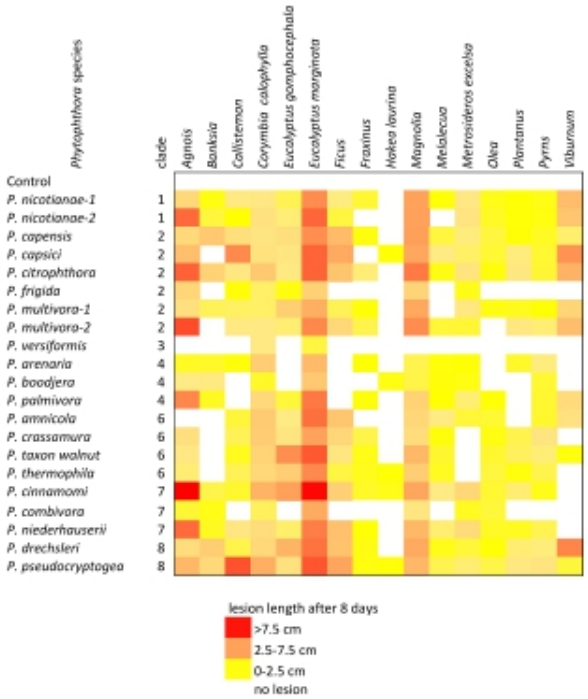


**Figure 1.** Heat map showing pathogenicity of nineteen *Phytophthora* species (21 isolates) toward sixteen plant species.

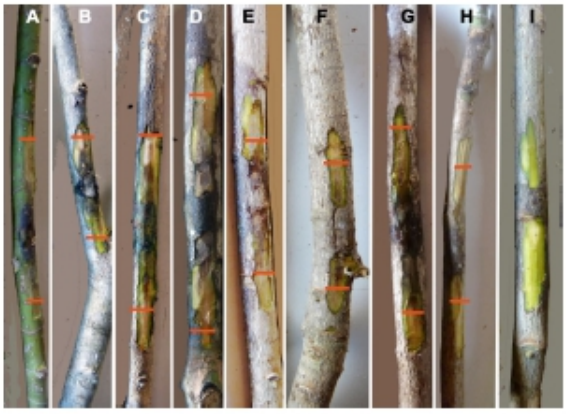


**Figure 2.** Lesion development on stems of *Magnolia* inoculated with (A) *Phytophthora drechsleri*,(B) *P. palmivora*,(C) *P. cinnamomi*, (D) *P. multivora*,(E) *P. pseudocryptogea*,
(F) *P. cambivora*,(G) *P. nicotianeae* (H) *P. capensis* compared to the (I) non-inoculated control

## Conclusions

- *Phytophthora pseudocryptogea, P. cinnamomi* and *P. multivora* were the most pathogenic species causing the largest lesions in most of these trees species, with *P. pseudocrytogea* pathogenic on all 16 plant species.
- Lesions developed in *Eucalyptus marginata* and *Corymbia calophylla* with all *Phytophthora* species, while *Hakea laurina* was resistant to 16 of the 19 *Phytophthora* species.
- All 19 *Phytophthora* species screened have the potential to be significant pathogens to the health of urban forest trees in Australia and worldwide.
- The excised stem pathogenicity bioassay is a good preliminary screen prior to more detail *in situ* pot trials

# Microbiota present in rhizosphere of *Austrocedrus chilensis* and their potentiality to control *Phytophthora austrocedri* disease.

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***Austrocedrus chilensis*** (Patagonian cypress) is a keystone tree species in most of the austral montane regions of Argentina and Chile. It is important because of its ecological functions and it is one of the few native tree species with high potential to be planted for timber production. *Phytophthora austrocedri* has been identified as the causal agent of significant mortality of *A. chilensis* wa in several areas. Disease progression appeared to be fast leading often to 50% mortality, or more, of trees of all ages. Recent work has identified *Phytophthora austrocedri* as the causal agent. The severity of the disease has led the species to a serious threat of conservation. Many studies have analysed the role of microorganisms as biocontrol agents in agricultural but no work has been done on the possibility of using microorganisms to control *P. austrocedri*-caused disease. This work aimed to evaluate the potential of isolates of bacteria and fungi, previously isolated from cypress rhizosphere, as biocontrol agents against *P. austrocedri*. Isolates were cocultured in microcultures with *P. austrocedri* and the effect on the hyphae growth and morphology of *P. austrocedri* evaluated daily. *P. austrocedri* growing alone was the control. Five fungal and 4 bacterial isolates differentially inhibited the growth of *P. austrocedri* which grew slowly (fungistatic effect) in the presence isolates of bacteria and actinomycetes (isolates 2A1E15 and A1E1), or *P. austrocedri* growth ceased (fungicidal effect) of in the presence other isolates from actinomycets, bacteria and fungi (bacterial isolates. 6 and 10 and fungal isolates 20A3E1,. 15A4E1 and 21A1E2). These findings allow progression towards the development of a biocontrol strategy.

# Microbiota present in rhizosphere of *Austrocedrus chilensis* and their potentiality to control *Phytophthora austrocedri* caused disease.

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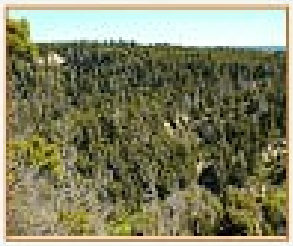
## Introduction

*Austrocedrus chilensis* (Patagonian cypress) is an endemic tree in the Cupressaceae, found in the southern Argentina and Chile. This native species is important because of its ecological functions and it is one of the few native tree species with high potential to be planted for timber production. Significant mortality of *A. chilensis* was reported in several areas. Disease progression appeared to be fast leading often to 80% mortality, or more, of trees of all ages. Recent work has identified *Phytophthora austrocedri* as the causal agent. The severity of the disease has led the species to a serious threat of conservation. *Phytophthora* species are causal agents of devastating diseases in different agricultural systems. Many studies have analysed the role of microorganisms as possible biocontrol agents. However, no work was conducted to study the possibility of using microorganisms to control *P. austrocedri*-caused disease. The aim of this work was to evaluate the potentiality of isolates of bacteria and fungi, previously isolated from cypress rhizosphere, as biocontrol agents against *P. austrocedri*.



## Material and methods

*In vitro assay*

Bioprospection of fungi and bacteria was made sampling 12 healthy trees in two sites with high presence of the disease, and sampling 6 trees from a site without presence of the disease. Isolations were made in PDA media (with and without antibiotics), Sh1 media and Rose Bengal Media. Fungal and bacterial isolates were cocultured in microcultures with *P. austrocedri*. Microcultures were observed under optic microscope on a daily basis and the effect over the hyphae growth and morphology of *P. austrocedri* was evaluated in each case. As control *P. austrocedri* growing alone was used.

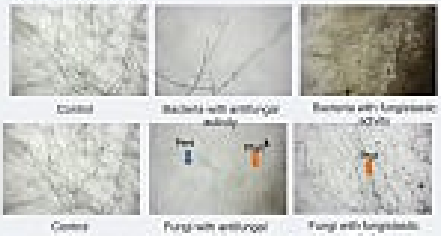

*In vivo assay*

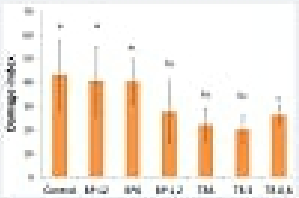
We inoculated the rhizosphere of 2 year-old plant with a suspension of *Bacillus subtilis* (BP) with $1x10^7$ ufc/ml (apox. ) using 12 ml, 6 ml and 1.2 ml per plant (BP12, BP6 and BP1.2 respectively) or with a combination of *Bradyrhizobium japonicus*,*Pseudomonas fluorescens* and *Azospillum brasilense* (TB), using different aumount of a combination of $1x10^7$ ufc/ml. We used 6 ml, 3 ml or 0.6 ml per plant (TB6, TB3 and TB0.6 respectively). Plants were inoculated 30 and 15 days before the inoculation of the pathogen. Bark cores (5 mm diameter) were aseptically removed and a disc of *P. austrocedri* were placed in the holes. Controls received uninfested discs. Assessment was carried out after 1 month. To evaluated the lesion a index was used:
**Damage Index: Length of the lesion/Width of the lesion² banding percentage**

## Results

*In vitro assay*

We found that 5 fungal and 4 bacterial isolates can differentially inhibit the growth of *P. austrocedri*. We observed that *P. austrocedri* grew slowly (fungistatic effect) in the presence of isolates of fungi, bacteria and actinomycetes (isolates 2A1E15 and A1E1 and *Penicillium* sp. 1T A4E2, *Penicillium* sp. 2S A5E2, *Penicillium* sp. A4SE1), or their growth was totally avoided (fungicidal effect) by other isolates from actinomycets, bacteria and fungi (bacterial isolates, 9 and 18 and *Penicillium* spp. isolates 2SA3B1, 1S4A2 1 and 2 1A1E2).





