IUFRO

PHYTOPHTHORA IN FORESTS & NATURAL ECOSYSTEMS

Conference Program and Abstracts

IUFRO Working Party 07.02.09 *Phytophthora* in Forests and Natural Ecosystems Conference

Paihia, New Zealand

8 – 13 September 2024

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Common Māori words you may hear during the conference.

āe	yes, agree, OK
Aotearoa	pre-European (and hopefully future official!) name for New Zealand
hapū	sub-tribe, kinship group
hui	meeting
iwi	tribe
kai	food
kaitiaki	guardian, custodian
kaitiakitanga	guardianship
karakia	prayer, grace
kaumātua	elder, person of status
kaupapa	topic, plan, purpose, agenda
kauri	kauri. Giant tree, *Agathis australis*
kauri ora	kauri health
kia ora	hello, good morning/afternoon, best wishes, thank-you
koha	gift, present, donation
kōrero	talk, discussion
kuia	female elder, grandmother
mahi	work
mana whenua	territorial rights, authority, Iwi/hapū of an area
manuhiri	visitor, guest
Māori	Indigenous people of Aotearoa-New Zealand
manaaki	To support, to take care of
marae	meeting house
mātauranga	knowledge
mōrena	good morning
ngā mihi (nui)	thank you (very much)
ngahere	bush, forest
Pākehā	person of European or non-Māori descent
pātai	question
pōwhiri	welcome ceremony, especially onto marae
rākau	tree

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Rākau Rangatira	chiefly trees
rohe	boundary, district
rongoā	traditional medicine, herbal remedies
tangata whenua	indigenous people
taonga	treasure, something of value
Te Ao	the world
Te Ao Māori	The Māori world
tēnā koe	hello (formal) to one person
tēnā koutou katoa	hello (formal) to three or more people
tikanga	protocol, correct pocedure
tūpuna, tīpuna	ancestors
waiata	song
whakapapa	geneology, heritage
whakatau	to welcome formally
whanau	family
whenua	land

Common greetings and pronunciation

Hello	kia ora (key or-ra)
Hello - 2 people	kia ora kōrua (key-or-ra caw-roo-ah)
Hello - 3+ people	kia ora koutou (key-or-ra ko tow)
Thank-you	ngā mihi (nah-mee-hee)
Good morning	mōrena (more eh nah) or atamārie (ah-tar-mar-ee-ay)
Sorry, apologies	aroha mai (ar-roar-ha my)

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Ian Horner	Plant and Food Research
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Pre-conference Program

Saturday 7th September

Conference delegates catching the bus from the Sudima Auckland Airport to gather on or before Saturday 7th September.

Sunday 8th September

8.30 am **Preconference transport Auckland to Paihia departs Sudima Auckland Airport**

9.00 am **Preconference transport Auckland pick up Sky City Bus station, Auckland City**

1.00 pm **Arrive Paihia**

5.30 pm **Welcome Reception**

Included in full registration. Gather at the Hobson's Memorial Entrace, Upper Grounds Waitangi Treaty Grounds at 5.15pm to be ready to be 'called on' the site at 5.30pm. After the Pōwhiri drinks and nibbles will be served.

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Conference Program

Monday 9th September

8.30 am
Conference whakatau (welcome address).

Session 1: *Phytophthora* research in a New Zealand context

Session Chair: Nari Williams

9.00 am
Kauri ora and the response to the threat of *Phytophthora agathidicida*

Matua Hori Parata

9.15 am
New Zealand's response to *Phytophthora* in Natural Ecosystems

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The kauri tree is a national treasure of Aotearoa, New Zealand and the third largest conifer in the world. Māori see the health of kauri as an indicator of the wellbeing of the forest and the people, so their protection is of utmost importance. However, kauri are being threatened by the soil-borne *Phytophthora agathidicida*, leading to the fatal kauri dieback disease. In response, the New Zealand government has commissioned a management agency, Tiakina Kauri, to allocate $32 million over five years to implement a National Pest Management Plan aimed at stopping the spread of this harmful pathogen. In this talk, representatives from Tiakina Kauri will describe how they work in partnership with the Indigenous peoples living in kauri lands to protect kauri by integrating biosecurity practices into forest management and industry practices.

9.45 am
New Zealand Forest Owner's Association perspective

Brendan Gould

NZ Forest Owner's Association

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10.00 am

Auckland Council's response to PA: *Phytophthora agathidicida* and other harmful plant pathogen management from an operational land manager and local government perspective

Sarah Killick

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Sarah Killick, Jane Meiforth, Hugo Geddes, Alastair Jamieson, and Lisa Tolich

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Auckland Council manages 28 Regional Parks and over 4000 Local Parks across Tāmaki Makarau (the Auckland Region). Some of the most significant kauri (*Agathis australis*) stands in the region are within either Auckland Council parkland or managed with support from Auckland Council. Following the detection of the virulent kauri pathogen *P. agathidicida* in mainland Auckland, the Council has adopted a greater operational focus on plant pathogen management in indigenous forest systems.

In 2021, Auckland Council conducted a large-scale kauri ora (kauri health) and *P. agathidicida* soil sampling survey in the Waitākere Ranges Regional Park in partnership with Te Kawerau ā Maki, mana whenua and kaitiaki (indigenous authority and caretaker) of Te Wao Nui ā Tiriwa / the Waitākere Ranges. In 2023, a large-scale kauri ora survey was undertaken in the Te Ngaherehere o Kohukohunui / the Hunua Ranges, a large kauri forest system not previously known to contain *P. agathidicida*. This was a joint project between Auckland Council, the Department of Conservation, Ngāi Tai ki Tāmaki, Ngāti Tamaoho, Ngāti Te Ata, Ngāti Whanaunga, and Ngāti Tamaterā. The results of these major surveillance projects, other external research, the legislative framework relating to kauri ora, and the relationships between Auckland Council and our mana whenua partners and other key stakeholders have informed and enhanced the management of kauri in Tāmaki Makarau (Auckland).

In this presentation we will describe how we have operationalised research, outline key remaining barriers to effective management of kauri forests, and share learnings that may guide researchers and assist other land managers.

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10.15 am
Building a water treatment plant in kauri forest: lessons from a consent application.

Sarah Flynn

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Sarah Flynn[1], Paul Jones[2], Edward Ashby[3], Giles Hardy[4], Lee Hill[5]

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The Huia Water Treatment Plant treats 20 per cent of Auckland's water, sourced from the Upper and Lower Huia Dams and Upper and Lower Nihotupu Dams in the Waitākere Ranges (Te Wao Nui o Tiriwa), and is a vital part of the city's water supply network, The existing plant was built in 1928 and is near the end of its operational life. In 2019, Watercare Services Ltd submitted an application for resource consent to replace the water treatment plant. The project also includes reservoirs to increase the volume of water stored locally, improving the resilience of the wider water network.

The site of the proposed replacement water treatment plant is on land designated for the purpose, adjacent to the existing plant. Much of this site was cleared of vegetation at the time the existing plant was constructed, but a cover of native forest has subsequently re-established. The surrounding catchment is residential but largely covered in regenerating forest with remnant stands of kauri, as is typical of the southern Waitākere foothills

A technical report (Hill et al 2017) on the distribution of kauri dieback in the Waitākere Ranges drew attention to evidence that human activity was causing the spread of *Phytophthora agathidicida*, by then recognised as the primary causal agent of kauri dieback disease. In response, Te Kāwerau a Maki placed a rāhui over the whole of the Waitākere Ranges in December 2017, followed by an upswell of action from Auckland Council, government agencies and science organisations to compile information and coordinate a response.

The need for comprehensive kauri dieback risk management as part of the large-scale infrastructure work proposed had been identified from the outset of Watercare's technical assessments, but became a critical aspect of the consent application over the course of the project. This talk presents a review of how the project unfolded, including highs and lows, reflections on the importance of cross-disciplinary collaboration, and a good deal of wisdom borne of hindsight.

References:
Hill, L, Stanley, R, Hammon, C, Waipara, N (2017) Kauri dieback disease: An investigation into its distribution in the Waitakere Ranges Regional Park. Auckland Council

10.30 am – 11.00 am Morning Tea

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Session 2: Biogeography

Session Chair: Bruno Scanu

11.00 am
Keynote presentation: Phytophthora impact across agricultural, horticultural and forestry landscapes

Ana Pérez-Sierra

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Phytophthora is a genus of plant-pathogenic oomycetes impacting on various landscapes and causing devasting diseases, each presenting unique challenges and requiring specific management strategies. *Phytophthora* has devastating effects on crops, causes significant economic losses in production and retail nurseries, and affects forest and natural ecosystems leading to biodiversity decline and altering forest structure. The transition of *Phytophthora* species from agricultural or horticulture settings to forestry and natural ecosystems or vice versa presents significant challenges. Understanding how these pathogens spread and affect different environments is crucial for developing effective management strategies to mitigate the impact on plant health and preserve the integrity of natural ecosystems. The spread mechanisms of these organisms involve human activity such as trade in infected nursery plants, agricultural runoff or restoration projects, and natural dispersal through soil and water movement. Effective management requires a holistic approach, incorporating monitoring, biosecurity measures, cultural practices, and integrated management strategies. Conducting risk assessments to identify vulnerable areas and the potential pathways for *Phytophthora* spread will help to prioritize where management efforts should be focussed. These include good biosecurity measures such as quarantine regulations, regular inspections and sanitation practices; use of advanced diagnostic tools to identify *Phytophthora* species accurately and quickly; robust monitoring programmes to detect the presence of *Phytophthora* early; public awareness and outreach programmes to raise awareness about the risks and prevention measures for *Phytophthora*; and research on the best practices to mitigate the damages caused by these pathogens. The movement of *Phytophthora* species from agriculture to forestry and vice versa is a complex issue and poses a significant threat to crop health and agricultural productivity, and biodiversity. Therefore, coordinated efforts are needed across multiple sectors to prevent or reduce the threat posed by these pathogens. Engaging with farmers, government agencies, stakeholders and researchers is essential. Furthermore, the knowledge and resources need to be shared across borders to manage *Phytophthora* globally. Collaboration between agricultural, horticultural and forestry sectors is essential to protect both agricultural productivity and natural ecosystems from the devastating effects of *Phytophthora*.

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11.30 am

Sporulation speculations: Probing Phytophthora pluvialis' path to forest pathogenicity

Emily McLay

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Emily McLay and Stuart Fraser

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Phytophthora pluvialis causes the foliar disease red needle cast (RNC) in New Zealand's radiata pine forests. Although RNC tends to follow a seasonal pattern with peak expression of symptoms in winter, the timing, severity, and location of outbreaks can be highly variable. The variation in expression makes RNC difficult to predict and therefore manage. This is confounded by a lack of knowledge on the primary inoculum for RNC outbreaks. Whilst oospores are produced in vitro, they do not readily germinate and are yet to be observed in planta. Without other survival structures such as chlamydospores, the ability of stress-tolerant inoculum to build up at a site is unknown. In addition, on radiata pine, *P. pluvialis* only infects needle tissue, which is cast following symptom expression, thus both pathogen and host can be rapidly removed from the crown as part of the disease cycle. With highly localised spread through water splash, this seems counterintuitive for dispersal of spores. Despite its ability to produce large-scale epidemics and damage, *P. pluvialis* appears to have traits that hinder maintenance of epidemics within the foliage of radiata pine. This assessment is contingent on our current level of understanding, which is incomplete. To better understand the disease cycle, and improve decision making for RNC control, we completed research under lab and field conditions on the environmental drivers of infection and sporulation of *P. pluvialis* on radiata pine with the goal of developing a process based epidemiological model. These studies suggest typical curves for temperature and wetness requirements for infection and sporulation, however, also revealed interesting behaviour at temperature limits. This included increased release rather than retention of sporangia at higher temperatures, and a preference for direct germination over the release of zoospores. Aggregation of zoospores appeared to vary with temperature also. As with other *Phytophthora*, starvation and interactions with microbes were required for sporulation (in vitro). Bacteria isolated out of these non-sterile solutions were able to induce sporangia production, however, this appeared to be isolate dependent. We present some of our peculiar observations on the sporulation behaviour of *P. pluvialis* to the forest *Phytophthora* community to compare with other pathosystems and discuss the possible reasons why these pathogen characteristics may present an ecological advantage.

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11.45 am
Global pathways for Phytophthora “Trojan horse” establishment in native ecosystems

Peter Scott

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The *Phytophthora* genus is associated with significant plant diseases in natural ecosystems, production, and global urban environments. *Phytophthora* pathogens pose formidable biosecurity challenges as they are increasingly spread worldwide and often cause major diseases within newly invaded environments. The global spread of *Phytophthora* species is closely linked to the insurmountable anthropogenic forces of globalization, urbanization, population growth, and the decimation of natural ecosystems observed since the genus was first established in 1870. Therefore, research into the global epidemiology of the genus provides a greater understanding of the interdependent relationship between human expansion and the natural world. Extrapolation of the genus biology highlights that many *Phytophthora* pathogens have yet to be discovered, and additional work is required to protect the biodiversity of hosts and pathogens alike. Probabilistic and Bayesian analyses of the traits of *Phytophthora* species and their global diseases have provided new tools to determine the risk of new species to different land use types. A new analysis of the host associations of global *Phytophthora* records highlights the likely pathway for 'Trojan', highly invasive species into natural ecosystems and the need for additional biosecurity and biodiversity conservation.

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12.00 pm

A meta-analysis of global Phytophthora surveys reveals patterns in detection method and land-use types

Mireia Gomez Gallego

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Shannon Hunter,[1,2,3] , Mireia Gomez-Gallego[4], Peter Scott[5] , Nick Waipara[2] , Bruce Burns[3] and Nari Williams[1,3,6]

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Forest ecosystems have evolved diverse flora, fauna, and microbial communities, including abundant bacterial, fungal, and oomycete plant pathogens. Recently, an increasing number of previously undescribed oomycetes (particularly those in the genus *Phytophthora*) have been detected in forests globally. In many instances, these oomycetes may be co-evolved natives, but still present a risk to plants as little is known about their biology, distribution, and how they will respond to environmental changes. A range of *Phytophthora* species have also been inadvertently spread widely, in response to globalisation, often causing devastating impacts in response to new host pathogens and environments interactions. This study aimed to conduct a meta-analysis of published survey papers conducted in different land-use types, including nurseries, natural, urban, and agricultural environments. The abundance of species in relation to the detection method (plating and baiting compared to Environmental DNA) and substrate tested (soil, water, tissue, and roots) were analysed. Nearly 200 papers were included in the database. There were 180 reports of unknown species across the surveys. *Phytophthora cinnamomi* was the most common species found in 75 surveys across 26 countries. The distributions of *Phytophthora* species in low-income countries of Africa, Asia, South America and the Pacific have been poorly studied. This talk will discuss the implications of this study for *Phytophthora* surveillance, its importance in disturbed environments, with a specific focus on approaches for studying these pathogens within developing countries. Surveys in diverse and yet, unexplored environments will help determine the origins of invasive *Phytophthora* pathogens and improve disease management. Significant work is required to determine how these highly adaptive and mobile *Phytophthora* communities will respond to increasing environmental changes and stress, including pressure from climate change.

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12.15 pm

Phytophthora Communities Associated with *Agathis australis* (kauri) in Te Wao Nui o Tiriwa/Waitākere Ranges, New Zealand

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Studies of *Phytophthora* impact in forests generally focus on individual species without recognition that *Phytophthora* occur in multispecies communities. This study investigated community structure of *Phytophthora* species in the rhizosphere of *Agathis australis* (kauri) in Te Wao Nui o Tiriwa/Waitākere Ranges, New Zealand, in the context of kauri dieback disease expression. Soil sampling and tree monitoring were conducted on 767 randomly selected mature kauri trees. *Phytophthora* species were detected using both soil baiting and DNA metabarcoding of environmental DNA (eDNA). Four species were detected with soil baiting (*P. agathidicida, P. cinnamomi, P. multivora,* and *P. pseudocryptogea/P. cryptogea*) and an additional three species with metabarcoding (*P. kernoviae, P. cactorum/P. aleatoria* and an unknown clade 7 species). *Phytophthora cinnamomi* was the most abundant species and was distributed throughout the forest. Both *P. multivora* and *P. agathidicida* were limited to forest edges, suggesting more recent introductions. *P. agathidicida* presence was strongly correlated with declining canopy health, confirming its role as the main driver of kauri dieback. The limited distribution of *P. agathidicida* and infrequent detections (11.0% samples) suggests that that this species is spreading as an introduced invasive pathogen and provide hope that with strategic management (including track upgrades and closures, restricting access to uninfected areas, and continual monitoring) uninfected areas of the forest can be protected. The frequent detections of *P. cinnamomi* and *P. multivora* from symptomatic trees in the absence of *P. agathidicida* suggest more research is needed to understand their roles in kauri forest health.