*In vivo assay*

We found that *Bacillus subtilis* (BP) in the lowest concentration, as well as the combination of *Bradyrhizobium japonicus*, *Pseudomonas fluorescens* and *Azospillum brasilense* (TB) in all dose were able to reduce the lesion caused by *P. austrocedri*. In these cases lesion and banding percentage were smaller than in control.

## Conclusions

- Some microorganism present in the rhizosphere of *A. chilensis* have antifungal and fungistatic activity
- PGPR bacterias are able to reduce the impact of the pathogen, probably by the induction of a sistemic response in the plant.
- These findings are very promising because they allow the progression towards the development of a useful biocontrol strategy.

# GIS modelling hazard dispersal for soil-borne *Phytophthora* diseases in Western Australia – Using a risk based approach when data is uncertain.

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*Phytophthora cinnamomi* data for South Western Australia is not perfect. Modelling hazard and applying results through a risk based approach to manage uncertainties can assist in reducing the likelihood of *P. cinnamomi* spread. As part of the Western Australian State Government funded Project Dieback, a Geographical Information System (GIS) *P. cinnamomi* Hazard Dispersion tool was developed to help quantitatively and qualitatively link hazard pathways of a known plant disease occurrence to a susceptible biodiversity value. The model is not a biological disease occurrence model, but a scenario hazard assessment tool. The Hazard dispersion tool estimates areas geographically linked to a known soil-borne disease point via spread pathways over time.

The model requires local expertise to set spread inputs including root-to-root rate of spread; human vector distance spread estimates for roads, downstream distance spread estimates for waterways; and time duration of dispersion scenario in years. Once inputs are set, using GIS, the model then runs dispersal calculations for the expert estimates. The final modelled *P. cinnamomi* hazard mapping outputs are divided into five even hazard categories which are pentiles of scenario time duration and distance to a disease point. These outputs are analysed to identify hazard roads, water ways, and quantify hazard areas. It is the responsibility of the user to interpret outputs in context to other available disease information, environmental conditions, susceptibility of values threatened, and the type of activities being undertaken along hazard pathways.

The model has been applied to the Esperance Ramsar Lakes Catchments located on the South Coast of Western Australia (see Figure 2 on poster). The Lake Warden and Gore Wetland Systems are wetlands of international significance listed under the Ramsar convention. They provide important habitat to waterbirds and native vegetation. These values are threatened by *P. cinnamomi*.

A study of existing local data and literature relevant to the model's inputs was conducted to provide technical rational for dispersion estimates:

- Known Department of Parks and Wildlife (DPAW) Vegetation Health Services (VHS) *P. cinnamomi* disease points locations (1982 to May 2016);
- Road distance = 300 m/annum;
- Drainage Maximum distance = 300 m/annum;
- Root to root Maximum distance = 3 m/annum; and
- Time scenarios = 50 years.

Analysis of the hazard dispersion scenario resulted in 15 per cent of the Esperance Ramsar Catchments and 2,800 km of road, track and trail networks identified as disease dispersion hazard pathways in the absence of risk reduction measures. For the Lake Gore and Warden Ramsar sites, respectively, the proportion of hazard area is 55 per cent and 99 per cent of the Nature Reserves. The Catchment areas road names and associated hazard rating were identified and provided to local stakeholders to help plan risk reduction procedures.

The hazard dispersion modelling analysis provides a tool to assist Stakeholders to:

- Recognise the likelihood of undertaking activities in a hazard area; and
- Part quantify the threat of *P. cinnamomi* disease to susceptible biodiversity values.

The model can be updated using new disease points, especially if located in a data gap area. Other confirmed soil-borne Phytophthora species locations can be included in the model as part of a broader biodiversity plant disease hazard assessment approach. All data collated as part of this project is available to registered users of the online "Dieback Information Delivery Management System" (DIDMS) tool. Registration for a free DIDMS user account can be made at www.dieback.net.au

Reference

Massenbauer, T. (2016). "Esperance Ramsar Wetlands Biodiversity Threat Plant Disease Decision Support Tools – A Technical Report", Internal Unpublished report for South Coast NRM

# GIS modelling hazard dispersal for soil-borne Phytophthora diseases in Western Australia – Using a risk based approach when data is uncertain.

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[1]South Coast NRM Inc. Albany, Western Australia, 39 Mercer Road, Albany, 6330, tilom@southcoastnrm.com.au

This project is implemented by South Coast NRM as part of 'Project Dieback – Western Australia' and supported by funding from the Western Australian Government's State NRM Program, along with contributions made by The Australian Government National Landcare Programme. South Coast NRM is a not for profit organization that facilitates and delivers natural resource outcomes with the community for biodiversity, rivers, wetlands, coastal, marine, agricultural and cultural values. http://southcoastnrm.com.au/

## Abstract

*Phytophthora cinnamomi* data for South Western Australia is not perfect. Modelling hazard and applying results through a risk based approach to manage uncertainties can assist in reducing the likelihood of *P. cinnamomi* spread. A Geographical Information System (GIS) *P. cinnamomi* Hazard Dispersion tool was developed to help link hazard pathways of a known soil-borne plant disease occurrence to a susceptible biodiversity value. The model was built using ESRI Arcmap 10.2 and Spatial Analyst.

The model is **NOT** a biological disease occurrence model, but a scenario hazard assessment tool. The model requires local expertise to set spread inputs for root-to-root rate of spread; distance spread estimates for roads, waterways; and time duration of dispersion scenario in years. Once inputs are set, using GIS, the model runs dispersal calculations for the expert estimates. The final modelled *P. cinnamomi* hazard mapping outputs are divided into five even hazard categories which are pentiles of scenario time duration. These outputs are further analysed to identify hazard roads, water ways, and quantify areas.


The model has been applied to the Esperance Ramsar Lakes Catchments located on the South Coast of Western Australia (Figure 1).

Figure 1 Esperance Ramsar Catchments and Wetland Systems

## Esperance Ramsar Catchments Case Study

The Lake Warden and Gore Wetland Systems are wetlands of international significance listed under the Ramsar convention. They provide important habitat to waterbirds and native vegetation. These values are threatened by *P. cinnamomi*. The project aims to:

*'Provide a P. cinnamomi dispersion hazard tool to help land managers assess threat to the Esperance Ramsar wetlands' susceptible values.'*

A study of existing local data and literature relevant to the model's inputs was conducted to provide technical rational for dispersion estimates:

- Known Department of Parks and Wildlife (DPAW) Vegetation Health Services (VHS) *P. cinnamomi* disease points locations (1982 to May 2016);
- Road distance = 300 m/annum;
- Drainage Maximum distance = 300 m/annum;
- Root to root Maximum distance = 3 m/annum; and
- Time scenarios = 10 years, 25 years, 50 years.

Figure 2 shows *P. cinnamomi* hazard dispersion results for the outlined scenario.

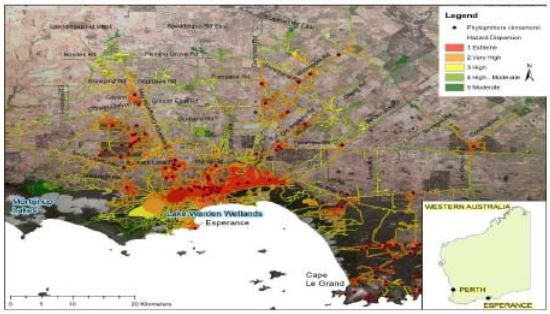


Figure 2 Lake Warden Ramsar Catchment P. cinnamomi hazard dispersion model 50 year scenario, 1982 - May 2016 VHS data

Analysis of the Hazard Dispersion scenario resulted in 15 per cent of the Esperance Ramsar Catchments and 2,800 km of road, track and trail networks as disease dispersion hazard pathways in the absence of risk reduction measures (Table 1).

Table 1 Summary of vector type length and modelled dispersion hazard for the 50 year scenario

| VECTOR TYPE | P. cinnamomi HAZARD DISPERSION RATING Length Km | | | | | |
|---|---|---|---|---|---|---|
| | Extreme | Very High | High | High Moderate | Moderate | Grand Total km |
| UNSEALED ROAD/TRACK | 951 | 502 | 332 | 281 | 200 | 2266 |
| SEALED ROAD | 213 | 141 | 66 | 33 | 26 | 480 |
| QUARRY | 12 | 3 | 4 | 2 | 1 | 22 |
| RAILWAY | 20 | 14 | 3 | 2 | 2 | 41 |
| Grand Total km | 1194 | 660 | 406 | 319 | 230 | 2809 |

The road names and its hazard rating have been provided to local stakeholders to help plan risk reduction procedures.

The *P. cinnamomi* hazard dispersion area for Lakes Gore and Warden Ramsar sites respectively proportion the total area of hazard at 55 and 99 per cent (Table 2). These modelled areas used in conjunction with other disease status data help quantify the threat of *P. cinnamomi* to the Ramsar wetlands values and can be used in further strategy development.

Table 2 Esperance Ramsar site P. cinnamomi hazard dispersion summary for the 50 year scenario

| | Ramsar Wetland System | | | | Total | |
|---|---|---|---|---|---|---|
| DISPERSION HAZARD RATING | Lake Warden System | | Lake Gore System | | Esperance Ramsar Sites | |
| | Area (ha) | Proportion (%) | Area (ha) | Proportion (%) | Area (Ha) | Proportion (%) |
| Extreme | 1805 | 90.4 | 550 | 13.7 | 2355 | 39.2 |
| Very High | 94 | 4.7 | 1022 | 25.4 | 1115 | 18.5 |
| High | 48 | 2.4 | 264 | 6.6 | 312 | 5.2 |
| High Moderate | 20 | 1.0 | 226 | 5.6 | 246 | 4.1 |
| Moderate | 12 | 0.6 | 166 | 4.1 | 178 | 3.0 |
| Total | 1978 | 99.1 | 2229 | 55.5 | 4207 | 70.0 |

## Conclusion

*P. cinnamomi* has been widely dispersed around the Esperance Ramsar wetlands and surrounding catchments for at least 30 years. There has been a lack of community awareness, and plant disease hygiene practices resulting in the ongoing spread of *P. cinnamomi*.

The hazard dispersion modelling analysis provides a tool to assist Stakeholders:

- to recognise the likelihood of undertaking activities in a hazard area; and
- In quantifying the threat of *P. cinnamomi* disease to biodiversity values.

It is the responsibility of the user to interpret outputs in context to other available disease information, susceptibility of values threatened, and the type of activities being undertaken along hazard pathways.

The model can be updated pending new VHS disease points, especially if located in a data gap area. Other confirmed soil borne Phytophthora species locations can be included in the model as part of a broader biodiversity plant disease hazard assessment approach.

The *P. cinnamomi* Hazard Dispersion Model will be refined further to better assist stakeholders with risk reduction assessments and maximise the benefit of often limited plant disease data.

All data collated as part of this project is available to registered users of the online "Dieback Information Delivery Management System" (DIDMS) tool. Registration for a free DIDMS user account can be made at www.dieback.net.au


## Reference

Massenbauer, T. (2016), "Esperance Ramsar Wetlands Biodiversity Threat Plant Disease Decision Support Tools – A Technical Report", Internal Unpublished report for South Coast NRM

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# Elicitin predictions from *Phytophthora pluvialis* and *P. kernoviae* and gene expression during infection of susceptible and resistant genotypes of *Pinus radiata.*

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Diseases caused by *Phytophthora* species that infect the foliage of *Pinus radiata* are relatively new and are impacting New Zealand's forest industry production. These diseases are red needle cast, caused by *Phytophthora pluvialis* and *P. kernoviae*, and Daño Foliar del Pino (DFP), caused by *P. pinifolia*. The ability of these species to infect the foliage is of particular interest, compared to infection by root-infecting species such as *P. cinnamomi* which has been a longstanding issue in New Zealand's *P. radiata* nurseries. Our objective was to identify elicitin genes that play a potential role during infection of *Pinus radiata* by different *Phytophthora* species. Elicitins are apoplastic effectors that have only been found in *Phytophthora* and *Pythium* species. Elicitin gene predictions were performed from the genome sequences of *P. pluvialis* LC-9 (USA), *P. kernoviae* CBS122049 (UK), *P. cinnamomi* (CBS 144.22) and *P. pinifolia* (CBS 122049). Elicitin predictions were performed using a modified bioinformatic pipeline. There were 20-51 putative elicitin-encoding genes across the four genomes, and did not correlate with genome assembly size. Resistant and susceptible genotypes of *P. radiata* needles were exposed to *P. pluvialis* and *P. kernoviae* zoospores and samples taken over time for transcriptomic analysis. To determine the gene expression profiles of the pathogen, transcripts showing similarity to predicted elicitin genes for both of these species were analysed. For *P. pluvialis*, despite highly variable gene expression patterns, gene expression increased from 0-5 days post-inoculation, for the majority of predicted elicitin genes, and differences were observed between the resistant and susceptible pine genotypes. Elicitin expression was not observed in the water controls. Further analysis of New Zealand strains of *P. pluvialis* and *P. kernoviae* and a Chilean strain of *P. pinifolia* is underway as well as analysis of *P. cinnamomi*. Gene expression data from the resistant and susceptible pine genotypes may also yield information on potential host targets and defense responses.

## Occurrence and pathogenicity of *Phytophthora × cambivora* on *Prunus laurocerasus* in Serbia.

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Cherry laurel is a native plant species in Serbian forests, but is also widely used for ornamental plantings. Following two extremely wet summers in 2014 and 2015, in spring and summer 2016 numerous cherry laurel plants with symptoms indicative for *Phytophthora* diseases, like wilt, chlorosis of leaves, dieback and bark necroses, were recorded in a park in Belgrade and in an ornamental nursery in central Serbia. From necrotic bark samples, *Phytophthora* isolates with woolly colonies were obtained. Due to the production of ellipsoid and elongated, nonpapillate sporangia and ornamented oogonia with two-celled antheridia, these isolates were identified as *Phytophthora ×cambivora*. In order to test the pathogenicity of the obtained isolates, one-year-old cherry laurel seedlings, grown in sterile perlite were inoculated under the bark, using a 6 mm cork-borer and same-sized agar disks from strains of *P. ×cambivora* from cherry laurel (PCCL) and beech (PCB). Isolates of *P. cactorum* (CAC), *P. cryptogea* (CRY), *P. plurivora* (PLU) and *P. ×serendipita* (SER) were included as comparison. In total 12 plants per isolate were used, including the control group which received sterile agar disks. One month after inoculation, first bark necroses appeared in most treatments. Two months after inoculation 4 plants in the PCB and 2 plants in PCCL treatment declined with longitudinal cankers and cholorosis, wilting and premature shedding of leaves. Three months after inoculation, 9 plants in PCB, 3 in PCCL, 2 in PLU and 1 in CAC declined showing the same symptoms. These results demonstrate the ability of *P. ×cambivora* to infect and cause decline of cherry laurel plants. The particularly high aggressiveness of the isolate from beech shows that *P. ×cambivora* poses a serious risk to cherry laurel in the rare natural communities of cherry laurel and beech in Serbia.

## Phytophthora Foot Rot of Black Pepper in Vietnam: Aetiology, Pathogen Population Structure and Disease Control.

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Black pepper, often termed the 'King of spices', is an important cash crop which earns valuable foreign exchange for many developing countries such as, Brazil, India, Indonesia, Sri-Lanka, Malaysia, Thailand and Vietnam. Currently, Vietnam is the world leader in black pepper production and export, but its production is unstable and invariably influenced by factors such as world market, drought and diseases.

Phytophthora foot rot, also known as 'quick dead', is one of the most serious diseases of black pepper. This disease has been reported to be a major problem causing significant economic losses throughout the world where crop is grown. Although the disease was first reported in 1952 in Vietnam, the identity of the causal organism was never been conclusively determined. The aims of the research reported in this thesis were to identify the causal agent associated with foot rot epidemics in black pepper, investigate the pathogen population structure and explore an effective measure for control of Phytophthora foot rot.

A national survey of the disease and collection of samples were conducted in four major black pepper-growing provinces. Results obtained from the survey showed that the percentage of diseased black pepper poles was 10.3 to 19.2 % in different provinces, causing significant black pepper yield loss on a national scale. *Phytophthora* isolates obtained from diseased roots and leaves and root-zone soil were identified by morphological characteristics and verified by ITS-RFLP analysis. Three *Phytophthora* species, *P. capsici*, *P. nicotianae* and *P. cinnamomi*, were isolated from diseased roots and leaves of black pepper and associated soil but *P. capsici* was recovered at the highest frequencies. This species was subsequently found to be the most aggressive species pathogenic to young black pepper vines, causing typical symptoms of foot rot. *P. capsici* was then determined as the main pathogen causing Phytophthora foot rot of black pepper in Vietnam based on disease symptoms, morphological characteristics, pathogenicity and ITS-RFLP analysis.