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12.30 pm

Phytophthora diversity in watercourses of the highly urbanized Swiss Plateau (Poster talk)

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Knowledge about diversity and composition of the *Phytophthora* community in European watercourses is still incomplete. In this study, we investigated the presence and diversity of *Phytophthora* species in watercourses flowing through the highly urbanized Swiss Plateau region, stretching from Geneva to St. Gallen. Over the period 2012–2016, we sampled 32 watercourses, including major rivers (i.e. Rhine, Rhone and Aare) and smaller streams. We isolated *Phytophthora* from our baits (rhododendron leaves) and sequenced the ITS region to identify the species. We recovered a total of 241 *Phytophthora* isolates, representing 11 species from five major clades. Species of the *Phytophthora* clade 6 were predominant, with *P. lacustris* being the most common, found in 94.7% of the watercourses. The number of *Phytophthora* species per watercourse ranged from one to five and did not correlate to the watercourse complexity. Our study reveals the presence of six previously unreported species in Switzerland (*P. bilorbang, P. gallica, P. hydropathica, P. lacustris, P. polonica, P. riparia*), while invasive species occasionally/regionally detected in Switzerland (e.g. *P. cinnamomi, P. multivora, P. ramorum*) were not found. Hence, watercourses appear less suited to detect invasive pathogenic *Phytophthora* species with a still limited distribution in the environment.

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12.35 pm

Survey of oomycetes and pathogenicity of *Phytophthora cinnamomi* associated with root rot of western white pine *Pinus monticola* mortality (Poster Talk)

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Western white pine *Pinus monticola* is an ecologically important species in western North America. The species persists across widely ranging environmental conditions and diverse habitats of western North America. The dramatic decline of this species over the past several decades is attributed mainly to introduction of an invasive alien pathogen *Cronartium ribicola*, the causal agent of the white pine blister rust disease. Planted western white pines with improved genetic tolerance to white pine blister rust disease are a valuable resource for management of this disease. In addition to biotic threat, the health of *P. monticola* is challenged by the adverse effects of ongoing climate change. Over the years, typical root-rot symptoms and sudden tree death have been observed in urban British Columbia's (BC) plantations of western white pine suggesting the presence of a "water mold" agent of the oomycete group. Our objectives were to survey one of the urban plantation located in south-west BC on Vancouver Island to confirm the presence of *Phytophthora* spp. and test their pathogenicity on western white pine seedlings by fulfilling Koch's postulates. We used direct plating of root samples on PARP(H)-CMA media and oomycetes baiting in soil and root samples. In addition, samples were assayed for oomycetes by sequencing PCR amplicons of the internal transcribed spacers (ITS) of the nuclear rRNA and they were identified using a phylogenetic approach. Ten oomycetes were isolated from roots and soil samples of diseased P. monticola trees including *Phytophthora cinnamomi*, *P. cactorum*, *P. cryptogea*, and *P. pseudocryptogea* and few species of the *Pythium*, *Phytopythium* and *Globisporangium* genus. Pathogenicity tests were validated for these *Phytophthora* spp. with controlled inoculations of 1-year-old seedlings, while none of the non-*Phytophthora* species caused disease. Mortality occurred as early as 15 days after inoculation when the seedlings were inoculated with *P. cinnamomi* and maintained in warm temperature condition (25°C). Molecular typing of *P. cinnamomi* cultures using Oxford Nanopore sequencing indicated that they belong to one of the two main clonal cluster of mating type A2 that have been recognized as the main drivers of the global *P. cinnamomi* epidemic. The discovery of the first record of *P. cinnamomi* in urban conifer plantation in BC supports the progressive expansion of this species towards higher latitudes, probably driven by climate change. Furthermore, anthropogenic activities (land use) in urban landscapes are shaping oomycete communities, creating a pathway of *Phytophthora* spp. spreading into natural forest ecosystems.

12.40 – 1.30 pm Lunch

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Session 3: Ecology 1

Session Chair: Andrea Vannini

1.30 pm
From tree to shrub: The fate of tanoak through disease and fire

Jacqueline Rose

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Jacqueline Rose

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Invasive plant pathogens have killed an uncountable number of trees and disrupted ecosystem processes throughout the world. Newly introduced pathogens may interact with historically occurring disturbances in unexpected ways to cause amplified mortality and change to ecosystem processes. Here we use the natural experiment created by three wildfires in the central coast of California, a region impacted by the nonnative pathogen *Phytophthora ramorum*, to forecast the future of a species susceptible to the pathogen. Tanoak (*Notholithocarpus densiflorus*) is killed by *P. ramorum* infection, which causes a disease known as Sudden Oak Death. Besides being susceptible to the pathogen, tanoak also allows sporulation of *P. ramorum* from its stems and is generally susceptible to fire until well after maturity. The combination of the novel pathogen and repeated fires resulted in near totality of tanoak stem mortality. These levels of mortality were not experienced by other species within the study area, including species considered fire-resistant but susceptible to *P. ramorum*, and species immune to *P. ramorum* but susceptible to fire. Our results demonstrate that tanoak may be reduced to shrub habit within the central coast region of California due to the interacting pressures of *P. ramorum* and fire.

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1.45 pm

Ecological impacts of Phytophthora agathidicida in the Waitākere Ranges Regional Park, New Zealand

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Soilborne pathogens directly harm their hosts by causing symptoms like root rot, canopy thinning, and eventual dieback and death. The decline in a particular tree species may impact associated species as well as drive changes in ecosystem functions like productivity, decomposition, and carbon and nutrient fluxes. Kauri (*Agathis australis*) is a large and long-lived conifer species endemic to New Zealand and of high ecological and cultural significance. The species' survival is now threatened by kauri dieback, caused by the oomycete pathogen *Phytophthora agathidicida*. Here, we measured litter chemistry, decomposition rate, root density and productivity in kauri forests affected by dieback across the Waitākere Ranges Regional Park, New Zealand. Kauri leaf litter carbon concentrations were higher under kauri where *P. agathidicida* was detected. Pathogen detection status also had a significant effect on the per cent mass loss of buried, decomposing Rooibos tea. We also found that kauri fine root density in the soil organic layer was lower where *P. agathidicida* was detected, while non-kauri fine root density was unaffected. In mineral soil, fine roots were unaffected by pathogen presence. Our findings suggest a slow but gradual change in ecosystem carbon and nutrient dynamics.

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2.00 pm
Diversity and distribution of *Phytophthora* species across different Italian forest ecosystems

Carlo Bregant

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In the last few decades, an impressive increase of emerging Phytophthora-related outbreaks has been observed in Italy in various environments and forest types, spanning from the Mediterranean maquis to the alpine and subalpine rhododendron, blueberry and dwarf pine ecosystems. Diseased plants showed a complex symptomology, suggesting multiple and often simultaneous attacks by soilborne and airborne Phytophthora species. Since little information is available about the Phytophthora species involved in these emerging syndromes, extensive field surveys were conducted between 2017 and 2024 in ten Italian regions to determine the impact and diversity of involved Phytophthora taxa. Morphological and molecular characterization revealed the occurrence of 40 Phytophthora species and hybrids, isolated from about 2000 rhizosphere and tissue samples collected from 77 plant species. The results clarified the complex aetiology of some emerging Phytophthora-induced diseases of forest trees and shrubs throughout Italy, providing new insights into their symptomology. The outcome was a surprising diversity of Phytophthora species, many of which were endowed with a different lifestyle, marked plasticity and ability to survive under extreme environmental conditions. Among these, *P. cinnamomi* appeared to be very widespread in Mediterranean formations, *P. plurivora* in temperate habitats and *P. pseudosyringae* in mountain environments. The occurrence of other invasive Phytophthora, such as *P. heterospora, P. multivora* and *P. pseudocryptogea*, poses a serious threat not only to forest ecosystems, but also to agricultural crops.

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2.15 PM

Unravelling variability: Exploring phenotypic and genetic characteristics of *Phytophthora cinnamomi* across hosts and regions in New Zealand

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Phytophthora cinnamomi is a pathogen with a widespread global presence that poses a significant threat to ecosystems, particularly in Mediterranean-type climates where it severely impacts biodiversity. Widely distributed in New Zealand, *P. cinnamomi* has been isolated from a range of exotic and indigenous ecosystems. However, little research has been done to understand the pathogen population in the country. With the growing impact of climate change and a shift in the environmental conditions towards warmer and wetter periods, *P. cinnamomi* infection, spread, and disease impacts are likely to be favoured. This research examines the genetic and phenotypic variation of the *P. cinnamomi* population in New Zealand in terms of growth temperature (minimum, maximum, and optimal), colony morphology, virulence, and mating types to gauge the ecological versatility and pathogenic potential of the species. Whole-genome (Illumina HiSeq) sequencing has been used to determine the genetic diversity of isolates and to determine whether there is a relationship between genetic and phenotypic variation being observed. In addition, understanding the phylogenetic relationship between *P. cinnamomi* will help to understand its invasion history and continued pathways of movement throughout the country, informing targeted management strategies and risk analyses aimed at mitigating the pathogen's impact on agriculture and natural ecosystems.

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2.30 PM

Potential alternative hosts of the kauri dieback pathogen *Phytophthora agathidicida*

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Phytophthora agathidicida causes decline and death of kauri (*Agathis australis*) trees in northern Aotearoa-New Zealand forests. To date, kauri is the only species known to be susceptible to *P. agathidicida* in the forest. However, it is expected that other forest species could potentially harbour or help proliferate the pathogen, while not necessarily showing obvious disease symptoms. Clean nursery-grown seedlings of various common species native to Aotearoa-New Zealand were planted into three *P. agathidicida*-infected kauri forests, to determine if roots were colonised and whether symptoms were evident. There were 50 replicate plots of each species tested, across the three sites. These trials were backed up by glasshouse inoculation studies with 10 plants of each species, and targeted sampling of roots from multiple native plant species naturally growing in *P. agathidicida*-infected stands. Assays to detect *P. agathidicida* included both direct plating and baiting of surface-sterilised roots. Glasshouse trials indicated that roots of most of the 29 species tested could be colonised by *P. agathidicida*. However, in the field plantings, apart from kauri only nine of the 28 species tested were colonised, and the frequency of detection was very low compared to that in kauri. In targeted sampling of naturally growing plants, only three of 60 species tested yielded *P. agathidicida*, again with a very low frequency of detection. The ecological significance of this will be discussed.

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2.45 PM
Kauri forest *Phytophthora* species and their pathogenicity

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Since 2006, more than 8,000 soil samples have been collected from kauri (*Agathis australis*) forests for analysis for the presence of *Phytophthora agathidicida*. Excluding the targeted *P. agathidicida* and easily characterised *P. cinnamomi*, other *Phytophthora* isolates not readily identified to species level based on morphological traits were detected and a selection stored in the Plant & Food Research culture collection. These 506 isolates previously unidentified to species level were each re-cultured and identified further by sequencing the ITS1 region of the ribosomal DNA and cox1 gene region. Seventy-nine percent (401 isolates) were found to be from Clade 2c, of which the majority (n = 364) were *P. multivora*. Of the remaining isolates, 18 were from Clade 6b (*P. megasperma* complex), 15 isolates from Clade 8a (*P. cryptogea* complex), and 14 from Clade 10b (*P. kernoviae*, *P. gondwanensis* and *P. boehmeriae*). Pathogenicity tests using a series of detached leaf, detached twig and root inoculations were conducted by inoculating kauri with a representation of isolates from each clade and species, in contrast to *P. agathidicida* and *P. cinnamomi*. *P. agathidicida* was confirmed as the most aggressive pathogen of kauri. Based on the pathogenicity testing, several *Phytophthora* isolates were found to be minor pathogens of kauri. Little is known of the importance of these species in kauri forest ecology.

3.00 – 3.30 pm Afternoon Tea

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3.30 pm

Effect of five *Phytophthora* species on seed germination and seedling performance in sweet chestnut

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The oomycete genus Phytophthora includes major pathogens of agricultural crops and woody plants worldwide. In forests, damage caused by Phytophthora species is well known on adult trees, but less on previous development stages (seed, seedling, sapling). In agriculture some Phytophthora species are known to decay seed or seedlings before they emerge or cause damping-off of young plants. In this study, we investigated the influence of five Phytophthora species commonly found in sweet chestnut (*Castanea sativa*) stands in Europe (i.e. *P. plurivora, P. cryptogea, P. cactorum* and the two causal agents of ink disease *P. cinnamomi* and *P.* x *cambivora*) on germination of chestnuts and seedling development. For this, we artificially infected a soil substrate with millet grains previously colonized by one of the selected Phytophthora species. We then used this substrate to grow chestnut seedlings out of nuts from four different Swiss chestnut cultivars. Germination rates, seedling status (alive/dead) and performance (height, dry mass, root length) were assessed. The results of this experiment will be presented and discussed in relation to a potential role of Phytophthora species as disturbance factor for natural regeneration of sweet chestnut.

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3.45 pm

Investigation on potential susceptible hosts for *Phytophthora pluvialis* in forests, wider environment and horticulture

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In September 2021 *Phytophthora pluvialis* was detected on western hemlock (*Tsuga heterophylla*) in the UK. Soon after it was detected on Douglas fir (*Pseudotsuga menziesii*) and Japanese larch (*Larix kaempferi*). This pathogen was described in 2013 and infects tanoak (*Notholithocarpus densiflorus*) and Douglas fir in the USA, and in New Zealand it infects radiata pine (*Pinus radiata*) which is a major host, a few other pine species and Douglas fir. The pathogen causes a needle cast disease in the USA and New Zealand. No other host plants have previously been associated with *P. pluvialis* which has also been detected in streams, soils and canopy drip in tanoak and Douglas fir forests. The majority of detections in the UK have been on western hemlock, which was a new host for *P. pluvialis*. The main symptoms observed on western hemlock and Douglas fir were multiple resinous cankers on branches, stem lesions, basal lesions, and root lesions. Needle symptoms including discoloration were also observed. As the first detection in the UK was on a new host and causing different symptoms, the aim of this study was to investigate the susceptibility of other potential hosts including conifer and broadleaved species of economic importance for forestry and woodlands; environmental hosts important to the biodiversity of flora; and horticultural hosts that could be potential pathways of introduction. Pathogenicity tests were performed on 26 different hosts using three *P. pluvialis* isolates from different origins (UK, USA and New Zealand). *Phytophthora ramorum* was used as a positive control and negative controls were inoculated with sterile agar or distilled water. Different inoculation methods were tested on whole plants and on detached foliage. In each trial eight plants of the selected host were inoculated with each of the isolates and plants, and then maintained under controlled conditions for three months before assessment. Lesions on stems were measured, isolations performed, and samples tested by real-time PCR for the detection of *P. pluvialis*. The sporulation capacity on foliage of different hosts was also studied. The results of these trials informed a pest risk analysis (PRA) of the pathogen relevant to the UK. These data suggest that *P. pluvialis* is a less aggressive pathogen than *P. ramorum*, another recent introduction to the UK, and also has a more limited host range than *P. ramorum*.

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Session 4: Community

Session Chair: Trudy Paap

4.00 pm

Best Practice Guidelines and Novel Approaches in Dieback-Free Basic Raw Materials (BRM)

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Mia Townsend

Dieback Working Group Inc., 89 Wood St, Swanbourne, Perth, Western Australia, Australia, 6010.

Basic raw materials (BRM) are materials such as gravel, sand, limestone and hard rock that are usually used in their unprocessed state, for example, in road construction and as fill. Their use poses a significant risk of spreading Phytophthora Dieback (dieback) if they are sourced from infested pits. The Dieback Working Group's BRM sub-committee has released the 2021 Best Practice Guidelines for Management of Phytophthora Dieback in the Basic Raw Materials Industries, which outlines how to minimise the risk of infestation at all stages of the exploitation of this important resource. Contracts for the supply of BRM may specify that it is dieback-free. However, the supply of dieback-free BRM is limited; it is a valuable resource which needs to be used with care. BRM is only recognised by WA's Department of Biodiversity Conservation, and Attractions as being dieback-free if it is sourced from an area of native vegetation assessed as being uninfested by a registered Phytophthora Dieback Interpreter, and the pit has been hygienically managed to maintain its uninfested status. Although this method gives land managers the greatest level of confidence that materials do not contain Dieback, there are several problems which make this method far from ideal: -Relies on clearing healthy vegetation, creating additional fragmentation and disturbance. -Strict hygiene protocols need to be adhered for all vehicles, personnel, and machinery accessing the pit, which is resource intensive. -Uninfested gravel pits are often some distance from the works required, meaning that carting, environmental offset and rehabilitation costs add significant financial overheads to projects requiring Dieback-free BRM. In response to these concerns, novel approaches to accessing dieback-free BRM have seen significant breakthroughs in recent years, through collaborative research projects conducted in collaboration with Curtin University and WA State Government bodies. The use of the fumigant Metham sodium to eliminate Phytophthora species from laterite gravel, more than 8 million cubic meters of which is required every year for civil works in Western Australia. Accessing dieback-free gravel continues to be a prominent issue for land managers across all tenures. Work published by Dr Elaine Davison on sterilisation of gravel using Metham sodium presents a novel and promising solution to this challenge, demonstrating that Phytophthora can be eliminated from over 94% of samples. The Dieback Working Group is working to facilitate a national and international pathway to market for this game-changing technology.