Population structure of *P. capsici* from black pepper was studied based on mating type analysis, random amplified microsatellites (RAMS) and repetitive extragenic palindromic (REP) fingerprinting. The mating type of 178 isolates was determined. Two mating types A1 and A2 were detected in four provinces in two different climatic regions, with A1 and A2 ratios ranging from 1.3 to 1.5. In several instances, both A1 and A2 mating types were found to co-exist in the same farm or black pepper pole. This finding indicates the potential of sexual reproduction of *P. capsici* in Vietnam. Furthermore, RAMS and REP DNA fingerprinting analysis of 118 isolates of *P. capsici* from black pepper showed that the population was genetically more diverse where two mating types were found, although the overall genetic diversity was low, with most of the isolates belonging to one clonal group. These findings imply that sexual reproduction may occur in the field in black pepper-growing regions, albeit infrequently. The low diversity among isolates suggests that the *P. capsici* population causing Phytophthora foot rot of black pepper may have originated from the same source. There was no genetic differentiation of isolates from different climatic regions. In addition, to the large clonal group, several isolates with unique RAMS/REP phenotypes were also detected. Most of these unique phenotypes belonged to the minority mating type, A1. This may have significant implications for a gradual increase in overall genetic diversity.

In the comparative analysis of *P. capsici* from black pepper and chilli RAMS and REP fingerprinting showed that isolates from chilli and black pepper were genetically different. However, cross-infectivity of *P. capsici* isolates obtained from these two hosts was demonstrated. The current study has shown that the overall *P. capsici* population in Vietnam is genetically complex and may pose a serious threat to black pepper production.

Potassium phosphonate was evaluated for the control of the disease in greenhouse and field trials. In greenhouse trials 3-month-old vines treated with phosphonate by soil drenching (10 to 20 g a.i./L) were significantly less affected by foot rot compared to non-treated vines. In field trials, mature vines were treated with phosphonate by soil drenching or root infusion. After 10 days, root, stem and leaf specimens were removed for bioassay by inoculation with zoospores of *P. capsici*. Samples from black pepper vines treated with phosphonate by soil drenching exhibited greater resistance to the colonisation of the pathogen as compared to those from non-treated vines. However, samples from vines treated with the chemical by root infusion did not display significant resistance to the pathogen. This study provides more evidence on the efficacy of potassium phosphonate in the management of black pepper foot rot caused by *P. capsici*. The excised leaf and stem bioassay used in this study was shown to be a rapid and useful technique for testing the efficacy of systemic fungicides for the control of this disease.

## Diversity and ecology of *Phytophthora* species on the island of Ireland.

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The island of Ireland has a long history with the plant pathogenic oomycete genus *Phytophthora.* Since the invasion of the late blight pathogen *Phytophthora infestans* in mid- 19th century, *Phytophthora* species have caused major changes to agriculture and plant health on the island of Ireland. In recent years, the scientific expertise built up in plant pathology and mycology on the island of Ireland has been drastically diminishing, while invasions of plant pathogens have been increasing in line with increasing globalisation and international trade in plant and plant based commodities. In order to draw attention to the increasing threat posed by *Phytophthora* to the plant health of the island of Ireland, we reviewed the records of *Phytophthora* species detected along with notes on the species' ecologies. Using published and unpublished records, we found that 27 species and two provisionally named taxa of *Phytophthora* have been recorded on the island of Ireland. The role of the horticultural trade in plants for planting in spreading invasive *Phytophthora* species was evident in the results. Areas in which future research should focus are given in order to add to the list of *Phytophthora* species detected on the island of Ireland are also discussed.

# Diversity and distribution of *Phytophthora* species on the island of Ireland¥

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## Plant pathology on the island of Ireland

- Historically, there was a strong research base in *Phytophthora* expertise on the island of Ireland, due in part to the large effect of potato late blight on the population in the 19th century. However this research base has diminished in recent years
- The island of Ireland includes Ireland and Northern Ireland. Northern Ireland is part of the UK, Ireland is not. The plant health regulatory authorities and the research communities in the two countries work very closely with each other on plant health related matters.

## Methods

- Aim: provide an up-to-date list of the *Phytophthora* species detected on the island of Ireland, with details of their ecology and host range
- A number of published and unpublished sources were reviewed
- Current nomenclature of the species was incorporated. Notes on the species ecology, and their native status is suggested

## Results and Discussion

- A total of 27 described species and 2 provisionally named species have been recorded (Table 1)
- 23 species are almost certainly invasive alien species while there is evidence that 4 may be native to Europe
- All 27 species are known plant pathogens
- 42 taxa have been recorded in Scotland (Cooke 2015)- *P.* × *multiformis*, *P. mississippiae*, *P. richardiae*, *P. uniformis* not found in Scotland
- Many more to be discovered...

## Important events in *Phytophthora* research in Ireland

1845 – signs of disease in Irish potato crops are first noted
1876 – Anton de Bary describes the causal agent of the potato famine as *P. infestans*
1913 – Pethybridge describes *P. erytroseptica* from potato
1919 – Pethybridge and Lafferty describe *P. cryptogea* as a pathogen of tomato
1919-1951 – McKay (1951) provides review of *Phytophthora* research on island of Ireland
1876-1976 – Muskett (1976) provides review of *Phytophthora* research on island of Ireland
1989 – International *Phytophthora* research community attend conference in Dublin
1995 – '*Phytophthora infestans* 150' conference draws international *Phytophthora* research community to Dublin
1976-2008 – Mangan (2008) reviews Phytopathology discipline in Ireland
2010 – Society of Irish Plant Pathologists highlight diminishing knowledge base in plant pathology and mycology
2011 – O'Hanlon and Harrington (2011) and Institute of Ecology and Environmental Management (2011) identify diminishing plant pathological and taxonomic expertise in Ireland


**Figure 1 (above)** Trade in plants for planting **[a]**, and illegal trade in cut foliage **[b]** are two likely routes for *Phytophthora* spread

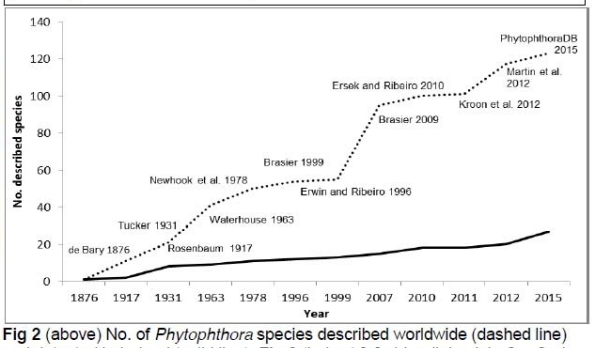
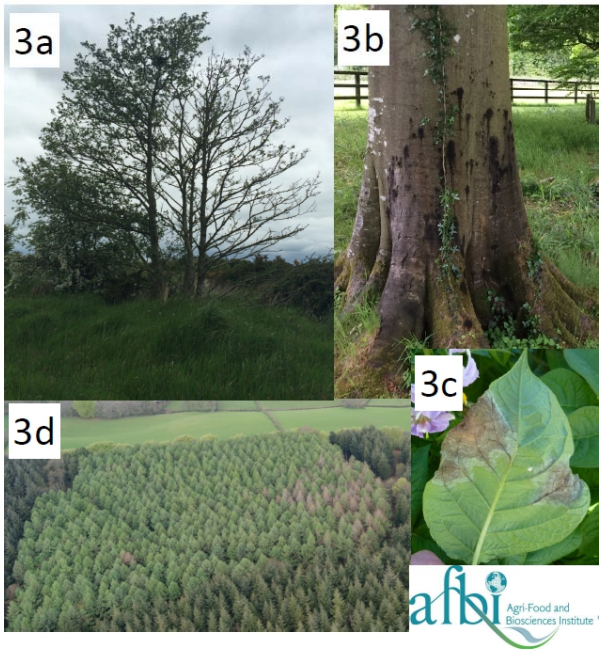



**Fig 2** (above) No. of *Phytophthora* species described worldwide (dashed line) and detected in Ireland (solid line). **Fig 3** (below) **[a]** alder dieback in Co. Cork caused by *P. alni*; **[b]** beech bleeding canker Co. Carlow caused by *P. cambivora*; **[c]** potato blight caused by *P. infestans* in Co. Antrim; **[d]** sudden larch death caused by *P. ramorum* in Co. Antrim (credit DAERA)

**Table 1 (below)** *Phytophthora* detected on island of Ireland with their sporangial shape (p, papillate; sp, semi-papillate; np, non-papillate), attachment (c, caducous; nc, non-caducuos) and host of concern. * Possibly native species

| Taxon | Clade | Sporangia shape | Sporangia attachment | Host of concern |
|---|---|---|---|---|
| *P.* × *alni* | 7a | np | nc | *Alnus* |
| *P.* × *multiformis* | 7a | np | nc | *Alnus* |
| *P. cactorum* | 1a | p | c | Tree species |
| *P. cambivora* | 8a | np | nc | *Fagus* |
| *P. chlamydospora** | 6 | np | nc | Woody ornamentals |
| *P. cinnamomi* | 7b | np | nc | Tree species |
| *P. cryptogea* | 8a | np | nc | *Solanum* |
| *P. drechsleri* | 8a | np | nc | *Rubus* |
| *P. erythroseptica* | 8a | np | nc | *Solanum* |
| *P. fragariae* | 7a | np | nc | *Fragaria* |
| *P. gonapodyides** | 6 | np | nc | *Fagus* |
| *P. hibernalis* | 8c | sp | c | Woody ornamentals |
| *P. infestans* | 1c | sp | c | *Solanum* |
| *P. kernoviae* | 10 | p | c | *Fagus, Vaccinium* |
| *P. lacustris* | 6 | np | nc | *Alnus* |
| *P. lateralis* | 8c | np | nc | *Chamaecyparis* |
| *P. megasperma** | 6 | np | nc | *Solanum* |
| *P. mississippiae* | | np | nc | - |
| *P. nicotianae* | 1 | p | nc | *Solanum* |
| *P. plurivora** | 2 | sp | nc | Woody ornamentals |
| *P. porri* | 8b | sp | nc | *Allium* |
| *P. pseudosyringae* | 3 | sp | c | *Fagus, Larix* |
| *P. ramorum* | 8c | sp | c | Tree species and woody ornamentals |
| *P. richardiae* | 9 | np | nc | *Arum* |
| *P. rubi* | 7a | np | nc | *Rubus* |
| *P. syringae* | 8d | sp | nc | *Malus, Fagus*, Woody ornamentals |
| *P. uniformis* | 7a | np | nc | *Alnus* |

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¥based on a manuscript published in *Biology and Environment* - O'Hanlon R, McCracken AR, Cooke LR (2016) Diversity and ecology of *Phytophthora* species on the island of Ireland

## *Phytophthora* spp. associated with nursery-grown native plants in the Pacific Northwest.

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Nursery plants have been responsible for the spread of several damaging *Phytophthora* species into forests and other wildland habitats. The risk is particularly great when nursery-grown plants infested with *Phytophthora* spp. are planted in restoration sites. We surveyed 11 native species widely planted in Oregon and Washington vegetation restoration to determine the incidence of *Phytophthora* infestation in nursery-grown plants: woody trees and shrubs *(Alnus rubra, Arctostaphylos uva-ursi, Ceanothus cuneatus, Cornus sericea, Mahonia aquifolium, Malus fusca, Thuja plicata)* and herbaceous perennials *(Delphinium trolliifolium, Lupinus polyphyllus, Mimulus guttatus, Potentilla gracilis)*. Ten plants of each species were purchased from a total of 15 nurseries in Oregon and Washington. Leachates from individual potted plants were baited with rhododendron leaves. Necrotic areas of baits were plated onto PARPH, and colonies were identified by morphological characters and Sanger sequencing of the ITS region and for some also COX region sequence. In Oregon, 22% of plants were infested with at least one species of *Phytophthora*. Thirteen *Phytophthora* species were detected: *borealis, cactorum, cambivora, chlamydospora, cinnamomi, citrophthora, cryptogea, hedraiandra, occultans, pini, plurivora, siskyouensis,* and *syringae*. *Lupinus, Alnus,* and *Ceanothus* were the most frequently infested plant species. In Washington, 25% of the plants were infested, with 10 *Phytophthora* species recovered including three *(P. uniformis, P. occultans,* and *P. gallica)* not previously detected in the state. *Alnus* had the highest frequency of contamination; 67% of plants were infested with one or more species of *Phytophthora.* These findings indicate the urgent need to develop outreach and education programs directed towards producers and buyers of native plants.

# *Phytophthora* spp. associated with nursery-grown native plants in the Pacific Northwest

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Marianne Elliott

## Introduction

The inadvertent spread of *Phytophthora* species into native ecosystems can have long-term environmental and economic impacts, as has been seen with *Phytophthora ramorum*, *P. lateralis*, *P. cinnamomi*, *P. tentaculata*, and other species. Nursery plants have been responsible for the spread of several damaging *Phytophthora* species into forests and other wildland habitats. The risk may be particularly great when nursery-grown plants infested with *Phytophthora* spp. are planted in restoration sites, introducing pathogens directly into native habitats. We surveyed 11 species of native plants widely planted in Oregon and Washington vegetation restoration efforts to determine the incidence of *Phytophthora* infestation in nursery-grown plants. Plant species included woody trees and shrubs and herbaceous perennials for forest, riparian, and prairie restoration plantings.


Sudden oak death spread from nursery plants infected with *Phytophthora ramorum*.


A nursery-grown native *Mimulus* sp. infected with *Phytophthora tentaculata*.

## Materials and Methods

| Common name | Species | Family | State |
|---|---|---|---|
| **Forest** | | | |
| Kinnikinnick | *Arctostaphylos uva-ursi* | Ericaceae | OR & WA |
| Ceanothus | *Ceanothus cuneatus* | Rhamnaceae | OR & WA |
| Western red cedar | *Thuja plicata* | Pinaceae | OR & WA |
| Oregon grape | *Mahonia* spp. (Berberis) | Berberidaceae | OR & WA |
| **Riparian** | | | |
| Red alder | *Alnus rubra* | Betulaceae | OR & WA |
| Pacific crabapple | *Malus fusca* (*Pyrus fusca*) | Rosaceae | OR & WA |
| Red osier dogwood | *Cornus sericea* | Cornaceae | OR & WA |
| **Prairie** | | | |
| Bigleaf lupine | *Lupinus polyphyllus* | Fabaceae | OR & WA |
| Monkey flower | *Diplacus* spp. (*Mimulus*) | Phrymaceae | OR & WA |
| Delphinium spp. | *Delphinium trolliifolium* | Ranunculaceae | OR |
| Slender cinquefoil | *Potentilla gracilis* | Rosaceae | OR & WA |
| Yarrow | *Achillea millefolium* | Asteraceae | WA |

Thirty potted plants of each species (ten each from three different nurseries) were purchased from eight commercial native plant nurseries in Oregon and seven nurseries in Washington. Tests of individual potted plants were conducted at OSU and WSU by pouring water through each pot and baiting the leachates with rhododendron leaves. Necrotic areas of the leaf baits were plated onto PARPH, and colonies were identified by morphological characters and Sanger sequencing of the ITS region with primers ITS4 & ITSDC6 (Cooke et *al.* 2000). The coxI and coxII regions were also sequenced for some isolates (primers FM84 & FM83 and FM75 & FM78 from Martin and Tooley 2003).

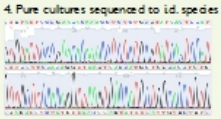

Potted plants in a native plant nursery.

1. Water poured through each pot

2. Leachate baited with rhododendron leaves

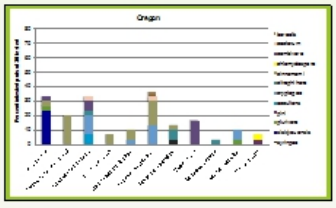
3. Baits plated onto PARPH

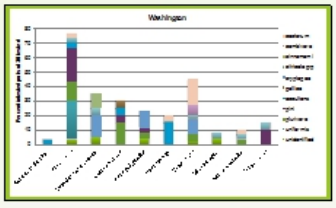
4. Pure cultures sequenced to i.d. species



## Results

### *Phytophthora* recovery in native plants purchased in Oregon and Washington




*Phytophthora* species were recovered from all plant species tested and from 13 of 15 nurseries sampled.

In Oregon, 17% of individual pots were infested with at least one species of *Phytophthora*. Twelve *Phytophthora* species were detected. Plant species with the highest incidence of *Phytophthora* recovery were *Lupinus* (37%), *Alnus* (33%), and *Ceanothus* (33%).