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4.15 pm

Mana Whenua Informed, Place-based Biosecurity Management – A Proposed Decision-making Framework to Address Plant Pathogen Threats in Aotearoa New Zealand

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Mana whenua relationships with their ancestral lands, waters, and nature, are living relationships based on genealogical connections to place, nature, and people. These deeply held familial connections reflect intergenerational empiricism with the natural world and are the embodiment of reciprocal relationships between land and nature and their interconnected human communities. As such, mana whenua communities are well positioned to lead biosecurity decision-making and management within their respective tribal areas. Despite recent efforts within New Zealand's biosecurity system to include and collaborate with mana whenua communities, particularly with regard to the plant pathogen *Phytophthora agathidicida*, mana whenua inclusion falls short of equitable power and resource sharing, and the importance of mana whenua relationships with place to inform biosecurity decision-making, response and management are not adequately acknowledged. This paper proposes a shift toward mana whenua informed, place-based biosecurity management of tribal landscapes, especially areas of Indigenous Forest, within New Zealand's post-border biosecurity system. A framework for mana whenua biosecurity decision-making is hence presented. The framework includes a number of characteristics that differ from current post-border biosecurity management. These characteristics include risk assessment and decision-making that is underpinned by mana whenua perspectives, reflecting long-term inter-generational thinking and intimate (whakapapa) relationships with land and nature; an approach that is inverse to New Zealand's 'single-threat, multiple location' post-border management, and instead reflects an approach of 'multiple threat, single location', thereby emphasizing the importance of mana whenua relationships with place; risk assessment that begins from the point of vulnerability (place), rather than a specific threat; and removing mana whenua from the cultural siloing that commonly occurs when Indigenous perspectives and participation are confined to the 'cultural' risk and impact category, recognizing that culture does not occur in isolation and therefore cannot be viewed separately from economic, environmental, social and human health factors. With anticipation, the framework presented here may benefit mana whenua communities, stakeholder groups, and government agencies, to collectively address plant pathogen and other biological threats to the forests, landscapes, and communities of Aotearoa New Zealand.

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4.30 pm
Community-led *Phytophthora* education and capacity building in Australia's biodiverse southwest

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The Dieback Working Group (DWG) is a community-led not-for-profit organisation based in the southwest of Western Australia, where more than 40% of our 5,570 vascular plant species are susceptible to the invasive and widespread pathogen, *Phytophthora cinnamomi*. DWG acknowledges that anthropogenic spread is the primary method through which *Phytophthora* species are spread through the landscape, and undertakes a wide range of activities to support our core mission: to raise awareness and build community capacity around *Phytophthora* Dieback and other environmental biosecurity issues impacting Australia's native flora. DWG was established in 1996, and has developed a series of flagship programs over the decades, including: Green Card training: A State Government endorsed training course on basic *Phytophthora* hygiene and management planning. This course has been historically delivered in a face-to-face format, but has recently been made available as an e-learning course through a partnership with southwest WA's largest electricity network provider Western Power. -Discovering Dieback Primary School Education Program: A WA curriculum-mapped education package which encourages *Phytophthora* awareness in Year 6 students. The program consists of a series of lesson plans available for delivery by teachers, with the option to include a DWG-led Dieback Busting Day in the field. Schools choose a local reserve in which to hold this event, and students experience phosphite tree injections as well as a DIY build your own boot cleaning kit. In a recent collaborative project delivered in partnership with the Binalup Aboriginal Corporation, these communications strategies have been combined with a review of signage and hygiene infrastructure, two-way knowledge sharing workshops with Traditional Owners to better understand the Aboriginal Cultural Heritage values at risk from *Phytophthora* infestation, and community awareness raising events to undertake an intensive education campaign in the South Coast NRM Region/Wagyl Kaip Indigenous Land Use Agreement area. The ultimate goal of this project is to protect a prominent National Park located within the UNESCO listed Fitzgerald Biosphere Reserve from the spread of *Phytophthora* through activities such as tourism, hunting and customary access by Traditional Owners, mining, and road construction. Project findings and successes are expected to translate well into nationally and internationally applicable communications strategies to assist in the management of soilborne pathogens. This multi-sector approach to *Phytophthora* education and training is expected to generate positive environmental behaviour change in residents and visitors to the Biosphere, which will be quantified through a series of research projects in partnership with WA Universities.

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4.45 pm

Binalup Aboriginal Corporation's Cultural Rangers work managing ecosystems threatened by Phytophthora pathogens

Shawn Colbung

The Binalup Aboriginal Corporation's Cultural Rangers are working throughout the Wagyl-Kaip region of Western Australia, protecting and preserving Culture, the land, flora and fauna. Experienced, and skilled, our cultural rangers are active throughout our Wagyl-Kaip (southwestern) region of Western Australia. Under the guidance and direction of Our Elders, we work hard to facilitate the participation of our people and culture in the management of our Boodja.

Binalup Cultural Rangers have obligations, commitments and a presence across some of the most culturally rich and biologically diverse landscapes in the West Australian Great Southern Region, including the Stirling Range National Park, Porongurup National Park and Fitzgerald River National Park. Working at the grass roots level under the direction of senior south coast Noongar elders, we thrive on making an impact.

Our Ranger Team comes from a variety of Noongar family groups with traditional connections to the Wagyl Kaip area, working to unite the Great Southern Noongar communities and connect younger generations to their culture and country. Our inclusiveness and respect for cultural protocol are founding principles of Binalup Aboriginal Corporation.

We know that sometimes all it takes to make a change is the right knowledge, a bit of hard work and a little support.

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5.00 PM

Kauri Rescue™ Citizen Science Evaluation of Kauri Dieback Treatment Tools

Ian Horner

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Kauri dieback caused by *Phytophthora agathidicida* is resulting in decline and death of kauri (*Agathis australis*) trees throughout northern Aotearoa-New Zealand. Many diseased or threatened trees are on private land, and landowners are left feeling helpless as their kauri trees decline around them. Kauri Rescue™ is a Charitable Trust working with private landowners to treat and monitor kauri trees suffering from kauri dieback disease on their properties. Landowners selected one of four phosphite injection treatment options for their trees (either 4% or 6% active ingredient, with 20 mL injections every 40 cm (low dose) or 25 cm (high dose) around the trunk circumference), or left the trees untreated. Tree health assessments were made pre-treatment and at various intervals up to 6 years post-treatment, either by landowners, volunteers, or Kauri Rescue personnel, with the latter doing an increasing proportion of assessments as time progressed. Considerable data cleaning was required because of obvious errors and inconsistencies in some citizen scientist-collected data, and the pool of trees for analysis was thus reduced. Because of this, data collection methodologies were changed to improve the robustness of future data collection. Ordinal regression testing of how different treatments influenced tree health indicated that phosphite at either 4% or 6%, with 20 mL injected at either 25 or 40 cm intervals around the trunk, reduces the activity of lesions caused by *Phytophthora agathidicida*. Comparative analysis of participant- and professional auditor-collected data suggests that lesion activity scores are likely to be the most accurate tree health indicators for citizen scientists to use.

5.15 pm Daily summary and discussion

Session Chair: Carmen Morales-Rodríguez

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Tuesday 10th September

Session 5: Ecology 2

Session Chair: Dr Simon Francis Shamoun. Natural Resources Canada, Canadian Forest Service

8.30 am

Keynote: The root rot pathogen *Phytophthora cinnamomi*: A long-overlooked threat to the Cape Floristic Region

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Phytophthora cinnamomi (*Pc*) is a globally important plant pathogen, causing root and crown rot, cankers, dieback and mortality of approximately 5000 woody plant species worldwide. *Pc* was first reported in South Africa in 1931, from avocado orchards. The first report of the pathogen causing mortality in South African natural ecosystems was van Wijk's 1973 account of "quick decline" of *Leucadendron argenteum* in the Cape Floristic Region (CFR) of the Western Cape Province. Subsequent studies highlighted the importance of *Pc* as a root rot pathogen of numerous native species, both in natural ecosystems and in cultivation. Despite this initial evidence for *Pc* being a notable threat to the flora of the CFR, very few studies have investigated the relative susceptibilities of Cape flora to this pathogen by artificial inoculation. A recent observation of *Pc* causing rapid mortality of *Sorocephalus imbricatus* (a Critically Endangered Proteaceae), highlights the urgent need to better understand the threat that *Pc* poses to conservation of the Cape flora. Considering the alarmingly high number of rare and threatened taxa occurring in the CFR, and with many of these taxa belonging to families known to be susceptible to *Pc*, an intensive local research programme should be initiated to determine the relative susceptibilities of Cape flora to this pathogen. Understanding the relative susceptibility of taxa by positioning them on a resistance-susceptibility continuum would help inform strategies for in-situ conservation. It would also inform conservation priorities and where recovery efforts should be directed.

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9.00 am

Understanding the presence and spread of *Phytophthora* spp. in garden ecosystems

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Public gardens host a diversity of plant material and rare collections. They are characterised by multiple and changing landscape displays, with the introduction of a high volume of incoming plant stock yearly. Phytophthora pathogens cause some of the most commonly found diseases in UK gardens. As part of the Euphresco 'Phyto-gard' project, involving ten international partners from six countries, we are trying to establish which are the main Phytophthora spp. found in selected UK public gardens, their spread patterns and whether their presence is linked to incoming plants. All partners in the project collect and analyse samples with the same set of shared protocols. The two UK public garden sites were located in two different geographical areas: northwest and southeast of England. Soil, water and plant samples were collected in early spring and summer, autumn and late winter from each garden, their nurseries and their plant quarantine areas. Soil samples were collected in areas with previously known Phytophthora cases or around declining host plants or important garden specimens. Water samples were collected from puddles across the garden sites, lakes and other water features. In the quarantine areas, samples were collected either as flow-through water from stock plants or as roots from the upper root ball of large specimens. Sample analysis consisted of DNA extraction from the samples and nested PCR to verify the presence (or absence) of Phytophthora DNA. All Phytophthora-positive samples were processed using an established metabarcoding approach involving Illumina sequencing (Green et al. 2021). The results will be used to understand whether incoming plants act as sources of introduction of Phytophthora, to map Phytophthora diseases in public gardens, improve the management of regulated species and enhance the biosecurity practices and operations in garden nurseries and gardens. The 'Phyto-gard' project will also enable a shared understanding of the impact of Phytophthora spp. in horticulture across different countries.

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9.15 am

Indirect impacts of fire and sudden oak death, caused by *Phytophthora ramorum*, on understory plant community structure

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Sam Balthazard and Richard Cobb

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The rapidly emerging disease sudden oak death caused by the non-native pathogen *Phytophthora ramorum* is fundamentally changing coastal California forests through severe tree mortality. We do not yet understand the full ecological implications of losing foundational tree species in these forests, which make up a significant part of one of the world's Biodiversity Hotspots. Furthermore, climate change, human development, and modern fire behavior interact with and may exacerbate the severity of disease impacts, especially through interactions with wildfire. Here, we leverage 20 years of vegetation dynamics and disease data in the highly diverse Big Sur region to determine how sudden oak death impacts plant community composition, diversity, and change in response to disease and wildfire. We found that conditions associated with the disease, including vigorous tanoak resprouting, dense Ceanothus cover, and redwood stand pureness, correlate with lower alpha diversity. We look further into causal relationships between disease and community change to find strategies to manage for ecological resilience.

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9.30 am

Endophytic fungal communities in tanoak leaves associated with *Phytophthora ramorum* infections in southwestern Oregon

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Tanoak (*Notholithocarpus densiflorus*) is a foundation species in the sclerophyll forests but is now threatened by Sudden oak death (SOD) in southwest Oregon and northern California. *Phytophthora ramorum*, the invasive pathogen that causes SOD, is spread by sporangia and zoospores. Infections are initiated in the canopy, eventually leading to bole cankers that kill mature tanoak trees. Most SOD research focuses on management or ecosystem impacts. However very little is known about what happens in tanoak canopies following infection, in particular impacts on the foliar fungal community of infected trees. To understand how SOD infections relate to the foliar microbiomes, and the endophytic community's assembly over the growing season at different canopy positions, 32 trees at 3 different sites in southwest Oregon were selected and sampled in July and November 2022. Tree canopies were measured and divided into 8 canopy segments and four cardinal directions. For each tree and each canopy segment, 10 leaves were sampled by punching out disks from both newly emerging leaves (n = 5) and mature leaves (n = 5). We labelled the sampled leaves in July and repeated collection in November when the growing season ended. Four leaf discs from each sample were selected for DNA extraction and ITS1 barcoding. From the sequenced samples 898 (July) and 750 (November) amplicon sequence variants (ASVs) were identified to the genus level and used for community analysis. Foliar fungal communities were different between sampling season time ($p < 0.001$), while there were no differences among healthy, suspect, and symptomatic trees ($p = 0.23$ and 0.07 for mature and young leaves, respectively). The microbial communities were different among canopy segments, ($p < 0.001$), and position within the crown ($p < 0.001$). Although, the foliar microbiomes of mature leaves were different between north and south facing aspects ($p= 0.006$), aspect had no effect on the microbiomes of young leaves ($p=0.09$). The most dramatic effect on fungal communities were the differences between young and mature leaves. *Curvibasidium* spp. were the most abundant within newly emerged leaves.

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9.45 am

Sudden Oak Death: EU1 and NA2 outbreaks of *Phytophthora ramorum* in Oregon forests

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Phytophthora ramorum, the cause of Sudden Oak Death, is an invasive pathogen in California and Oregon in the US and in several countries in Europe. There are four lineages of *P. ramorum* in the US and Europe (NA1, NA2, EU1, EU2) causing invasive forest infestations. In Oregon, tanoak (*Notholithocarpus densiflorus*) is the most susceptible species and the main driver of this disease. Forest infestations in Oregon were all caused by the NA1 lineage until 2015, when the EU1 lineage was discovered in wildland forests. In 2021, the NA2 lineage was discovered in Port Orford, Oregon infecting wild tanoak trees. This was the first report of the NA2 lineage in wildland forests. Infected tanoak trees develop lethal stem cankers and sporulate from infected leaves and branches. A variety of greenhouse and field experiments were conducted in order to compare the epidemiology of EU1 and NA1 lineages in Oregon forests and to determine the relative virulence of the NA2 lineage compared to the other lineages of *P. ramorum*. The accumulated evidence indicates that the EU1 lineage is able to infect more tree species and at a higher rate than NA1 with a potentially has higher sporulation under field conditions. In greenhouse experiments, on average, the NA2 inoculated trees had the longest lesions. Overall, the NA2 lineage has the potential to cause larger lesions than the NA1 and EU1 lineages and cause more severe disease on tanoak. This indicates that EU1 poses a greater risk to Oregon forests than the NA1 lineage and that the NA2 lineage has the potential to cause more severe disease than the NA1 and EU1 lineages which could pose even greater consequences for Oregon forests.

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10.00 am

Exploring viromes of *Phytophthora pseudosyringae* and *Phytophthora ramorum*

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Viruses are considered to be the most prevalent biological entities on Earth, found within almost all living organisms, including fungi and oomycetes. Although seemingly common in oomycetes, there are many unknowns about their origin, mechanisms of spread, interactions with, or possible effects on their hosts. *Phytophthora* is a genus of globally distributed oomycetes of major significance, with species capable of infecting a wide range of plant species, causing extensive damage in agriculture and forestry as well as in both aquatic and terrestrial natural ecosystems. In the past decade RNA seq technologies have enabled an expansion in detection of viruses, however there is still a considerable disparity between the knowledge of fungal and oomycete viral communities. The extent to which being a native or introduced pathogen influences the viral community is currently unknown. By comparing the viromes of native and introduced invasive pathogens that share a similar ecological niche, such as *P. pseudosyringae* and *P. ramorum*, we aim to explore host-virus relationships, how these have the potential to influence disease epidemics and inform future scoping studies for viral biological control agents. Additionally, the extent to which individual pathogen populations and geographic origins shape their viral communities are investigated.

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10.15 am

Can native plants and their microbiomes create a disease-suppressive environment for kauri? A long-term field trial in Waipoua Forest.

Monica Gerth

Monica Summers[1], Su Min Yeoh[1], Julie Deslippe[1], Ashely Davenport[2], Saphire Davenport[2], Libby Nathan[2], Hone Hoaia[2], Conrad Marsh[2], Taoho Patuawa[2], Chris Pairama[3], Ian Mitchell[4] and Monica Gerth[1]

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Plants naturally produce chemicals to defend themselves from pathogens and pests. Plant-associated microbes are also known to influence plant health, for example, by enhancing growth and/or increasing tolerance to disease. Combined, these natural defences could provide a holistic approach to controlling plant diseases in native ecosystems and/or agriculture.

In this project, we are exploring the impacts of selected native plants when used as ‘companion plants’ for kauri. Our hypothesis is that, in the long term, these plants and their associated microbiomes will establish a disease-suppressive environment, leading to improved resistance to kauri dieback disease (caused by the pathogen Phytophthora agathidicida).

To test this hypothesis, we have established a field trial in Waipoua Forest. Two companion plant species, karamū (Coprosma robusta) and māpou (Myrsine australis), were planted in different combinations with 4-year-old kauri saplings. Our previous research has shown that both karamū and māpou produce chemicals that inhibit P. agathidicida in vitro. Our field trial includes sites where P. agathidicida is known to be present and sites where it is assumed absent (based on the presence/absence of symptoms and/or soil testing).