In Washington, 25% of the pots were infested. Ten *Phytophthora* species were recovered including three (*P. uniformis*, *P. occultans*, and *P. gallica*) not previously detected in the state. *Alnus* had the highest frequency of recovery with 67% of the plants infested with one or more species of *Phytophthora*.

## Discussion and Conclusions

- Potted plants from Oregon and Washington native plant nurseries are frequently infested with plant pathogenic *Phytophthora* species.
- These findings indicate the urgent need to develop outreach and education programs directed towards producers and buyers of native plants.
- Further research is needed to determine the occurrence, persistence, and impact of *Phytophthora* species in restoration sites before and after planting nursery-grown native plants.

## Acknowledgements

This research was funded through a grant to the USDA Forest Service PSW Range and Forest Experiment Station from the USDA Farm Bill.

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## Horizon 2020: POnTE - Establishing a *Phytophthora* baseline in British soils.

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Tree diseases caused by *Phytophthora* have increased in recent years in Britain and metabarcoding approaches suggest that many previously undetected *Phytophthora* species could be present. To gain a better understanding of the range of Phytophthoras, including new and potentially unknown taxa, a study is underway to set a baseline of *Phytophthora* occurring in soils in Britain. Both land use and planting history may influence the likelihood of *Phytophthora* occurrence, so survey sites include those categorized as 'disturbed' i.e. those frequently visited by the public, with recent and new plantings that can be linked to nurseries versus forest and woodland sites with little disturbance or interventional management ('undisturbed'). The aim is to identify these Phytophthoras and study the extent to which these might pose a risk to trees in Britain, especially those not previously thought to be existent and therefore possibly cryptic or undetected. The project will explore any connection between Phytophthoras discovered in nurseries with those found in recent plantations and more disturbed sites, and will compare with *Phytophthora* diversity in more natural or less disturbed sites. Traditional baiting methods will be used for isolation of *Phytophthora* from the soil, but simultaneously DNA will be extracted from the same soil samples, amplified with modified genus-specific primers and then massively parallel sequenced through Illumina MiSeq platform. The study covers twelve sites (four in Scotland, six in England and two in Wales), each site comprising ten locations to be sampled at least once a year. The locations within each site represent different environments, including soil types, land use, new plantings, and areas of recreation. An update of the results will be presented and the findings discussed.

## *Phytophthora* communities in a western Oregon (USA) river.

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Horticultural nurseries typically harbor multiple *Phytophthora* species. One potential source of contamination is untreated water from ponds and rivers used for irrigation. We sampled river water used by a commercial nursery in the Willamette Valley in western Oregon (USA) to detect plant pathogenic *Phytophthora* species and to determine if waterborne *Phytophthora* communities changed over time. Water samples (1 L) were collected from Palmer Creek at approximately 2-week intervals from June, 2015 to April, 2016 and filtered through 5µm Millipore filters. A subset of unfiltered water samples were used to bait rhododendron leaves. DNA was extracted directly from filters and baited leaves, and oomycete-specific primers were used to amplify the ITS1 region for Illumina MiSeq 250 bp paired-end sequencing. The paired sequences were first cleaned, quality filtered and separately queried against a custom oomycete reference ITS database using a nucleotide megablast search. Query sequences with ≥99.5% similarity to the reference were identified as positive matches while those with 99 to 99.5% similarity to the reference were identified as a closest match. More than 120 positive-match taxa and 100 closest-match taxa were found in both forward and reverse sequence analyses. More than 75 species of *Phytophthora,* including several plant pathogenic species as well as aquatic opportunists, were detected from filter extracts. Additional oomycete taxa belonging to *Achlya, Aphanomyces, Brevilegnia, Peronospora, Pythium, Phytopythium* and *Saprolegnia* were detected. *Phytophthora* communities from leaf baits were distinct from the communities detected on filters. Communities differed by sampling period, reflecting seasonal changes. Between-sample diversity was less for the communities derived from the filters as compared to the leaf-baits. Amplicon sequencing is a sensitive and semi-quantitative method for detecting diverse *Phytophthora* species present at low concentrations in water.

# *Phytophthora* communities in a western Oregon (USA) river

SCRI - CLEAN WATER³ REDUCE. REMEDIATE. RECYCLE

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USDA NIFA

## Introduction

Horticultural nurseries typically harbor multiple *Phytophthora* species. One potential source of contamination is untreated water from ponds and rivers used for irrigation.

## Objectives

- To detect pathogenic oomycetes species in a western Oregon river using Illumina MiSeq amplicon sequencing .
- To compare the communities detected using water filtration and leaf-baiting techniques.
- To determine seasonal effects on the oomycetes diversity.

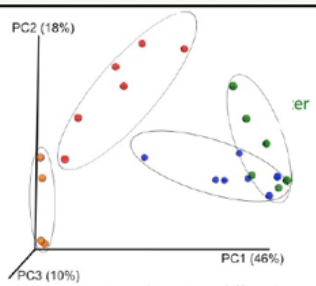
## Sampling to sequencing

We sampled river water approximately every 2 weeks from April 2015 to May 2016 and filtered 1L through 5µm Millipore nylon membranes. A subset of unfiltered water samples was used to bait rhododendron leaves. DNA was extracted directly from filters and baited leaves, and oomycete-specific primers[1] ITS6 and ITS7 were used to amplify the ITS1 region for Illumina MiSeq 250bp paired-end sequencing.

## Data analyses

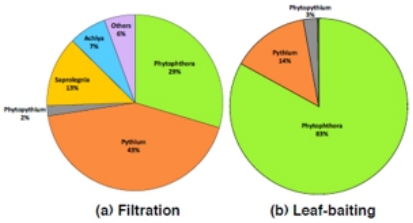
The paired sequences were first cleaned, quality filtered and queried against a custom oomycete reference ITS database[2,3] using a nucleotide megablast search. Operational taxonomic units (OTUs) were identified based on percent sequence similarity to the reference sequence of known oomycetes species. OTU abundance and diversity analyses were performed using QIIME[4]. Mock communities composed of known oomycetes were used in this study to estimate cutoffs.

## A. Species diversity in river water




Oomycetes species diversity differed across seasons, as depicted in the Principal Component Analysis plot (above) for filter samples. Species richness (Chao index[4]) increased from spring to winter (peak), while fall samples showed more diversity (Shannon-Weiner[4] and Simpson's index[4]) (data not shown). Bray-Curtis[5] between sample diversity was estimated using 1,900 sequences per sample (depth).

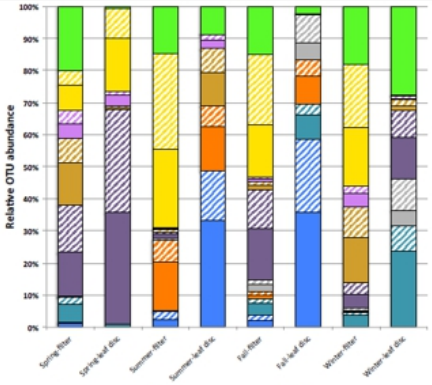
## B. Oomycetes detected in river water




(a) Filtration (b) Leaf-baiting

With filtration (a), *Pythium* was the most abundant oomycete genus found in river water, followed by *Phytophthora*, *Saprolegnia, Achlya,* and *Phytopythium*. Primarily *Phytophthora* species grew on leaf-baits (b), followed by *Pythium* and *Phytopythium*, but species richness was quite low for leaf-baits. Although a greater number of species were detected on filters, the leaf-baiting technique was useful to detect active plant pathogenic species.

## C. Seasonal variation in *Phytophthora* species




- *P. irrigata* dominated on leaf-baits in summer and fall; while the *P. riparia* complex and *P.* taxon pgchlamydo dominated in spring and winter respectively.
- *P. citricola* complex was observed on filters in every season, but only in spring did it amplify on leaf-baits.
- *P. parsiana* complex was only identified in summer and spring; while *P.* taxon oaksoil complex was only identified in fall and winter.
- *P. gonapodyides* did not grow on leaf-baits in fall.

**Solid box** = 100% sequence similarity to reference
**Dashed box** = > 90% sequence similarity to reference

P. irrigata | P. taxon pgchlamydo | P. parsiana complex | P. riparia complex | P. citricola complex | P. taxon oaksoil complex | P. gonapodyides | P. megasperma complex | Other Phytophthora sp.

solid box = positive match dashed box = closest match

**Limitations:** Illumina MiSeq amplicon sequencing technology is very effective and sensitive towards detecting community structure. However, shorter (250 bp) MiSeq sequences are unable to differentiate between two or more species in some cases, and often result to unresolvable species complexes.

**Conclusions:** All the *Phytophthora* species identified in this study were found in the filtered water samples, while only a subset of these species were present on leaf-baits. The ability of *Phytophthora* species to grow on leaf-baits greatly varied across seasons.

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## Acknowledgments

This research was funded by Specialty Crop Research Initiative (SCRI) program at USDA-NIFA.

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**8th Meeting of IUFRO Working Party 7.02.09**
**Phytophthora in Forest and Natural Ecosystems**

## Susceptibility of different Swedish alder populations to *P. xalni.*

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The *Phytophthora alni* species complex is causing major impacts on riverbank alder populations across Europe. In Sweden, *P. uniformis* has been found all across Southern Sweden, while *P. alni* is located in the warmest areas of the country. Changes of the climatic conditions can favor *P*. x alni to spread northwards, and colonize colder areas currently occupied by *P. uniformis.* The consequences of such expansion remain unpredictable. We hypothesized that alder trees growing in *P. uniformis* infested stands have undergone weaker selection than those growing in *P. xalni* infested stands, owing to lower aggressiveness of *P. uniformis.* We thus expected a higher susceptibility to *P.* x *alni* of alder trees growing in *P. uniformis* infested stands than of trees growing in *P.* x *alni* infested stands. To test this, seeds from alders growing in *P. xalni* and *P. uniformis* infested stands were collected, grown for three weeks, and inoculated by immersing their root systems in a zoospore solution of *P.* x *alni*. Wilting symptoms and mortality was recorded for each seedling every 24 hours for 10 days. Preliminary results showed a higher mortality after 10 days of seedlings coming from *P. uniformis* sites than *P.* x*alni* sites. Among the surviving seedlings 10 days after inoculation, the frequency of wilting symptoms was higher in those from *P. uniformis* sites than in those from *P.* x *alni* sites. The differences in susceptibility between alder genotypes and their link to selection pressure will be discussed.

## Screening *Agathis australis* (kauri) genotypes for resistance to *Phytophthora agathidicida.*

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*Phytophthora agathidicida* is responsible for a devastating decline of *Agathis australis* (kauri) within its endemic range in the North Island of New Zealand. This significant soilborne pathogen causes primary, secondary and tertiary root damage and significant stem bleeding basal cankers on host trees. The pathogen is easily spread through the movement of contaminated soil water and organic material. Work is currently being conducted to protect non-infested forests and reduce the spread of *P. agathidicida*. Further research is required to understand the vulnerability of the host species and, where appropriate, identify genotypes of kauri that can be made available for restoration. Kauri seeds from infected and non-infected forests have been collected in collaboration with local iwi (Maori groups). This germplasm will be used for conserving and propagating genetic material in tissue culture libraries, optimizing pathogenicity screening protocols, identifying genetic signatures of resistance, and scaling up of a resistance screening programme. To accelerate pathogenicity screening, root and leaf inoculation assays were conducted for over 35 unique kauri genotypes. A spectrum of susceptible to comparatively more resistant genotypes was identified. This shows promise that resistance to *P. agathidicida* will be identified. The next key stage is to understand the level of variability in resistance within kauri families and across populations/regions, and to determine how robust these screening assays are with mature trees.

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# Screening *Agathis australis* (kauri) genotypes for resistance to *Phytophthora agathidicida*

Peter Scott, Echo Herewini, Martin Bader and Nari Williams
(peter.scott@scionresearch.com ) Scion, Private Bag 3020, Rotorua 3046, New Zealand

## Introduction

*Phytophthora agathidicida* is responsible for a devastating decline of *Agathis australis* (kauri) within its endemic range on the North Island of New Zealand. This significant soil borne pathogen causes primary, secondary and tertiary root damage; and significant stem and basal bleeding cankers on host trees (Figure 1). The pathogen is easily spread through the movement of contaminated soil, water and organic material. While work is being conducted to protect non-infested forests and reduce the pathogens spread, the long-term future of this keystone species relies on the identification of resistant hosts.

This work aims to:

- investigate the vulnerability of kauri to *P. agathidicida* and identify resistant genotypes; and
- compare variation in resistance to foliar and root inoculations across kauri genotypes to determine if pre-screening of leaf material provides a non-destructive predictor for susceptibility.

This screening will inform risk analyses of the susceptibility of kauri populations to disease. Families showing higher resistance may be made available for selective breeding programs and/or restoration of the species.

**Figure 1.** Dieback and collapse of *Agathis australis* caused by *Phytophthora agathidicida*. The top of the bleeding lesion which extended beyond six meters up the exposed trunk is indicated (arrow). Basal bleeding lesion of kauri dieback (inset)

## Materials and methods

Families of kauri were initiated by embryogenic tissue culture with clonal ramets of each line potted into pasteurised potting media and grown in the glasshouse (Gough et al 2012).

From more than 1600 isogenic lines produced in tissue culture, roots were initiated on 50 with 18 lines producing sufficient root mass for inoculation studies.

Paired inoculations of excised leaf and intact roots were conducted on each plant, respectively (Horner at al 2014). Leaf lesion area and the length of roots less than 0.5 mm in diameter and 0.5-4.5 mm in diameter were quantified (Scott et al 2012).

## Results and discussion

A spectrum of differential responses to infection by *P. agathidicida* were observed using both leaf and root inoculation assays.

Variation in the impact of root infection across genotypes was observed on the length of fine roots less than 0.5 mm in diameter. However, no significant differences were observed in the length of thicker roots (Figure 2). Fine root growth appeared to be either stimulated, not impacted or supressed in response to *P. agathidicida* inoculation. This suggests complex resistance traits that may be exploited through selection.

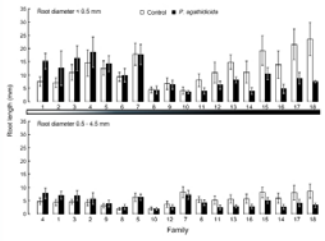


**Figure 2.** Variation in the length of root diameters ≤ 0.5 mm (upper panel) and from 0.5 - 4.5 mm (lower panel) for control and *Phytophthora agathidicida* inoculated *Agathis australis* genotypes. The shaded line under the upper panel family numbers represent differential response to infection. Lighter colours correspond to genotypes where *P. agathidicida* inoculation stimulated root growth. Darker colours correspond to families where inoculation supressed root growth.

Leaf damage and fine root length weakly correlated regardless of inoculation. However, a higher correlation was observed ($r^2 = 0.34$) in the *P. agathidicida* infected plants (Figure 3). Some control inoculated leaves produced a wound response impacting over 40% of the leaf area. Differentiation of leaf lesions was further impacted by highly variable leaf pigmentation within and between genotypes.

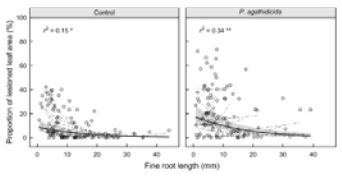


**Figure 3.** Comparison between leaf inoculation, expressed as the proportion of lesioned leaf area (y axis) and root inoculation, expressed as fine root length (x axis) for *Agathis australis* families including plants that were shame inoculated with non-colonized media (Control – left panel) and with *Phytophthora agathidicida* (right panel). Thick black line indicate the model predications for the relationship between lesioned leaf area and fine root length across all genotypes with the 95 prediction intervals shaded in grey. Dashed lines show the model predications for the relationship between lesioned leaf area and fine root length within genotype. Correlations between lesioned leaf area and fine root length for control and *P. agathidicida* inoculated plants are indicated. $^{**}P \leq 0.01$, $^{*}P \leq 0.05$.

## Conclusions

Kauri genotypes showed variable response in fine roots length to *P. agathidicida* inoculation ranging from stimulation of root growth to loss of fine roots. This variation may be used to identify resistance traits in broader populations of kauri.

Leaf and root inoculation assays did not closely correlate across families in part due to the high variation in leaf pigmentation observed across kauri leaves. This has shown that leaf assays are not likely to be informative for determining susceptibility to disease.

The next key stage is to understand the level of variability in resistance within kauri families, across populations and regions.

Kauri seeds from infested and non-infested forests have been collected in collaboration with local mana whenua (Maori groups) and are being grown up for further wide-scale screening; identification of biochemical and genetic signatures of resistance and to improve understanding of the mechanisms of resistance which is important given the 2000+ year life span and ecological importance of these majestic trees. This information will be applied to scale-up the resistance screening programme.

### Acknowledgements

Healthy Trees Healthy Future (MBIE C04X1305 ), Kauri dieback Tāngata whenua rōpu, Kauri Dieback Program, Bio-Protection Research Centre, Massey University.