Since the start of the field trial in 2021, we have been monitoring kauri growth and survival across these sites, as well as studying the microbial communities that have naturally established on the kauri companion plants. Though this trial is ongoing, I will present some of our preliminary data, which shows that these plants establish distinct microbial communities, and these communities contain beneficial microbes that inhibit P. agathidicida. These results support the continued exploration of companion plants and their microbiomes as part of a holistic approach to kauri forest health.

10.30 – 11.00 am Morning Tea

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Session 6 – Detection

Session Chair: Dr Iryna Matsiakh, Swedish University of Agricultural Sciences

11.00 am

Detection of viable *Phytophthora* and other oomycetes from forest nursery soils using eRNA and its potential use a diagnostic tool

Rebecca McDougal

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High-throughput DNA sequencing (HTS) of environmental RNA (eRNA) can provide information on soil communities that differ from those observed with environmental DNA (eDNA). RNA has a very short half-life compared to DNA and is found in living and active organisms, whereas DNA can persist in environmental samples long after an organism has died. In this way, eRNA gives a snapshot of the living organisms in an environment which may be more biologically relevant than relying on eDNA. In a warming climate it has been predicted that fungal pathogens will increase in prevalence, including soil-borne pathogens. Detection of soilborne pathogens is hampered by inefficient and often ineffective diagnostics which rely heavily on soil baiting, can take weeks to perform, and do not provide an accurate diagnosis when unculturable organisms are involved. These essential tools underpin current and future biosecurity systems. In this study comparative eDNA/eRNA HTS approach was employed to determine the diversity and viability of *Phytophthora* species and other oomycetes following fumigation of a *Pinus radiata* nursery bed. These results were compared to those from soil baiting. We found that the detected oomycete populations differed between eRNA, eDNA and soil baiting. Notably, some *Phytophthora* were detected in the eRNA samples that were not detected in the eDNA. In addition, this study revealed that soil fumigation was not completely effective at eliminating oomycetes from nursery soil. This work demonstrated the utility of eRNA for identifying viable oomycetes in environmental samples. This will be expanded upon to develop new and much needed tools for soil diagnostics. Innovative new tools will contribute to early detection and improved risk assessment for pathogens leading to better outcomes for healthier forests.

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11.15 am
A Comparison of Methods for Quantifying *Phytophthora lateralis* in Soil

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Port-Orford-cedar (*Chamaecyparis lawsoniana*) is an ecologically important tree in Oregon and California mixed-conifer forests. It is also globally utilized as an ornamental plant and for its timber. In the 1920's, the non-native root-rot pathogen, *Phytophthora* lateralis, was introduced to the native range of Port-Orford-cedar, dealing a devastating blow to this ecologically important species. *P. lateralis* is largely a soil- and water-borne pathogen, spreading through riparian environments and along roadways (Zobel et al 1985). Motile zoospores in soil infect roots of susceptible trees and girdle the phloem at the root collar. Little is known about pathogen density in infested soil and how it can be influenced by various environmental factors. The development of *P. lateralis*- resistant lines of Port-Orford-cedar (Sniezko et al. 2000) raises additional questions regarding what happens to pathogen population dynamics in the soil once there is no longer a susceptible host. Culture-based methods of measuring pathogen density are simple and inexpensive but have yet to be tested rigorously in the *P. lateralis* system to develop relationships between inoculum concentration and disease. In recent years, molecular tools have been developed that effectively detect the presence of *P. lateralis* (Feau et al 2019), but these methods have not been evaluated for quantifying pathogen density in soil and cannot differentiate between viable inoculum and pathogen DNA. In this study we compare culture-based and molecular methods with the aim of developing a standardized protocol that can be used to examine *P. lateralis* dynamics in soil. With many more soilborne *Phytophthora* species in need of management, similar methods could be applied.

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11.30 am
Developing and validating a method for the direct detection of *Phytophthora agathidicida* from soils

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Jade Palmer, Leticia Castro, Jochem Vink and Monica Gerth

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Phytophthora agathidicida is the causative agent of one of New Zealand's most infamous plant diseases; kauri dieback. Despite the devastating impacts of kauri dieback, its management has been hampered by a lack of rapid and cost-effective diagnostic tools. The primary method in use today relies on a combination of soil baiting and loop-mediated isothermal amplification (LAMP). While the use of LAMP was an important breakthrough, the combined method still takes over 10 days. Also, due to the baiting step, there is currently no way of obtaining results directly from soils, meaning no quantitative data are available on pathogen load. My PhD research is focused on developing DNA-based diagnostics for the direct detection of *P. agathidicida* from soils. There are two key challenges to this approach: the difficulty in separating oospores from soil, and the difficulty in extracting DNA from the oospore stage of the *Phytophthora* lifecycle. Oospores are thick-walled "survival spores" found in contaminated soils; detection of viable oospores is the foundation of existing baiting methods, but lysis and DNA extraction from these spores has proven challenging. Here, I will present our results in optimising oospore separation and lysis, as well as PCR-based diagnostics. Our size-based separation of oospores away from bulk soil has allowed for increased soil to be sampled when compared to typical extraction protocols, while also improving PCR detection by removing other organisms' DNA and inhibitors. Our subsequent improved lysis protocol obtains a 99% lysis rate in pure oospore samples. While our separation and extraction protocols have been designed for *P. agathidicida*, they are thought to be easily adaptable to the spores of other species and sizes. Finally, we have developed a primer pair for PCR/qPCR that exclusively amplifies *P. agathidicida*, differentiating it from other known soil *Phytophthora* species, with a qPCR detection threshold of 1.2 fg genomic DNA. I will also present our progress toward validating these methods using naturally infected soil samples from kauri forests. Our overall goal is to provide communities and management agencies with a faster, quantitative, and more affordable method for detecting *P. agathidicida* in kauri forests.

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11.45am pm

Low-cost, novel high throughput field testing strategy for *Phytophthora agathidicida*

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Shamini Pushparajah and Marion Wood

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New Zealand kauri (*Agathis australis*) is a significant taonga species threatened by the root rot causing oomycete *Phytophthora* agathidicida (PA). Continuous monitoring and early detection through increased testing for new infections are crucial for the protection of kauri for future generations. Conventional soil baiting assays commonly employed are labour-intensive, requiring transportation of infected soil samples to specified testing laboratories. Additional use of the commercially available, field deployable lateral flow stick (LFS), marketed as *Phytophthora* ImmunoStrips® (Agdia, IN, USA) fails to identify *Phytophthora* spp. to species level and we have shown does not readily work with soil samples directly. In this study we are developing and evaluating an alternative, cost-effective and timely novel method to increase soil testing throughput without the need to transport infected soil samples across the country. Whatman® qualitative filter paper, Grade 2 discs were used as the matrix to collect PA from both mycelial suspensions and soil slurry artificially infected with PA. DNA was extracted from the filter paper discs and the TaqMan assay preformed using the standard protocol of Than et al., 2013. TaqMan analyses demonstrated that as little as 5ng/uL of PA was readily detected and further attempts to improve sensitivity are currently underway. Co-development of this sampling strategy and ongoing transference of this knowledge to mana whenua strongly supports and empowers local communities to evaluate their ngahere (forest) directly. The ability to undertake intensive on-site testing and monitoring will continue to contribute significantly to effective disease management and biosecurity efforts. This work was funded by Biological Heritage's Ngā Rākau Taketake: Theme 5 Control Protect Cure led by Dave Milner (Kahu Environment) and Marion Wood (Plant and Food Research).

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12.00 pm

Phytophthora podocarpi, a recently described pathogen and its recent and historical association with Podocarpus spp. (tōtara)

Rebecca McDougal

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The tōtara needle blight pathogen, *Phytophthora podocarpi*, was first discovered in 2011, and was found in small stands of native remnants in commercial exotic forests. The disease has been found primarily on *Podocarpus totara*, but also *Podocarpus laetus* to a lesser extent and has been sporadic in appearance possibly due to climatic conditions. Due to the recent discovery of this pathogen, very little is known about its impact on tōtara as a host, and it has not been found associated with other hosts. The genome of the pathogen was sequenced and is thought to have an ancestral relationship to the downy mildews, a biotrophic class of oomycete plant pathogen distinct but related to *Phytophthora* species. The pathogen was officially described as a new species in 2022, an important step toward raising awareness and understanding more about this disease. Investigating the history of *P. podocarpi* may help us to understand if it is native or introduced, and therefore its potential as a threat to tōtara in natural forests, as well as tōtara as a forest species for wood products of distinct value for New Zealand. The National Forestry Mycological Herbarium collection at Scion holds historical plant-fungal specimens. Tōtara specimens of interest, were identified by visual examination, and dated back to 1950s. These samples are being analysed for the presence of *P. podocarpi* prioritising analysis for certain locations around New Zealand. Development of a new qPCR assay for the specific detection of *P. podocarpi* differentiates this *Phytophthora* species from other species impacting New Zealand forests and will not only support detection of the pathogen in the herbarium samples but will also support Scion's Forest Health diagnostic service. Partnership with mana whenua from an affected region has provided the opportunity to visit a forest location, and to raise awareness of this disease which is critical for protection of tōtara. The identification of *Phytophthora podocarpi* from the herbarium specimens will help us to understand how long this pathogen has been associated with tōtara in New Zealand. Further DNA sequencing will be required to determine the genetic diversity in the population to provide important information on whether this pathogen is native or introduced.

12.15 – 1.30 pm Lunch

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Session 7: Management

Session Chair: Stephen Whisson

1.30 pm
Alternatives to copper oxide for control of *Phytophthora pluvialis*

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Red needle cast (RNC) disease of *Pinus radiata* is caused by *Phytophthora pluvialis* which has been present in Aotearoa New Zealand since at least the mid-2000's[1]. Recent research has shown that copper is a promising treatment against RNC in *Pinus radiata*[2]. The long-term outlook regarding the use of copper in foliar disease management is uncertain due to environmental impacts as well as forecasted material scarcity. The Forest Stewardship Council (FSC) has highlighted the toxic effects of large quantities and prolonged use of copper on soil and aquatic ecosystems. Additionally, copper has been identified as one of eight geologically scarce metals[3]. These concerns prompted the need to look at alternative chemicals, including metal salts, as control tools to replace copper. The disease-controlling properties of the copper salt Cu^{2+} are attributed to nonspecific inhibition of enzymes and denaturation of proteins by copper ions that penetrate the cell and react with thiol or amino groups. Other types of metal salts may share these same properties, though any suitable substitute for copper salt Cu^{2+} would have to be similarly inexpensive and have lower environmental toxicity. To understand the impact of alternative chemicals against RNC, this research builds on a 2023 trial which tested 14 metal salts against copper oxide for the treatment of *Dothistroma septosporum*. This project will focus on the top-performing alternative treatments, investigating overall efficacy of treatments to reduce *P. pluvialis* growth, and necessary label rate (0.5x, 1x, or 2x) to reach parity with copper oxide. The results of this trial will allow land managers to make more informed decisions with regards to foliar disease management in their plantation stands.

References: [1]Dick, M. A., Williams, N. M., et al. 2014. Pathogenicity of *Phytophthora pluvialis* to *Pinus radiata* and its relation with red needle cast disease in New Zealand. N. Z. J. For. Sci. 44:6.
[2]Rolando, C., Somchit, C., et al. 2019. Can Copper Be Used to Treat Foliar *Phytophthora* Infections in *Pinus radiata*? Plant Dis. 103:1828–1834.
[3]Henckens, M. L. C. M., Driessen, P. P. J., and Worrell, E. 2014. Metal scarcity and sustainability, analyzing the necessity to reduce the extraction of scarce metals. Resour. Conserv. Recycl. 93:1–8.

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1.45 pm

Widespread dieback and mortality of wild olive trees involving multiple *Phytophthora* taxa and implementation of management and control strategies

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Wild olive represents one of the most iconic woody plant species in the Mediterranean basin. It is a slow-growing evergreen tree characteristic of the sclerophyll communities, extremely tolerant to drought, salinity and diseases, thus commonly used as rootstock for grafting cultivated olive varieties. Since 2022, a severe and widespread dieback and mortality of wild olive trees has been observed in central Sardinia (Italy). Affected plants showed leaf chlorosis, wilting, defoliation of the whole crown, shoot blight and epicormic shoots, frequently associated with root rot and necrosis on the feeder roots, suggesting a possible involvement of soil-borne pathogens in the genus *Phytophthora*. Preliminary studies revealed the occurrence of *P. bilorbang* and *P. pseudocryptogea*, and their pathogenicity on wild olive seedlings has been proven. Subsequent, more in-depth research revealed that the aetiology of this epidemic phenomenon was more complex with several other *Phytophthora* taxa involved. Using the baiting technique, eleven *Phytophthora* species belonging to three distinct phylogenetic clades (2, 6 and 8), were isolated from 60% of the 91 rhizosphere soil samples collected around both symptomatic and asymptomatic wild olive trees, and two additional *Phillyrea latifolia* and *Myrtus communis* plants. The identified species included *P. asparagi, P. bilorbang, P. crassamura, P. cryptogea, P. inundata, P. kelmanii, P. oleae, P. pseudocryptogea, P. sansomeana, P. syringae* and a yet undescribed taxon here named *P. oreophila*-like. To confirm Koch's postulates pathogenicity trials were conducted on 1-year-old wild olive potted trees using the soil-infestation method. Remote sensing and field data were used for modelling and predicting the spatio-temporal spread of tree declines and mortality. Treatments with different fungicides, biostimulants and microbial formulations were undertaken in the field to evaluate their efficacy to reduce the impact of *Phytophthora* root infection. Preliminary results are herein discussed.

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2.00 pm
Targeting soil *Phytophthora* to protect native trees and plantation forests

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Gayan Heruka De Zoysa and Viji Sarojini

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Highly virulent soil-borne *Phytophthora* cause catastrophic damage to New Zealand's native trees and planation forests. The radiata pine, which makes up 90% of plantation forestry valued at $5 billion and New Zealand's iconic kauri forests are in significant danger because of *Phytophthora* caused diseases.[1] Our interdisciplinary research is focused on developing environmentally friendly solutions to eradicate aggressive *Phytophthora* pathogens.[2] One particularly challenging aspect of this is the eradication of oospores embedded in the soil and zoospores that are chemotactically attracted to susceptible roots causing infection of new hosts thus spreading the disease. Our recent results using novel compounds and formulations that target oospores and zoospores of *Phytophthora* will be presented in this talk.

References: [1]Weir, B. S.; et al, A taxonomic revision of *Phytophthora* Clade 5 including two new species, *Phytophthora* agathidicida and *P. cocois* Phytotaxa 2015, 205 (1), 21-38.
[2]De Zoysa, G. H.; Schwendenmann, L.; Waipara, N.; Sarojini, V., Evaluating the potential of environmentally friendly compounds to deactivate different life stages of *Phytophthora* species. Plant Pathology 2023, 72 (5), 912-923.

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2.45 pm
Can biochar be included in an integrated strategy to control *Quercus suber* decline?

Manuel Trindade

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Portugal has over one-third of the *Quercus suber* world area, a natural treasure threatened by a progressive decline, which has been witnessed during the last decades as an increasing tree mortality, and where *Phytophthora cinnamomi* is considered the main factor. Some research outputs on *P. cinnamomi* control have been published and, similarly to other crops and forest phytosanitary problems, integrating various control strategies could be a viable approach at present. Biochar has proven to be a product of great interest for application in agriculture due to its benefits in terms of soil chemical and physical properties. Evidence of its contribution to improve the phytosanitary status of plants/crops has also been reported in relation to some pathogens, including *P. cinnamomi*, namely inducing resistance in plants. Three biochars with different compositions were tested under greenhouse conditions: one prepared from Acacia sp., another from a mixture of woody plant species, and a third from macroalgae Oedogonium sp. Two different concentrations of each were used, 1% and 5% (v/v). The aim was to evaluate the effect on root rot disease caused by *P. cinnamomi* infection on young *Q. suber* plants. At the same time, under lab conditions, we also tested the effect of the three biochars, and their respective concentrations, on the growth of *P. cinnamomi* mycelium. Our observations allow us to conclude that biochar could be an option to include in an integrated strategy plan to control the root rot disease in cork oak caused by *P. cinnamomi*.

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3.00 pm

Impact of phosphite on the *Phytophthora* community and inoculum abundance in treated kauri (*Agathis australis*) in New Zealand native forests

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Phosphite treatments have been shown to significantly reduce the quantity of Phytophthora inoculum in the rhizosphere of the threatened keystone species *Agathis australis* (kauri). *Phytophthora* are causing declines in forests worldwide, and the chemical control treatment, phosphite, is increasingly being used to protect natural ecosystems from *Phytophthora* diseases. In New Zealand, the threatened, endemic keystone species, kauri (*Agathis australis*), is suffering from a dieback disease primarily caused by *Phytophthora agathidicida*. Phosphite is applied to kauri as a trunk injection and has been shown to improve symptoms (such as reducing the expansion of basal lesion bleeds). We investigated the *Phytophthora* species assemblages and inoculum abundance in response to phosphite treatments in the field in two infected kauri stands at Huia and Waitoki. At Huia, phosphite was applied in 2012 as part of another study, and we conducted soil sampling and tree health surveying in November 2023. At Waitoki, soil sampling and tree health monitoring were conducted before a phosphite treatment in June 2022 and again 6 and 18 months post-treatment. *Phytophthora* species were detected using soil baiting and DNA metabarcoding of soil eDNA. A multiplex qPCR assay was used to quantify the inoculum abundance of *P. agathidicida* and *Phytophthora* clade 7 in the roots and the rhizosphere. At both sites, three species were detected with soil baiting (*P. agathidicid*a, *P. cinnamomi*, and *P. multivora*) and an additional two species with metabarcoding (*P. pseudocryptogea*, and a *P. europaea*-like taxa). *Phytophthora cinnamomi* was the most abundant species, followed by *P. agathidicida*. These two species were more likely to occur together than by chance alone, and they were both associated with declining tree health (increased canopy thinning and poor root health). The abundance of *P. agathidicida* (and *Phytophthora* clade 7) inoculum was lower in the rhizosphere around the phosphite-treated trees compared to the untreated control trees after 1.5 years at Waitoki. The phosphite treatment at both sites reduced basal bleed expansion (around the circumference) and bleed activity. A reduction in *Phytophthora* inoculum load in the soil because of phosphite treatments would have a direct impact on the epidemiology of *Phytophthora* diseases. Further field trials at disease fronts could investigate if phosphite treatment barriers prevent the spread of inoculum from an infested site and protect uninfected areas.

3.15 pm

The environmental and cultural impacts of Phytophthorain the Bunya Mountains

Adrian Bauwens

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3.30 – 4.00 pm Afternoon Tea

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Session 8: Surveillance 1

Session Chair: Dr Mireia Gomez-Gallego, INRAE

4.00 pm
Improved management of red needle cast through an integration of proximal and remote sensing with epidemiological modelling

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Red needle cast (RNC), primarily caused by *Phytophthora pluvialis*, is one of the most important diseases of *Pinus radiata* (radiata pine) in New Zealand. The disease causes reddening and premature cast of needles, leading to growth loss. The disease predominantly affects the lower crown but extends to the whole of the crown in severe cases. Disease expression generally begins in autumn, with disease severity developing rapidly and peaking in winter or spring. Remote sensing approaches are being used to investigate several aspects of RNC, including epidemiology, growth impacts, and control options, with an aim to support the development of management options. High-resolution imagery from fixed cameras, UAV, fixed-wing aircraft, and satellites is used to manually score field trials. Autonomous canopy sensor networks have been established to monitor temporal and spatial variation in microclimate in near real-time. Frameworks have also been developed for the use of high- and low-resolution satellite imagery to automatically map and monitor outbreaks of RNC and provide data to investigate climatic drivers of epidemics. Simultaneously, data on the environmental tolerances of the different pathogen life stages have been used to develop a process-based infection risk model, for which a range of remote sensing data will be used for calibration and validation. The benefits of this multi-disciplinary approach and the specific challenges of this system will be discussed.

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4.15 pm

The impact of *Phytophthora agathidicida* infection on kauri (*Agathis australis*) water relations

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Kauri (*Agathis australis*), an ancient giant tree species, holds substantial ecological and cultural significance in Aotearoa (New Zealand). Although slow-growing, mature kauri trees have large trunks with deep sapwood, providing substantial water storage capacity. This stem water supply, coupled with other mechanisms such as deep roots, consistently low stomatal conductance, and nocturnal transpiration, protects kauri trees from drought stress.

Kauri infected with *Phytophthora agathidicida* (kauri dieback disease) produce symptoms similar to drought stress, including leaf yellowing and defoliation, branch dieback, and slow tree mortality. Infection causes root rot, often accompanied by basal resinosis. Despite the mortality observed in relation to many *Phytophthora* diseases, the specific physiological process of death following infection remain poorly explored.

Our research employed a dual approach − *in-situ* observational and *ex-situ* experimental − to investigate the effects of *P. agathidicida* infection on kauri physiology, with a focus on water relations. We compared sap flow and canopy water relations between infected, non-infected, and artificially droughted kauri in two Auckland forests. The results show that sap flow in infected kauri is similar to droughted kauri, although stomatal conductance increased. Juvenile kauri were then inoculated with *P. agathidicida* and compared with non-infected control juveniles for water use, stomatal conductance, stem hydraulic conductance, and terminal branchlet water potential. Infected juveniles displayed root rot, which was associated with decreased water uptake, more negative branchlet water potentials, and increased stomatal conductance. These findings suggest that altered water relations following *P. agathidicida* infection contribute to kauri mortality.

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4.30 pm

The potential of remote sensing tools to detect early decline of kauri (*Agathis australis*) infected with *Phytophthora agathidicida* and the use of foliar phosphite to treat infection in the glasshouse

Matthew Arnet

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Phytophthora agathidicida is a cryptic pathogen that primarily infects kauri (*Agathis australis*) roots, causing kauri dieback. There is a very long latent period from infection to symptom expression. Current methods of detection involve collecting soil samples for baiting or DNA analysis of *Phytophthora* species, but confirming pathogen presence through soil testing and implementing management is typically only done on symptomatic trees. Determining infection at an earlier stage could improve the management of kauri dieback. This study aimed to determine if *P. agathidicida* infection could be detected using hyperspectral and thermal imaging in potted kauri saplings in a glasshouse, and if foliar phosphite application could be used as a method for controlling disease. Stomatal conductance, water use, hyperspectral and thermal imaging were all used to corroborate changes in plant physiology following infection by *P. agathidicida* and treatment with phosphite. Application of Foschek® with Du-Wett® resulted in a significant reduction in plant water use and stomatal conductance for the following three weeks. Further results will be discussed.

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4.45 pm
Taking positive action on forest health using airborne remote sensing and AI

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A wide range of abiotic and biotic disorders pose a serious threat to the viability and carbon sequestration potential of urban, plantation and native forests, and the biodiversity services they provide. Current ground-based methods for monitoring tree health/condition, and the impacting factors, are very time-consuming, expensive, and subjective in nature. Most forest managers do not have the resources and budgets available to undertake such monitoring on a regular (annual/biennial) basis. Managing the health of forests is essential if they are to achieve the ambitious targets being set for their growth and yield. Airborne remote sensing and Artificial Intelligence are revolutionising the approach to managing forest health, including biosecurity threats. These technologies enable the development of spectral vegetation indices at the individual tree crown and sub-crown level to rapidly measure baseline and change in tree condition at scale. We will present case studies detailing a combination of airborne remote sensing, AI and our forest pathology expertise, to provide early warning of the abiotic and biotic threats to urban, plantation and native forests, leading to targeted management strategies.

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5.00 pm

Integrating remote sensing information and environmental parameters to modelling *Phytophthora cinnamomi* disease risk at local scale

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Alien Invasive Forest *Phytophthora*s (AIFPs) are among the most relevant threats for evergreen oak and chestnut ecosystems in the Mediterranean basin. Among these AIFPs the most destructive agent of root and stem cankers, responsible of extensive decline and mortality of broadleaves species is *Phytophthora cinnamomi*. Nowadays in Italy, Spain, and Portugal, tree mortality associated with ink disease of chestnut and evergreen oak decline is mainly driven by this oomycete, causing relevant economic and ecological losses. The control of this disease in agroforestry systems is based on preventive and control measures that must be applied at both regional and local scale. Therefore, development of effective management strategies requires information on the spatial distribution of risk posed by the disease. In this context, the present study was developed with a double objective: (i) to identify the main factors of disease spread and; (ii) to develop a modelling tool to improve the accuracy of monitoring of this disease and the preparation of risk maps. As a result, an ensembled classification model was developed, generated from six machine-learning algorithms. Classification models were calibrated in an area located in Villanueva de Córdoba (Córdoba, Spain) where several foci of holm oak root rot with positive diagnosis of *P. cinnamomi* were identified and delimited. The selected response variable (risk of infection) was generated from the mortality observed in these foci between 2016 and 2022. Tree mortality was identified through comparison of aerial images from the Spanish National Plan of Aerial Orthophotography (PNOA) after segmentation process. Different topographic indices, distance to road and drainage networks, and vegetation indices obtained through spectral information from aerial images of the PNOA were used as predictor variables. Of these factors, distance to foci and topographic moisture indices (e.g., topographic wetness index and compound topographic index) were the most relevant factors in the model. The model was used to predict the spread of *P. cinnamomi* from the already located foci, showing a high level of success in the selected evaluation tests. This tool could help in focusing management strategies, providing effective control through better understanding of how the disease spreads over the host landscape. Moreover, the methodology developed could be effective at predicting the spatial spread of other Phytophthora species with similar dispersal mechanisms.

This talk will present results of the project LIFE-2021-SAP-CLIMA-101074466 Fagesos.

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5.15 pm

A structured approach to investigating the *Phytophthora agathidicida* outbreak in Auckland, New Zealand: Ongoing efforts to protect New Zealand's iconic kauri

Karyn Froud

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Kauri (*Agathis australis*) is an iconic northern Aotearoa New Zealand keystone species. Kauri are regarded as rākau rangatira (chiefly trees) and living tūpuna (ancestors) to Māori. *Phytophthora agathidicida* (PA), a soil-borne pathogen, causes severe dieback and death in kauri. Since 2020, Auckland Council has worked in partnership with the Mana Whenua in the Auckland Region. With Te Kawerau ā Maki investigating the distribution of PA within the Waitākere Ranges (Froud et al., 2022b) and with Ngāi Tai ki Tāmaki, Ngāti Tamaoho, Ngāti Te Ata, Ngāti Whanaunga, and Ngāti Tamaterā to assess pathogen freedom within the Hunua Ranges.

A nine-step structured outbreak investigation approach was followed: i) verifying the outbreak, ii) establishing a case definition, iii) identifying the population at risk, iv) identifying and counting cases, v) describing the outbreak in place and time, vi) developing and testing hypotheses on causes and controls, vii) implementing disease control interventions, viii) monitoring the response to interventions, and ix) revising interventions.

An emerging outbreak of kauri dieback was verified in 2006 as a significant risk to longterm kauri survival. A case definition was collaboratively developed with researchers and stakeholders for pathogen infected sites (PA sites) and kauri health (symptomatic) in relation to PA infection (basal lesions, canopy dieback or leaf colorisis).

Remote sensing identified the host population at risk. We undertook two ground surveys to identify and count cases, describe the outbreak in space and time and develop and test hypotheses on causes and controls. The Waitākere survey used a randomised cross-sectional study to describe the spatial distribution of PA; collect baseline kauri health and risk factor data for pathogen presence and disease development; and diagnostic sensitivity and specificity parameters for soil bioassay. In Hunua a hybrid risk-based and random survey design proved pathogen freedom and collected baseline kauri health data.

In Waitākere we found PA spread was discreet, and the risk of PA increased at lower elevations, with proximity to historic timber sites and coastal areas, and in denser regenerating or disturbed forest. Kauri dieback symptoms were significantly associated with PA. This raised the hypothesis of historical introduction from the coast and human-assisted movement of PA through logging and other disturbances.

The diagnostic sensitivity of the soil bioassay (63.2%) was used to assess confidence of PA freedom in Hunua at a prevalence of 1% or higher. Hunua had lower risk values than Waitākere, raising the hypothesis PA may be absent from Hunua due to lower historical risk exposure.

Disease control interventions of defending non-infected areas, hygiene, pest management and strategic track connections, closures and upgrades are continuing. Ongoing monitoring against baseline tree health for incidence will test the response to these interventions, and inform revisions to disease control measures over time.

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5.30 pm Daily summary and discussion

Session Chair: Andrea Vannini

Thursday 12th September

Session 9: Management 2

Session Chair: Simone Prospero

8.45 am

Toward resilient Mediterranean forests against *P. cinnamomi*: bridging the gap from laboratory studies and outcomes to practical application in the forest.

Carmen Morales-Rodríguez

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Phytophthora cinnamomi is a destructive pathogen that causes root rot of many plant species worldwide, including Mediterranean forests where it poses a significant threat to biodiversity and ecosystem health. *Phytophthora cinnamomi* impacts a wide range of plant species, among which many trees and shrubs native of Mediterranean forests. Some susceptible tree species include oak (Quercus spp.) and chestnut (*Castanea sativa*). Since the 90s researchers and forest managers in the Mediterranean region have been actively studying *P. cinnamomi* and developing management strategies to contrast its spread. This includes efforts to identify resistant plant species, improve soil health, and implement biosecurity measures to prevent further introductions and spread of the pathogen. In September 2022, the European 101074466 — LIFE21-CCA-IT-LIFE FAGESOS project was launched. The project has among its objectives the development of IPM protocols for the mitigation of damage caused by *P. cinnamomi in Quercus ilex, Q. suber,* and *C. sativa* as well as its large-scale application. By the end of the project, we expect to treat up to 1,070 ha (7 demonstrative sites) and to protect up to 18,119 ha of vulnerable areas in total. The transition from laboratory to fieldwork in managing forest plant pathogens, such as *P. cinnamomi* in forests presents several challenges such as environmental complexity; large-scale applications; access and logistics; availability of active molecules and regulation; collaboration and stakeholder engagement. Addressing these difficulties requires integrated approaches that consider the complexity of natural systems, promote collaboration between different stakeholders, encourage innovation and continuous adaptation, and strengthen the capacity of stakeholders and local communities to manage plant diseases effectively and sustainably. This work collects the experiences and problems that we have faced in this leap into reality.

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9.00 am

Field treatment with Brassica seed-based products efficiently controls *Phytophthora cinnamomi* in ink diseases in cork oak forest in Italy. Which products impact on the symbiome community?

Andrea Vannini

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Wajid Aurangzeb, Carmen Morales-Rodriguez, Leonardo Guidoni and Andrea Vannini

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The efficacy of biofumigation by *Brassica carinata* defatted seed meal in suppressing several soilborne pathogens including oomycetes has been demonstrated by several studies. However, specific studies employing different formulations and doses are needed to support the release of a standard protocol of use. In this study, the efficacy of two commercial products based on *B. carinata*, against *P. cinnamomi* and the impact on symbiome was explored in mesocosm and open-field experiments carried out in an ink-diseased cork oak forest in Central Italy. Dry pellets and a liquid formulation were first tested in mesocosm in natural soil added with *P. cinnamomi* inoculum demonstrating the higher efficacy of the latter. Further experiments carried out in mesocosm and open-field evidenced that the liquid formulation at a final concentration of 2% was able to completely kill the inoculum down to 15 cm deep. At the same concentration, the product was able to efficiently protect cork oak seedlings in artificial inoculation experiments. Finally, the cork oak symbiome was analysed in soils before and after the treatment with the liquid formulation. Preliminary results evidenced a negative effect in terms of CFUs on the fungal community and a positive effect on the bacterial one. HTS analysis is currently ongoing and will provide specific taxonomic and functional data of the symbiome before and after the treatment.

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9.15 am

Biocultural landscapes, indigenous communities and integrated knowledge systems - Te Roroa perspectives of forest health and PA management

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Biocultural landscapes refer to areas of land with generational human-nature interactions. These interactions allow for the development of knowledge systems based on the observations of the natural processes which occur within and how the collective human impact affect these processes and resources. Much of the landscape of Aotearoa New Zealand has changed over the last 200 years, with increasing human populations, advancements in technology, and the introduction of exotic plants, animals, and pathogens, among those with enduring, inter-generational impacts. This talk will briefly examine some of these impacts with a focus on the social implications of the management of the kauri pathogen, *Phytophthora agathidicida*, in the Waipoua Forest on the west coast of Te Taitokerau, Aotearoa New Zealand.

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9.30 am
Ten years of phosphite trials to control kauri dieback

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Phosphite (phosphorous acid) is a potential treatment for kauri (*Agathis australis*) trees infected or threatened by the kauri dieback pathogen *Phytophthora agathidicida*. Trials established in 2012 on ricker trees in four infected forests showed that phosphite (Agri-fos® 600) at concentrations of 7.5% or 20% injected (20 mL) at 20-cm intervals around the trunk resulted in healing of all basal trunk lesions, usually within one year of application. Ten years after treatment, all above-ground lesions in treated trees remained inactive, contrasting with untreated control trees where many lesions remained active, often resulting in trunk girdling and tree death. Phytotoxicity was noted in some trees, and severely diseased trees sometimes declined more rapidly following treatment, indicating that phosphite rates were too high. Trials established in 2016 using lower rates showed that on infected ricker trees, 4% phosphite, with 20 mL injected at 20- or 40-cm intervals, also effectively healed trunk lesions, without causing obvious phytotoxicity symptoms. However, control was observed to wane slightly after 6 years, particularly with the lowest rates. On large kauri trees (0.5 to 2.5 m trunk diameter), 4% phosphite injected at 40- or 80-cm intervals around the trunk was only partially effective at healing lesions, and some remained active. A second application after 2 years improved lesion healing, but for large trees higher doses may be required. Dose rates based solely on trunk circumference may not be appropriate for treating very large trees.