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## Measuring the *Phytophthora* infestation level in a *Ceanothus thyrsiflorus* crop from a California restoration nursery.

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In a California restoration nursery that was relatively well maintained, but with little information or training for staff on managing for pathogens, and no set of best management practices (BMP's), it was determined at least some *Phytophthora* infested plants were present including from a *Ceanothus thyrsiflorus* crop. Only a few plants had been tested, though, and it was unknown if *Phytophthora* infestation was extensive- indicative of past epidemic spread in the crop, or limited. If only a few plants were infested, the out-planted crop might not be a threat to ecosystems, but if infestation was extensive the chance of wildland infestations spreading would likely be greater, and a set of BMP's might be necessary to reduce the chance of similar crop infestations later. We made use of the entire *C. thyrsiflorus* crop of 500 plants. We assessed symptoms before moving, randomly assigned plants into five blocks, and randomly sampled 25% of them by destructive means. We isolated, cultured, and phenotypically and molecularly determined species present. Questionnaires were given to nursery managers to assess the 'cleanness' of this and four other similar nurseries to determine areas for improvement. The results showed that symptomatic plants were found throughout the crop and were not radiating from any one particular area. Of the 125 plants, about 30% of the plants were infested, with variability by block. *P. cactorum* was the dominant species, but there were others. The potential contributing factors based on the nursery surveys will be discussed, and BMP's suggested based on crop and nursery survey outcomes.

## Comparisons of *Phytophthora* species found based on isolation technique, nursery, and plant type in California, USA.

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Sampling is underway in restoration plant nurseries in California for *Phytophthora* species from woody plants belonging to one of eight families: Sapindaceae, Rosaceae, Rhamnaceae, Phrymaceae, Platanaceae, Salicaceae, Ericaceae, or Fagaceae. Comparisons will be based on isolation technique, nursery, and plant type. Specifically, comparisons of the *Phytophthora* species recovered using a three-bait-system vs. direct plating of fine root pieces, from differing plant families, within families across nurseries, and from differing plant varieties within nurseries. Preliminary isolation technique results suggest the same *Phytophthora* species are recovered from baited root systems as from fine roots, but more species are recovered by the three-bait-system. It appears that isolation results from direct-plated roots are sometimes poor especially from highly degraded root systems whereas from the same root systems baiting still works well. Differences in *Phytophthora* species based on plant type within a nursery also occurred. *Phytophthora cryptogea* was isolated from the *D. aurantiacus* "trish" hybrid cultivar whereas the very close relative of *P. cryptogea,* "*P*. taxon kelmania", was isolated from the California native *D. aurantiacus* type.

## The first report of *Phytophthora nicotianae* isolated from Roselle (*Hibiscus sabdariffa*) in Shahdad, Kerman, Iran.

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Roselle (*Hibiscus sabdariffa*, Malvaceae) an annual shrub widely cultivated in tropical Asia, Australia, and West and Central Africa. In different countries, various parts of this plant are being used for cooking, oil extraction and its fibers are used for making ropes. Further, the swollen flower calyces of Roselle are mainly used to colour food and beverages. Additionally, Roselle has many pharmaceutical benefits. *Phytophthora* and some fungal agents have been recorded as limiting factors to the production of Roselle, worldwide. In the middle of 2015, declining *H. sabdariffa* plants were observed during a survey near to Shahdads "Kalouts"; one of the most spectacular natural phenomena (the largest accumulation of sand and clay clods in the world) of Shahdad, sub-urban city of Kerman province, Iran. Samples including plants and soil materials were collected and processed through baiting soil and plating roots and were enriched on *Phytophthora* semi-selective medium (PARPH). *Phytophthora* isolates were incubated at 28 °C in the dark. Based on the morphological features, isolates were identified as *Phytophthora nicotianae* and the identification was confirmed by sequence analysis of ITS region. A pot trial was established in the greenhouse using *4*-week old *H. sabdariffa* seedlings and 2-week well-grown *P. nicotianae* on millet seed as an inoculum source. One week post inoculation, all plants inoculated with *P. nicotianae* started showing wilting symptoms and after 12 days almost 70% of them died compare to healthy control plants. *P. nicotianae* was re-isolated from wilted plants confirming Koch's postulate. According to the Agroforestry Database Roselle is severely affected by *P. nicotianae*. This pathogen has also been reported on *H. sabdariffa* from other parts of the world including Nigeria, Malaysia and Indonesia; however, this is the first report of *P. nicotianae* on *H. sabdariffa* from Shahdad, Iran.

## Characterization of genetic diversity of *Phytophthora plurivora* isolates in Nepal.

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*Phytophthora plurivora* is widespread in different environments in Europe and USA and is sporadically present in Australia and South Africa. In Europe, this species is associated with declines of European beech (*Fagus sylvatica*) and oak species (*Quercus* sp.) in forests, and dieback of ornamental plants in gardens and nurseries. During an expedition in a remote forest in Western Nepal, isolates of *P. plurivora* were recovered from the rhizosphere of mixed broadleaf forests and single broadleaf trees. The isolates were subsequently genotyped at microsatellite loci previously developed for *P. plurivora*. The main aims of this study were (1) to characterize the structure of the *P. plurivora* population in Nepal, (2) to compare the local genetic diversity with that of European and North American *P. plurivora* populations, (3) and to determine eventual gene flow among the Nepalese and other *P. plurivora* populations. Here, we will present and discuss the results of this study.

## Longevity of active *Phytophthora ramorum* in terminal tree hosts following the removal of primary sporulating hosts.

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A Forestry Commission forest in south west England was one of the first locations in 2009 identified with *Phytophthora ramorum* infected Japanese larch (*Larix kaempferi*). In the 398 ha forest, >30% of larch had catastrophic levels of infection with trees of all ages affected. As larch proved to be the primary sporulating host, all larch was removed from the forest between 2009-2011 for disease management. Prior to this, spores released from infected larch foliage had already initiated cankers and dieback on many non-sporulating conifer and broadleaved hosts usually growing within 100m of infected larch. In 2015, more surveys were undertaken in areas adjacent to larch-cleared areas and trees with visible *P. ramorum* cankers were still readily identifiable. Affected hosts included *Fagus sylvatica, Abies grandis, Pseudotsuga menziesii* and *Tsuga heterophylla*. Some had sunken and calloused stem cankers but infection had apparently arrested recently; in others callus growth had completely occluded old cankers. However, several trees still had active cankers, evidenced by resinous exudation (all conifers) and black bleeds (*F. sylvatica*), and these almost entirely girded some tree stems. Samples taken from active cankers on all affected tree species were tested for *P. ramorum* by isolation and rt PCR. *Phytophthora ramorum* (EU1) cultures were obtained from all except *A. grandis* where only rtPCR confirmation of the pathogen was obtained. This suggests that even when the spore generating larch is removed preventing successive years of re-infection, original *P ramorum* stem lesions continue to expand in some terminal hosts for at least five years. This has biosecurity implications for timber processing if the pathogen can remain viable in infected tissue over several years. It also contrasts with the rapid death of larch trees in just 2-3 years, whilst other conifer hosts can remain alive for >5 years despite established, active stem lesions.

## Genome evolution of the clonal lineage *Phytophthora ramorum* NA1.

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*Phytophthora ramorum* NA1 is an invasive pathogen that has spread and diversified throughout the West Coast of the United States since its introduction in the mid-1990s. Despite limited genetic diversity in the founding population, recent work using read depth analysis and Single Nucleotide Polymorphisms (SNPs) have shown considerable variation between isolates in the form of Gene Conversion and Structural Variations (SVs). SVs are of interest because such genetic variation can add another layer of evolutionary potential for the rapidly expanding population. Chromosomal aberrations were first observed in *P. ramorum* associated with the genus *Quercus*. However, we have recently found *P. ramorum* NA1 isolates associated with transmissible hosts from nurseries and forests that have SVs. Whether these large alterations in the genome contribute to adaptation to a new environment is unknown. To test if SVs contribute to the evolution of the pathogen, we sampled 17 isolates from nursery and forest hosts and reconstructed their phylogeny using SNPs. With the phylogeny of *P. ramorum* NA1, we identified SVs that idependently arose between isolates suggesting either convergent evolution and/or sites in the genome prone to mutation. We also observed alleles that are amplified within SVs or Gene Conversion and compared these alleles between isolates. The same set of alleles appear to be favored between isolates further supporting our hypothesis of convergent evolution. However, further sampling of the population and identification of convergent mutations are needed to know if SVs contribute to the invasive pathogen's adaptation to new niches.

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