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Poster Talks

Session Chair: Fryni Drizou

9.45 am
Development of ecofriendly agents to sequentially target *Phytophthora* life stages (Poster Talk)

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Phytophthora are among the most notorious pathogens threatening natural, agricultural and forest ecosystems. In Aotearoa, around 30 different Phytophthora species have been isolated so far, including *Phytophthora agathidicida* causing kauri dieback disease and *Phytophthora pluvialis* responsible for the red needle cast of radiata pine trees. Phytophthora species have a complicated life cycle with multiple life stages such as mycelia (vegetative growth stage), zoospores (inoculation of new hosts) and oospores/chlamydospores (long-term survival) which are responsible for the invasion and spread of the pathogen, thus eradication of Phytophthora is notoriously difficult. The current chemical treatment methods for managing Phytophthora outbreaks include the use of antifungal agents such as phosphite (phosphorous acid, H_3PO_3), metalaxyl, fosetyl-Al, etc. However, their phytotoxicity and the risk of resistance development are some of the disadvantages associated with their extensive use. This warrants the development of novel anti-Phytophthora compounds that target multiple Phytophthora life stages. We have identified several synthetic compounds and natural products that target multiple life stages of Phytophthora pathogens. This poster will further highlight our ongoing research towards developing sustainable treatment options with improved activity against multiple life stages of Phytophthora pathogens.

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9.50 am

Investigating the impact of foliar phosphite (Foschek®) and Du-Wett® application on the water relations of kauri (*Agathis australis*) (Poster Talk)

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Phosphite is an essential tool for managing Phytophthora diseases around the world and is often applied by trunk injection or foliar spray. Kauri (*Agathis australis*) can be infected by *Phytophthora agathidicida* causing kauri dieback, which is usually fatal. Kauri can be treated by phosphite trunk injection to effectively manage the symptoms of kauri dieback but involves someone physically visiting and wounding the tree to administer the treatment. Foliar application of phosphite may provide a useful alternative for treating infected kauri without wounding or the need for ground-based disturbance, particularly in sites that are difficult to access. Results from a previous experiment showed that foliar application of 7.5 g/L Foschek® + 0.2% v/v Du-Wett® significantly reduced water use and stomatal conductance of kauri saplings but it was unclear whether this was caused by the Du-Wett® or phosphite. The current experiment aimed to investigate the leading cause and mechanism of reduced stomatal conductance following foliar application of Foschek® and Du-Wett® on kauri seedlings in the glasshouse. Seedlings were treated with foliar phosphite (Foschek®), Du-Wett®, phosphite + Du-Wett®, or distilled water. Stomatal conductance and water use were measured, as well as stomatal aperture. Results of this study will be discussed.

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9.55 am

Composting of biowaste infected by *Phytophthora cinnamomi*: study of pathogen survival comparing different diagnostic techniques (Poster Talk)

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Leonardo Guidoni, Andrea Vannini and Carmen Morales-Rodriguez

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Aerobic composting represents an important practice that supports the environmental sustainability of human activities by reducing the amount of organic waste sent to landfills, greenhouse gas emissions and land consumption. A high-quality compost enriches soil fertility, promotes plant growth, and serves as a valuable substitute for chemical fertilizers. However, if not produced correctly, compost can harbour harmful pathogens for both animals and plants, particularly when the stock material is infected. Common issues leading to unsuccessful composting include improper thermal phase, unbalanced C/N ratio, high moisture, low pH and anaerobic conditions. Wood chips are widely used for composting due to their ability to balance nutrient availability and ensure optimal conditions for aerobic microorganisms. Nurseries could represent a great source of woody material for composting but are known to harbour a significant amount of plant pathogens such as Phytophthora spp. which could be transmitted to compost and subsequently to the environment where compost is applied. Therefore, specific standards are needed to ensure the biosafety of the final product, especially when contaminated stock material is used. The study aims to assess the survival of *P. cinnamomi* inoculum throughout the entire composting process. *Castanea sativa* plants infected with *P. cinnamomi* were used as a source of woody chips. During composting, the presence of the pathogen was monitored using baiting technique and high-throughput sequencing (HTS). HTS analyses detected the presence of *P. cinnamomi* DNA only for the first 20 days of composting. However, negative baiting results demonstrated that the pathogen was immediately inactivated by a thermophilic phase of 2-3 days, with temperatures above 50°C. The present findings might help to solve the problem of pathogen transmission via compost by establishing minimum requirements in compost production to ensure the biosafety of the final product, even when using contaminated material.

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10.00 am

Global warming and *Phytophthora cinnamomi* invading Fagaceae ecosystems along an altitudinal gradient in the Mediterranean basin (Poster Talk)

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Global warming is generating significant changes in ecosystems, with particularly notable effects on species distribution along latitudinal and altitudinal gradients. The Monti Cimini forest area is characterized by a succession of Fagaceae ecosystems at an altitude range between 373 to 1054 meters above sea level. Deciduous oaks dominate at lower altitudes being substituted by sweet chestnut in an altitude range between 550 and 950 m and European beech at the highest altitudes. Ink disease represents one of the limiting factors for chestnut sustainability in the area. Surveys carried out in mid ’90 up to early 2000 evidenced that *Phytophthora x cambivora* was the main pathogen responsible. However, in the last 15 years, *P. cinnamomi* has been the prevalent species isolated, while *P. x cambivora* seemed to gradually disappear. The hypothesis is that global warming is favouring the establishment of *P. cinnamomi* at low and medium altitudes relegating *P. x cambivora* at higher altitudes where sweet chestnut meets European beech. In this work, we studied the changes of soilborne Phytophthora community along an altitudinal gradient in Fagaceae ecosystems of the Monti Cimini area using HTS analysis and classical baiting to demonstrate the above hypothesis

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10.05 am

Exploring *Phytophthora* community associated with severe decline of cork oak forests in Tunisia: distribution and potential impact (Poster Talk)

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Cork oak *(Quercus suber)* and African oak *(Q. canariensis)* forests in Tunisia cover an area of about 7600 ha in the northwest of the country. Over the last few years, a severe decline of these forests has been reported with symptomatology recalling the impact of Phytophthora diseases, including crown decline and extensive mortality mainly affecting cork oak. An intensive survey was carried out by an international team in April 2023 in the distribution range of cork and Algerian oak in Tunisia. Up to 50 soil samples were collected and baited. Twenty-three of them resulted positive for Phytophthora spp. Six Phytophthora spp. were isolated from samples throughout the whole distribution range of cork and Algerian oak. *Phytophthora cinnamomi* was the most represented species together with a taxon of the *P. cactorum* species complex. Other unusual Phytophthora species for oaks were also detected but with lower frequency. Pathogenicity tests in mesocosm on cork oak seedlings of Tunisian germplasm are currently ongoing.

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10.10 am

Monitoring ink disease epidemics in chestnut and cork oak forests in central Italy with remote sensing data (Poster Talk)

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Alessandro Sebastiani[1], Matteo Bertozzi[1], Andrea Vannini[2], Carmen Morales-Rodriguez[2] and Gaia Vaglio Laurin[1,2]

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Forests provide multiple ecosystem services including water and soil protection, biodiversity conservation, carbon sequestration, and recreation, which are crucial in sustaining human health and wellbeing. Global changes represent a serious threat to Mediterranean forests, and among known impacts, there is the spread of invasive pests and pathogens often boosted by climate change and human pressure. Remote sensing can provide support to forest health monitoring, which is crucial to contrast degradation and adopt mitigation strategies. Here, different multispectral and SAR data are used to detect the incidence of ink disease driven by *Phytophthora cinnamomi* in pure forest sites in central Italy, dominated by chestnut and cork oak respectively. Sentinel 1, Sentinel 2, and PlanetScope data, together with ground information, served as input in Random Forest to model healthy and disease classes in the two sites. The results indicate that healthy and symptomatic trees are clearly distinguished, whereas the discrimination among disease classes of different severity (moderate and severe damage) is less accurate. Furthermore, the crown dimension is important in the selection of the better sensor; better results are obtained for the larger chestnut crowns with Sentinel 2 data. In both sites, the red and near infra-red bands from multispectral data resulted well suited to monitor the spread of the ink disease.

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10.15 am

Citizen science and outreach: *Phytophthora ramorum* education in southern Oregon (Poster Talk)

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Sudden oak death (SOD), caused by a non-native pathogen *Phytophthora ramorum* has killed hundreds of thousands of tanoak (*Notholithocarpus densiflorus*) trees in Curry County Oregon since it was first detected in 2001. Despite efforts to slow the spread, the pathogen that causes SOD continues to spread in the moist and windy conditions of the southern Oregon coast. New detections of the European lineage (EU1) in 2015 and the North American lineage (NA2) in 2021 has increased the need for outreach education of local landowners about SOD and state quarantine regulations. Since 2018, Oregon State University Extension has collaborated with Oregon's SOD program and the OSU LeBoldus Lab on a coordinated citizen science and outreach program to teach local residents about disease recognition, early detection methods, and effective treatment options. Outreach and education sessions including in-person workshops and webinars have resulted in over 600 educational contacts. A citizen science project, initiated in 2019, focuses on early detection at the leading edge of the disease. Citizen scientists learn standard sampling protocol to set bucket and stream bait stations, collect, record and submit samples. Citizen scientist volunteers have submitted 456 samples since the project's initiation. 38 percent of the volunteers deployed samples over multiple project years. 27 samples were found positive for *Phytophthora* species. We will present project design, results and lessons learned.

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10.20 am

Improved qPCR sensitivity for *Phytophthora pluvialis* detection using a mitochondrial target (Poster Talk)

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Current detection of *Phytophthora pluvialis*, forest pathogen and causal agent of red needle cast (RNC) in *Pinus radiata*, relies on isolation and culture, baiting or PCR using single-copy gene targets with limited sensitivity at low levels of detection. To inform disease management, epidemiology and research objectives, a more sensitive species-specific molecular detection assay is necessary for the detection of *P. pluvialis* in forest samples. This study aimed to identify multiple-copy mitochondrial gene regions with the same or improved specificity and sensitivity than the currently available single-copy nuclear gene target, ras-related GTP-binding protein 1 (ypt1), for the detection of *P. pluvialis* in forest samples. We assessed six mitochondrial gene regions for suitability of real-time PCR (qPCR) primer design, developing candidate assays for each. Assays targeting mitochondrial cytochrome c oxidase subunit 2 (cox2) were developed the furthest based on success with in-vitro testing for sensitivity followed by specificity testing using DNA from related and unrelated species. These assays were then tested on DNA collected from infected plant material until a final candidate assay was selected and validated for successful diagnostic application. Despite the challenges of primer design targeting mitochondrial gene regions, specificity in the cox2 region was sufficient to allow the design of a sensitive, species-specific diagnostic qPCR assay. The resulting assay has a detection limit of 12.8 fg mycelial DNA and detected *P. pluvialis* DNA on average 6.12 qPCR cycles before the ypt1 assay. In forest samples, the cox2 assay was found to consistently detect *P. pluvialis* similarly ahead of the ypt1 target for all stages of needle disease symptoms from early lesion infection through to fully cast red needles. We also found that the increased sensitivity of this new assay allowed for the asymptomatic detection of *P. pluvialis* in *Pinus radiata* plantation forest which only began to develop visual symptoms of disease 4 weeks after positive detection by qPCR. The availability of a highly sensitive diagnostic assay has also enabled rapid and confident diagnostics for recent new detection of *P. pluvialis* in the United Kingdom. The assay has had demonstrated use on high-throughput scalable robotic diagnostic platforms and could also be applied to environmental (water) sampling, making it a useful tool for effective early detection of *P. pluvialis* and management of disease in plantation forests.

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10.25 pm
Evaluation of prescreening and monitoring methods using sequencing technologies for *Phytophthora* and oomycetes. (Poster Talk)

Guillaume Bilodeau

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Metabarcoding can provide insightful information on species diversity for surveillance of introduced species; while isolation and bating could underestimate species diversity, high throughput sequencing can be used for early detection of oomycetes. This is particularly true for *Phytophthora* species responsible for major plant diseases, whether or not they are regulated or listed as invasive alien species. For their detection, real-time qPCR is sensitive and specific but requires in-depth knowledge of the organism and is limited to a few species at a time. High throughput sequencing (HTS) technologies, however, allow us to investigate different types of samples, process large numbers of samples, and produce even greater amounts of genomic data. Metagenomics tools with different genetic regions and combining Ion Torrent and Oxford Nanopore sequencing and custom bioinformatic pipelines can be used to evaluate potential sampling methods for pathogens in forestry and agriculture and contribute to identifying spreading pathways. However, few *Phytophthora* monitoring schemes have been assessed to understand biomonitoring technology's limitations. Nevertheless, exploiting eDNA isolated from air, soil or tissue can be a powerful tool for revealing sources of oomycetes likely to be problematic. Consequently, we aim to provide a framework combining sampling tools with HTS methods, appropriate bioinformatics pipelines, and qPCR assays suitable for different sample types for the early detection of emerging and invasive alien species. Evaluation of those methods will help improve early warning, promote public awareness, and support regulatory activities.

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10.30 am

Use of high-throughput automated qPCR system for rapid diagnostic processing for detecting Phytophthora agathidicida from environmental samples

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Phytophthora agathidicida (PA), the causal agent of kauri dieback, threatens the long-term survival of kauri (*Agathis australis*) in Aotearoa. Currently, PA presence is typically confirmed via a soil test, which includes a soil baiting step. The protocol is a lengthy, labour-intensive, and costly. Additionally, several factors can affect the accuracy of this test, resulting in false negative and/or false positive detection. These factors include the presence of other oomycetes, in particular *P. cinnamomi*, which has been shown to mask the detection of PA. Several molecular techniques are being explored in Aotearoa, for PA diagnostics. These include a PCR-based technique that can detect PA directly from soil and soil water and qPCR assays for detection of PA and a Loop-Mediated Isothermal Amplification (LAMP) assay that can detect PA from soil baits. These molecular techniques can be costly, time consuming and are limited by the number of samples they can process at any one time. Automation of PCR-based assays could substantially speed up diagnostics from soil baiting for PA. Slipstream Automation provides both robotic, higher-throughput and improved consistency in DNA extractions and qPCR as well as being an automated service. The aim of this project was to test the possibility of using this high-throughput system as a viable, cost effective and efficient method for testing large numbers of samples for PA detection. We used two qPCR assays to detect PA in infected soil baits as a model system. In addition, we assessed the effect P. cinnamomi has on the detection of PA using the same qPCR assays. Initial results are promising, and with further optimisation of the qPCR's, Slipstream automation's, automated high-throughput qPCR system could become a more reliable, cost effective and efficient way of testing soils for PA compared to current techniques.

10.35 – 11.00 am Morning Tea

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Session 10: Molecular pathology

Session Chair: Carmen Morales-Rodríguez

11.00 0m
Discovery and processing of pathogenicity effectors in Phytophthora

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Phytophthora and other oomycete plant pathogens cause diseases that threaten both food security and natural environments globally. Disease development is driven by secreted effector proteins that can act outside plant cells (apoplastic effectors) or be translocated inside plant cells (cytoplasmic effectors) to exert their action. Cytoplasmic effectors include those that possess an N-terminal RXLR (arginine-any amino acid-leucine-arginine) peptide motif, often followed by an EER (glutamic acid-glutamic acid-arginine) motif. The mechanisms involved in RXLR effector secretion and translocation are not fully resolved. To study these processes, we have used the potato and tomato late blight pathogen *Phytophthora infestans* as a tractable model. Using tagged RXLR effectors expressed in transformed *P. infestans*, we have found that the RXLR class of translocated effectors undergoes multiple proteolytic cleavage events before secretion and translocation into plant cells. While much research has focused on the roles of RXLR effectors in disease, less is known about the functions of the diverse array of other secreted Phytophthora proteins. To reveal new candidate apoplastic effectors that may have enzymatic activity, we have used a combination of plant cell culture infections, proteomics, and transcriptomics. Silencing of the genes encoding these effectors in *P. infestans* has demonstrated their importance for successful infection, and tagging with fluorescent proteins has revealed when and where they are localized during infection. Structural modelling in AlphaFold, and other bioinformatic tools, are being used to generate hypotheses about the function of candidate effectors.

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11.15 am

Unravelling the role of *Phytophthora pluvialis* RxLR effector proteins during pine needle infection

Claudia Meisrimler

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Sophie Eccersall[1], Leann Vinson[1], Hazel McLellan[6], Preeti Panda[3], Michael Currie[1], Rebecca McDougal[4], Ren Dobson[1,2], Paul Birch[5,6] and Claudia Meisrimler[1,2]

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Phytophthora pluvialis, the causative agent of red needle cast, is a destructive foliar disease of pine. Like other oomycetes, it utilizes host-translocated effector proteins to promote successful infection. For the elucidation of differentially expressed effector genes associated with *P. pluvialis* infection, we used quantitative RNA sequencing to compare mycelium and pine needles of susceptible and resistant pine lines infected with *P. pluvialis* at 3 and 5 days post-infection. Overall, 311 effector genes were identified as differentially expressed (136 Crinklers, 115 RxLRs, and 60 Elicitins). Further downstream analysis focused on RxLR effectors differentially expressed in susceptible and resistant pine lines at 3 days post-infection. RxLR effectors are host-translocated effector proteins that play crucial roles in facilitating infection and suppressing host plant defense mechanisms. First, we employed a complementary computational approach to acquire an understanding of protein sequence and structural conservation, visualized as networks and phylogenetic trees. Structural modeling showed that more than 90% of the early expressed RxLR effectors contained at least one WY motif. Transient expression in *N. benthamiana* for eight selected candidates showed diverse localization in planta. Successful protein expression was confirmed by Western blotting. Furthermore, cell death and cell death suppression assays have been completed for all RxLR effector proteins. Identification of effector targets was pursued by a Yeast-2-Hybrid approach using a well-established *Phytophthora infestans* potato library and a novel *P. pluvialis*-pine library. Identified targets have been further analyzed with computational approaches, followed by effector-target co-localization by transient expression in *N. benthamiana*. Three of the effector candidates, PpR03, PpR06, and PpR07, were successfully expressed in *E. coli*. Purified proteins have been characterized using size exclusion chromatography, analytical ultracentrifugation, and bio-SAXS. Results confirmed that PpR03 forms dimers, whereas PpR06 and PpR07 predominantly exist as monomers. We will further discuss the importance of RxLR effectors in plant-oomycete interactions, targeting host proteins to modulate cellular processes, thereby determining the outcome of the infection and contributing significantly to the virulence of the pathogen.

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11.30 am

Moving away from model hosts: understanding the role of effectors in the kauri dieback pathosystem

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Phytophthora agathidicida is the causative agent of kauri dieback, a destructive disease threatening kauri endemic to the northernmost regions of New Zealand. Relatively little is understood of this pathogen and its interaction with kauri, but advances in the understanding of other *Phytophthora* pathogens, and the completion of a detailed *P. agathidicida* genome sequence, now allow for this interaction to be studied in greater detail. Core components of any pathogen-plant pathosystem are effectors, which are molecules released by the pathogen during infection that promote virulence. A well-characterised type of effector from *Phytophthora* are glycoside hydrolases (GHs), a broad group of extracellular effectors including XEG1 from the soybean pathogen *Phytophthora sojae*. In the model host *Nicotiana benthamiana*, XEG1 is recognised by a receptor-like protein (RLP) that associates with the co-receptor SOBIR1 to begin the signalling cascade needed for plant immunity. In line with this, the presence of SOBIR1 strongly impedes the ability of P. agathidicida to grow on *N. benthamiana*. This suggests that RLPs may play a critical role in immunity against kauri dieback disease that could involve recognition of the XEG1 homolog from *P. agathidicida*. In this study, we aimed to characterise the role of XEG1 in kauri dieback, to determine if the same immunity observed in *N. benthamiana* can also be found in kauri and how this influences the host-pathogen interaction. Kauri from families with differing levels of tolerance to kauri dieback were kindly provided by Te Roroa, with the requirement that XEG1 tested in kauri leaves was purified protein. Experiments were first conducted in *N. benthamiana* to confirm that the *P. agathidicida* XEG1 protein elicits a plant defence response in the form of localised cell death and that this response is dependent on the SOBIR1 co-receptor. The purified protein was then infiltrated into kauri leaves where it caused a similar localised cell death defence response as was seen in *N. benthamiana*. The next step involves assessing how the XEG1 protein influences the ability of *P. agathidicida* to infect kauri by inoculating protein-infiltrated leaf tissue with the pathogen and determining if there are differences in responses between the kauri families. Whilst this work is ongoing, the identification of effector-specific RLP immune receptors in kauri could potentially provide a screening tool for kauri dieback resistance. If different responses are seen between different kauri families, it may indicate that some have more efficient variants of those RLP immune receptors.

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11.45 am

Defining the role of Crinkler effectors on the infection process of *Phytophthora cinnamomi*

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Phytophthora cinnamomi is a highly destructive plant pathogen causing significant damage to an impressively wide range of plant species (e.g., avocado, pine, lettuce). *P. cinnamomi*, like other plant pathogens, secrete effector proteins including RxLRs and Crinklers (CRNs), which enhances their infection potential in diverse aspects. CRN effectors were originally identified from *P. infestans* and are known to be responsible for inducing leaf crinkling, necrosis in plant cells, and suppression of plant defence mechanisms. Several studies report the importance of CRN effectors as crucial candidates for the infection process of *P. cinnamomi* . However, to date, no studies have been conducted on the structural and functional characterization of *P. cinnamomi* CRNs. Therefore, this study attempts to identify the structural and functional characteristics of *P. cinnamomi* CRNs that will assist in unravelling the virulence roles of these effectors. CRN sequences reported from the most recently published *P. cinnamomi* genome in 2021 from South Africa were analysed using online prediction tools including SignalP, LOCALIZER, EffectorP, and InterProscan5. Accordingly, out of the forty-eight CRNs reported, concerning the presence of a signal peptide, the Crinkler effector protein N-terminal domain, and the conserved LFLAK domain, seven were selected for the downstream analysis. Initially, a computational pipeline was adopted to study the sequence and structure conservation of the CRNs of interest. Similarly, the tertiary structures of the protein were analysed using AlphaFold2 and structural similarities were determined using the DALI server. The transient expression of the selected seven CRNs in *N. benthamiana* using N- and C- terminal fluorescent tags indicated their localization in the nucleus and/or plasma membrane. Western blotting confirmed successful expression in planta and the stability of the proteins. Further, cell death assays and infection assays were performed for these CRNs to gain further insight into their infection mechanisms. The 9.5kDa, PcCRN4 was further characterized and analysed for its structural details and host protein targets. Computational prediction tools suggest the occurrence of N- and O-type glycosylation sites and phosphorylation sites in PcCRN4 along with two cysteines producing a disulphide bond. Combining computational approaches, with cell biology, biochemistry and phytopathology allowed us to gain new insights on CRN effectors and their function during host infection. This knowledge will be crucial for rapidly implementing effective control measures to reduce the impact of *P. cinnamomi* and thereby protect agriculture, forestry, and ecological equilibrium.

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12.00 am

Comparing gene expression and genomic features among lineages of *Phytophthora ramorum*

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Three decades have passed since the plant pathogen *Phytophthora ramorum* became established in forests on the west coast of the United States. Since then, a variety of management strategies have been attempted in an effort to control a series of isolated introductions in wildland forests. Though *P. ramorum* has a broad host range, the epidemic in Oregon is mainly driven by tanoak (*Notholithocarpus densiflorus*), a co-dominant to dominant tree in coastal forests that is culturally important and a key source of forage for mammals. Spread is dominated by airborne asexual sporangia. Although the main mode of reproduction is asexual, cryptic genetic diversity has been observed. The mechanisms leading to this diversity may include mitotic recombination and host-induced genomic modification. Large regions of the *P. ramorum* genome can be completely homozygous, and patterns of these runs of homozygosity (ROH) vary among isolates and lineages. Whole genome Illumina sequences of isolates belonging to the NA1, NA2, and EU1 lineages from Oregon forests, nurseries in the U.S. and Canada, and the NCBI Sequence Read Archive database were mapped to the NA1 (PR-102_v4) and EU1 (PR-15-019) reference genomes. SNPs were called to infer ROH. ROH that were not conserved among lineages made up a larger proportion of the NA1 genomes compared to the other two lineages. The NA1 genomes were also the most variable with respect to ROH content. Isolates with a higher percentage of non-conserved ROH had a slower-growing phenotype and produced fewer spores compared to other isolates. To further compare NA1 and EU1, given their different phenotypes and genomic features, genes differentially expressed by *P. ramorum* both during infection of tanoak and in vitro were identified using RNAseq. mRNA was isolated from tanoak branches infected by an NA1 or EU1 isolate at 2, 7, and 14 days-post-inoculation. Negative binomial generalized linear models were used to identify genes differentially expressed over time and between lineages. At each time point and condition, genes encoding for XRCC4, a protein implicated in DNA double-strand break repair, were among the top three genes with the highest log fold change in NA1 versus EU1. The function of this protein in relation to genomic feature patterns unique to lineages will be discussed.

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12.15 pm

Understanding key weapons of the kauri dieback pathogen *Phytophthora* agathidicida

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Phytophthora agathidicida, a clade 5 oomycete, is a soil-borne pathogen that causes root and collar dieback of New Zealand kauri (*Agathis australis*), which is one of the world's largest ancient Araucariaceae conifer species and has immense cultural significance for Māori. *Phytophthora* pathogens produce both intracellular and extracellular virulence factors, termed effector proteins, that they use to infect their hosts and to suppress the host's immune system. The most well-studied class of these are the intracellular RXLR effectors. Many *Phytophthora* RXLR effectors have a role in virulence by targeting different host molecules to suppress host immunity. A recent study identified 147 RXLR-encoding genes from the new *P. agathidicida* 3770 PacBio genome assembly. Among those, we identified a gene that encodes an RXLR effector, PaRXLR40, which localizes to the nucleus and is able to suppress plant immunity triggered by a range of other intracellular and extracellular *P. agathidicida* effector proteins. Using a 'yeast-two-hybrid' method that enables the identification of proteins that interact with each other, and a model host plant, we identified the putative plant target of PaRXLR40: a BTB domain-containing 'ARIA' protein that also localizes to the nucleus. No interaction was observed between the ARIA protein and a modified version of PaRXLR40 that lacks immune suppression activity. Curiously, the target ARIA protein also suppresses cell death elicited by another intracellular cell death-eliciting protein of *P. agathidicida*. Based on tests with the alternative host *Nicotiana benthamiana*, both the RXLR40 effector and the ARIA host target protein appear to have important roles in enhancing colonization by *P. agathidicida*. Future experiments include confirmation of the interaction of these two proteins in planta and transient silencing of the gene encoding the ARIA target in *N. benthamiana*. This study provides an important foundation for studying the molecular basis of plant–oomycete interactions in gymnosperm forest trees. Moreover, the identification of host targets might ultimately provide molecular markers for selection or breeding for disease resistance in forest trees. Alternatively, RNAi-based silencing of key pathogen virulence factors might be an effective pathogen control strategy.

12.30 – 1.30 pm Lunch

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Session 11: Surveillance 2

Session Chair: Guillaume Bilodeau

1.30 pm
The potential risks of *Phytophthora* species to woody plants in Sweden

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The genus *Phytophthora*, with 326 species in 12 phylogenetic clades currently known, includes many economically important pathogens of woody plants. Many *Phytophthora* species are known to cause various disease symptoms in different tree species, shrubs, and crops across the world, infecting plants from seedlings (annual crops) to mature trees. Despite the available knowledge of *Phytophthora* species in northern Europe, their impact on woody plants in different environments is still uncertain and requires further attention, especially since such impact can be affected by different abiotic factors, possibly impeding the establishment of some *Phytophthora* species. We have tried to analyze the occurrence of *Phytophthora* species associated with woody plants in Sweden. Seventeen *Phytophthora* species were detected from nurseries, natural and anthropogenic forests, urban areas, and rivers/water sources in Sweden. Nine *Phytophthora* species (*P. alni, P. cactorum, P. cambivora, P. cinnamomi, P. cryptogea, P. gonapodyides, P. quercina, P. ramorum, and P. plurivora*) are considered invasive in Sweden, while the status of the remaining species is uncertain, despite being present in the country. In Sweden, six *Phytophthora* species were found to be associated with different types of damage on woody plants. A citizen science platform established to harness the interest of the public to report on the location of diseased trees in urban, peri-urban, and recreational forests and serve to increase our detection of *Phytophthora* pathogens affecting oak, beech and horse chestnut trees, as well as orchard trees. Assessing the potential risks of *Phytophthora* spp. to woody plants in Scandinavian conditions is a highly complex task but from the disease management point of view, it is important to understand which *Phytophthora* species can be pathogenic and cause disease in a particular host.

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1.45 pm
A pathogen-centric approach to *Phytophthora* agathidicida surveillance

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To date surveillance activities for *Phytophthora agathidicida* have largely focused on the pathogen's presence surrounding or within kauri (*Agathis australis*). This is due to the importance of kauri to Aotearoa New Zealand and the proven threat posed by *P. agathidicida*. It is a known primary pathogen of kauri which is a culturally, ecologically, and economically significant species that has previously been decimated by logging and forest clearance. While the focus on identifying *P. agathidicida* presence at the site of a single host species is important for the protection of kauri, it is unclear how this impacts the greater biosecurity goals of the Unwanted Organisms' management. Instead of a host-centric focus to *P. agathidicida* surveillance this investigation took a pathogen-centric approach to investigate if the novel design would provide greater insight into presence and distribution. The study took place at the site of a planned replacement water treatment plant in the peri-urban suburb of Titirangi, Auckland. As the findings from the surveillance had direct impact on the design of *Phytophthora* management plans to be implemented during construction the investigation assessed not only the difference in known *P. agathidicida* distribution but also the management actions that the novel surveillance approach generated. The surveillance design took a multifaceted approach based on known and potential high-risk factors of *Phytophthora agathidicida*. The design utilized the concept of layering of risk factors to create higher levels of sampling within higher risk areas. In total 1,395 samples were processed and *P. agathidicida* presence was detected in 215. The surveillance enabled a comprehensive assessment of pathogen distribution, which included the first detections of *P. agathidicida* within natural watercourses and within non-kauri vegetation areas. The novel approach led to a greater understanding of *P. agathidicida* distribution across the proposed construction site and had a major impact on the subsequent *Phytophthora* management plan.

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2.00 pm
Mātauranga Māori framework for surveillance of plant pathogens

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Faced with growing biosecurity risks and threats, countries worldwide seek to protect their biodiversity from ecosystem degradation and loss. Biosecurity surveillance of plant pathogens and the diseases they cause is fundamental for management and eradication of these risks. To date, the surveillance systems in Aotearoa New Zealand have reflected empirical scientific principles and have been largely devoid of mātauranga and te ao Māori, which have seldom been regarded as valid or relevant knowledge systems to inform biosecurity. Because of this, mana whenua themselves have been disconnected from these systems. The inclusion of mana whenua and their mātauranga is important, not only to recognise their role and rights as indigenous peoples of Aotearoa New Zealand, but because it is through place-based approaches that better biodiversity and environmental outcomes can be achieved. Here, we describe a mātauranga Māori framework for surveillance (MMFS) of plant pathogens, which introduces the principles and methodologies that aim to elevate mana whenua and mātauranga research into the biosecurity and science systems. The MMFS facilitates the co-existence of mātauranga and empirical scientific knowledge without the need for inter-dependent validation, on the assumption that this will lead to better research and operational outcomes. It addresses issues around data ownership and sovereignty, informed consent, and cultural licence. We present a case study where the MMFS has been applied to research initiatives aimed at addressing myrtle rust and kauri dieback in Aotearoa New Zealand. The MMFS informed the development of a data storage platform, which anchors data to its place of origin, recognising its provenance and giving effect to Māori data sovereignty. This process ensures mana whenua have timely access and use of existing and emergent data. Following the principles of the MMFS, we developed and used a ‘proof of pathogen absence’ tool to co-design with mātauranga environmental experts a risk-based surveillance plan for the purpose of demonstrating freedom from disease in areas where a pathogen has not been detected. The MMFS provides a way of planning and implementing environmental surveillance that can be applied to the full range of environmental problems internationally where indigenous populations are involved.

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2.15 pm
Design and analysis of risk-based surveillance to demonstrate absence of *Phytophthora* agathidicida in New Zealand

Cecilia Latham

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Kauri forests are threatened by the deadly kauri dieback disease, caused by the microscopic soil-borne pathogen *Phytophthora agathidicida*. Despite it being more than 15 years since the pathogen was discovered on the New Zealand mainland, there is still a paucity of information on the distribution of symptomatic trees and/or of the pathogen itself, which makes it challenging to understand where the infection foci are and how fast the pathogen is spreading. The considerable cost of surveillance for *P. agathidicida* (both on the ground sampling and subsequent laboratory analyses, as well as the risks associated with people walking between infected and non-infected sites, necessitates careful prioritisation of sites to be selected for surveillance. This task requires an understanding of which part of the landscape and how much of it needs to be surveyed to effectively detect the disease if present. We use information on the distribution of kauri trees with visible stress symptoms in the canopy to develop a baseline relative risk map depicting areas where *P. agathidicida* is most likely to be present for three kauri forests in Northland, New Zealand. The relative risk map provides a simple, yet effective management tool to target trees for surveillance, monitoring, and protection. Using this insight, we demonstrate the application of a proof of absence statistical model to determine the surveillance effort required to be confident that if no *P. agathidicida* is found during surveillance in selected priority sites, these can be considered free from the pathogen. We also demonstrate how the approach can be scaled up to a regional scale.

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2.30 pm

Characterising the diversity of oomycetes in a multi-use landscape in Aotearoa New Zealand

Alana Thurston

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Te Kaha, rohe of Te Whānau-ā-Apanu, is a multi-use landscape that in recent years has invested in kiwifruit orchard expansion. This transition to a more horticulturally intensive crop will create a greater need for irrigation, and current schemes propose diverting water from the nearby Kereu River. Landowners and kaitiakitanga are cognisant that untreated irrigation water from natural bodies can lead to the dissemination of water-borne plant pathogen propagules, and thus, this research sought to characterise the diversity of oomycetes present in both aquatic and terrestrial sites in the Te Kaha catchment, with a focus on *Phytophthora*. In January 2023, water samples were collected and filtered at nine positions along a 9.3 km stretch of the Kereu River. Soil samples were also collected from nine sites that featured varying land usage — kiwifruit, maize, and ngahere | native forest. Oomycete specific ITS1 amplicons were generated from water and soil eDNA and sequenced using Illumina MiSeq. Kereu River sites were also baited in situ using a selection of native, exotic, and agriculturally relevant plant leaves. Exotic plant baits were more successful in yielding cultures with morphologies congruent with *Phytophthora*, whereas native plant baits had a higher incidence of producing isolates with sporangial exit tubes, a feature associated with *Pythium*. The exact identities of these *Phytophthora* isolates are being confirmed with Sanger sequencing. Similarity in Oomycota communities between river and soil samples was compared using ANOSIM analysis. Oomycete communities from water were almost entirely distinct from those present in soil (stress = 0.065, R = 0.99, p=0.001). The differences in Oomycota diversity in soils between land usage was also compared, which found significant differences with some overlap in taxa present (stress = 0.074, R = 0.42, p = 0.03). Of the 804 ASV produced, approximately 2% belonged to the Phytophthora genus. Only a third of terrestrial sites had *Phytophthora* present, whereas *Phytophthora* were detected in all locations along the Kereu River. *Phytophthora* spp. belonging to clades 2b and 12 were unique to soils, whereas species belonging to clades 6, 7, and 8 were exclusive to the Kereu River. Only one ASV, belonging to the Clade 2c '*P. citricola* complex', was found in both terrestrial and aquatic landscapes. While there is currently no evidence of *Phytophthora* 'spill-over' between terrestrial and aquatic systems in Te Kaha, our results suggest that continued monitoring of this catchment could prove beneficial as these neighbouring landscapes increasingly interact in the future.

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2.45 pm

Phytophthora: an ancient, historic, biologically and structurally cohesive and evolutionarily successful generic concept in need of preservation

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In response to evidence that all downy mildews (DMs) reside phylogenetically within *Phytophthora*, rendering *Phytophthora* paraphyletic, a proposal has been made to split the genus into multiple new genera. Despite a substantial increase in the number of described species and improvements in molecular phylogenetics the *Phytophthora* genus structure has remained stable since first characterized in 2000. After an assessment of more than 200 species for twenty morphological and behavioural criteria, the clades continue to show good biological cohesion. Saprotrophy, necrotrophy and hemi-biotrophy of woody and non-woody roots, stems and foliage occur across all clades. Phylogenetically less related clades often show strong phenotypic and behavioural similarities and no one clade or group of clades shows the synapomorphies that might justify a unique generic status. The extraordinary flexibility of the genus may account for its global 'success'. The 20 genera of the obligately biotrophic, angiosperm-foliage specialised DMs evolved from *Phytophthora* at least twice via convergent evolution, making the DMs as a group polyphyletic and *Phytophthora* paraphyletic in cladistic terms. The long phylogenetic branches of the DMs indicate this occurred rather rapidly, via paraphyletic evolutionary 'jumps'. Such paraphyly is common in successful organisms. The proposal to divide *Phytophthora* appears to address the issue of the convergent evolution of the DMs rather than the structure of *Phytophthora* per se. We consider this non-Darwinian, putting the emphasis on the emergent groups (the DMs) rather than the progenitor (*Phytophthora*) and ignoring the evolutionary processes that gave rise to the divergence. Further, the generic concept currently applied to the DMs is narrower than that between some closely related *Phytophthora* species. Considering the biological and structural cohesion of *Phytophthora*, its historic and social impacts and its importance in scientific communication and biosecurity protocol, a workshop was hosted by the American Phytopathological Society to assess community consensus on the global *Phytophthora* community. The community voted unanimously to retain the genus name *Phytophthora*.

3.15 – 3.45 pm Afternoon Tea

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Session 12: Nurseries and New Species

Session Chair: Simone Prospero

3.45 pm
Sustaining Native Plant Restoration in California: Implementing Effective *Phytophthora* Best Management Practices and Testing Methods in Container Nurseries

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Native plant restoration in California faces recurrent challenges due to *Phytophthora* introductions, leading to failed restoration projects. As a response, the *Phytophthora*s in Native Habitats Work Group developed Accreditation to Improve Restoration (AIR), an audit-based program to evaluate the implementation of Best Management Practices that strictly exclude *Phytophthora* from nursery operations. Initiated in 2018, the program currently serves 21 nurseries with nine fully accredited participants and 12 in progress. To date, *Phytophthora* has not been detected in AIR compliant nurseries. An important component of AIR is plant testing. To determine the most effective methods for accurate *Phytophthora* diagnostics, to the genus or species level, we compared five diagnostic methods: leachate baiting, Immunostrips®, direct root isolations, recombinase polymerase amplification (RPA, targeting genus specific *trnM-trnP-trnM* locus), and quantitative polymerase chain reaction (qPCR, targeting the genus specific *atp9-nad9* locus). These methods were tested on both artificially inoculated and naturally infested plants. Two common native California plants, *Frangula californica* (coffeeberry) and *Heteromeles arbutifolia* (toyon), were inoculated with three *Phytophthora* species: *P. cinnamomi*, *P. cactorum*, and *P. cryptogea* and sampled every six weeks post-inoculation for four months. Among the detection assays both RPA and leachate baiting had the greatest *Phytophthora* identification success rate, regardless of the host, sampling time, or species ($P < 0.01$). qPCR results are still underway. Further results from comparative diagnostic analyses of naturally infested plants collected at five different nurseries will provide a robust assessment of methodology. Upon completion, this study can guide the design of testing and diagnostic methods for effective and rapid *Phytophthora* detection, which can be incorporated in the AIR and other nursery health programs. To sustain plant health in restoration the use of strict nursery Best Management Practices, including container plant testing, is key to prevent inadvertent *Phytophthora* introductions.

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4.00 pm
Diversity of *Phytophthora* taxa in Mediterranean forest nurseries and application of new biosecurity management practices

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Luca Sarais[1], Salvatore Seddaiu[1], Antonio Alberto Mulas[1], Pino Angelo Ruiu[1], Giovanni Piras[1], Maurizio Frongia[2], Alberto Masci[2], Giovanni Pili[2], Andrea Brandano[3], Antonio Deidda[3] and Bruno Scanu[3]

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Species in the genus *Phytophthora* are well-known pathogens causing a significant impact on forest and natural ecosystems worldwide. The globalization and trade of plants have facilitated the widespread introduction and distribution of *Phytophthora* species, with nurseries playing a key role as hubs and potential spread of these pathogens. These organisms can survive abiotic adverse conditions in soil, and infected plants can often be symptomless, particularly in nursery environments. As a result, the use of infected nursery plants represents an important source for the upsurge of new outbreaks in orchards, gardens, parks and natural ecosystems. Based on the new EU forest strategy for 2030, at least 3 billion additional trees are being planted in Europe to enhance well-being, health benefits and climate change mitigation. Therefore, the start of tree restoration and urban afforestation activities represents a challenge to the Italian forest nursery sector, particularly in meeting the demand for healthy plants. Between 2019 and 2023, a survey was conducted in one private and 13 public nurseries in Sardinia to investigate the diversity and distribution of *Phytophthora* species. Almost 600 samples were collected, including roots and soil from seedlings, irrigation water, substrate piles and discarded plants. *Phytophthora* species were found in all investigated nurseries, from 41 different hosts, as well as substrate piles, irrigation water and discarded plants. In addition, a pilot nursery was established where new biosecurity management practices were implemented to reduce *Phytophthora* infection. During the entire production chain, seedlings and substrates were thoroughly analyzed for the presence of *Phytophthora*. The results obtained revealed that the application of biosecurity management practices can significantly reduce, if not eradicate *Phytophthora* in nurseries. To our knowledge, this is the first attempt in Italy, one of the main districts in Europe for plant production, to produce *Phytophthora*-free plants.

4.15 – 4.45 pm Panel discussion on Phytophthora in nurseries plus daily summary

Session Chair: Simone Prospero

4.45 – 5.15 pm IUFRO Business Meeting

Meeting Chair: Nari Williams

5.15 – 6.00 pm IUFRO *Phytophthora* Football Game

7.00 – 9.00 Conference Dinner

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Friday 13th September

Session 13: Diverse perspectives in tackling the challenges of Phytophthora pathogens in forests and natural ecosystems

Session Chair: Ana Perez-Sierra

9.00 am
Dieback Interpreter Registration and Auditing in Western Australia

Kylie Ireland

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Kylie Ireland[1], Ian Moore[1] and Nathan Ashburner[1]

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Phytophthora dieback (dieback) is the greatest disease threat to the flora of the south-west bioregion of Western Australia. Accurate mapping is integral to the management of the disease, underpinning the development of hygiene and management protocols to prevent and minimise disease spread. The Department of Biodiversity, Conservation and Attractions (DBCA; and its predecessors) has maintained a dieback mapping program since the 1980s. It is a requirement that mapping on DBCA-managed lands is undertaken by registered *Phytophthora* Dieback Interpreters (Interpreters). Interpreters are specially trained to detect, diagnose and map the presence of *Phytophthora*, particularly *P. cinnamomi*, according to the *Phytophthora* dieback interpreter's manual for lands managed by the department (2015; Interpreter's manual). The system for registering interpreters and monitoring standards of interpretation is administered by the Plant Diseases Program in the Ecosystem Health Branch. The system was recently reviewed (2023) to be guided by two documents: (1) the System Guidelines for Phytophthora Dieback Interpreter Registration (System Guidelines); and (2) the Procedure for the Auditing of Phytophthora Occurrence Assessments (Auditing Procedure). The Auditing Procedure has undergone the largest change, with those proposing disturbance activities that move soil in areas at risk of dieback spread now responsible for supplying dieback assessments and evidence from the interpreters they have engaged to the DBCA for auditing, effectively shifting the onus of responsibility to the risk creator for both the quality of the mapping and the management of their contracts with the interpreters they engage. A summary and review of the rollout of the system for dieback interpreter registration and auditing in Western Australia will be presented, including what the systems looks like in practice, as well as lessons learned.

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9.15 am

Phytophthora Research in Sub-Saharan Africa: Unveiling the Imperative Next Steps

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Phytophthora species are highly destructive plant pathogens, posing a significant threat to diverse ecosystems in sub-Saharan Africa. To date, 77 *Phytophthora* species have been identified in this region. While sub-Saharan Africa has been a focal point for many important studies, contributing greatly to our understanding of *Phytophthora* across various research fields, the pace of research here lags global trends. This is evident from the lack of records of *Phytophthora* in certain countries. We want to draw attention to these critical research gaps, emphasizing the urgent need for specific studies to address these deficiencies. The proposed research not only aims to protect the region's iconic floral biodiversity but also plays a crucial role in enhancing economic stability and ensuring food security. These initiatives serve as a call to action, urging collaborative efforts to bridge knowledge gaps and implement informed strategies for building more resilient landscapes in sub-Saharan Africa.

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9.30 am

Know Your Foe: Unraveling the Secrets of *Phytophthora cinnamomi* Resilience and Pathogenicity in the Face of Drought

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Phytophthora cinnamomi, a hemibiotrophic oomycete, presents a significant threat to global biodiversity with its vast host range, targeting plant species which impact agriculture, forestry and culturally significant plants. *P. cinnamomi* spread through soil runoff, streams, animal activities, and anthropogenic actions, which are aggravated under warm and wet weather conditions. Climate change introduces considerable shifts in global climate patterns, manifesting as increased temperatures in traditionally colder regions and decreased temperatures in warmer areas, consequentially impacting native flora and fauna, potentially triggering adaptation, migration, or, in severe cases, extinction. Climate-induced changes critically influence the interaction between *P. cinnamomi* and its hosts. Similar to other oomycetes, *P. cinnamomi* secretes effector proteins, molecules designed to manipulate host cell structure and function, to facilitate infection and counteract plant defences. These effectors are vital for *P. cinnamomi* infection capabilities. We hypothesize that *P. cinnamomi* efficacy and range are susceptible to climatic conditions, possibly affecting the pathogen's virulence and global spread. Our research aims to understand *P. cinnamomi* stress adaptation mechanisms to drought and salinity, and ultimately, its impact on performance, virulence and pathogenicity. *P. cinnamomi* mycelium was subjected to osmotic and salinity stress conditions using varying concentrations of polyethylene glycol (PEG) and sodium chloride (NaCl), respectively. We evaluated factors including mycelial growth, and reactive oxygen species (ROS) levels. We combined them with a systems approach using quantitative transcriptomic and proteomic analysis in a stress and time-dependent manner. Upon exposure to stress conditions characterized by 5% PEG and 100 mM NaCl, the pathogen demonstrated significantly elevated growth rates, surpassing those of control. Proteomic analysis revealed both the presence and increase of ROS-quenching proteins, such as peroxidases. Additionally, there was a noticeable increase in the abundance of pathogen effectors, specifically RxLR and Crinkler, observed at 12 hours post-treatment. This was further corroborated by subsequent assays involving ROS staining and the quantification of peroxidase activity. This indicates a potential linkage between the transcriptional responses and the proteomic adaptations observed under the specified stress conditions. These findings indicate *P. cinnamomi* molecular adaptation processes to water-limiting conditions underlining its invasive capabilities and resilience. We believe that such studies are vital for understanding the long-term effects of climate change on invasive *Phytophthora* species and their host infection processes to inform biosecurity strategies in regions like Aotearoa, New Zealand, where the preservation of native biodiversity against invasive pathogens is of paramount importance.

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9.45 am
Identification and characterisation of *Phytophthora* species associated with New Zealand apple orchards

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Phytophthora root and crown rot is a destructive and economically important disease of apple worldwide. Several *Phytophthora* species are implicated but there have been no recent detailed studies to identify and characterise the *Phytophthora* species associated with New Zealand apple orchards. Soil samples were obtained from apple orchards in Hawke's Bay (11 orchard blocks), Tasman (13 orchard blocks), Waikato (2 orchard blocks), Otago (2 orchard blocks) and Canterbury (2 orchard blocks), representing different cultivars and age groups. *Phytophthora* isolates were recovered by baiting using Himalayan cedar needles (*Cedrus deodara*), lupin radicles and apple cotyledons as baits. Lupin was the most effective bait, recovering 147 *Phytophthora* spp. isolates compared to apple cotyledons (72 isolates) and cedar needles (52 isolates). The *Phytophthora* isolates recovered were identified based on morphology and DNA sequencing. Seven species were identified, *Phytophthora cactorum, P. cambivora, P. megasperma, P. plurivora, P. rosacearum, P. chamydospora, P. cryptogeae* and isolates with unresolved identity (*Phytophthora* spp.). The most prevalent species was *P. cactorum* (203 isolates). A higher number of *Phytophthora* spp. and *P. cactorum* isolates were recovered from the old (> 20-year-old) and medium (6–19-year-old) orchards compared to the young (2–5-year-old) orchards. The genetic diversity of *P. cactorum* was determined using random amplified microsatellites (RAMS) and universally primed polymerase chain reaction (UP-PCR) primers and relative virulence of isolates of the recovered *Phytophthora* species assessed. The 59 *P. cactorum* isolates were placed into five major groups of 3, 16, 9, 9 and 20 isolates each with two isolates each placed in groups by themselves, with the *P. cactorum* population in the orchards dispersed in the different groups. From the genetic diversity analysis isolates of *P. cactorum* and representative isolates of the different species recovered were selected and their virulence assessed on different apple rootstocks using detached root and shoot assays and whole plant experiments. All *Phytophthora* species and *P. cactorum* isolates tested were virulent on the apple rootstocks tested, with rootstock MM106 being more susceptible than M9 and M26. There was no correlation between the genetic diversity and virulence. The prevalence and dominance of *P. cactorum* determined in the study denotes its significance within New Zealand's apple orchards. The broad diversity of *Phytophthora* species identified emphasizes the need for continued monitoring to safeguard orchards from these potential threats. These may also represent a reciprocal threat for other ecosystems if these species move from orchards to forest/native ecosystems and vice versa.

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10.00 pm

Sniffing out the problem: Canine detection of *Phytophthora ramorum*

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The introduction of non-native *Phytophthora* species is a major threat to forests and natural ecosystems worldwide. *Phytophthora ramorum* has found a wide range of highly susceptible hosts after being introduced to Europe and North America, affecting both native keystone species, such as oaks in the USA, as well as a commercially important plantation conifer, larch, in Great Britain (GB). *P. ramorum*, which is subject to statutory control in GB, has been found to spread within the nursery trade and from nurseries to natural ecosystems, either via infected plantings or by direct escape. Our study looked at the potential of using detection dogs for the detection of *P. ramorum* to support national phytosanitary control measures. Using established dog training techniques, involving positive reinforcement in which the dog was rewarded for indicating *P. ramorum*, we have proved that a detection dog previously trained to detect bed bugs and contaminated water can be retrained to detect *P. ramorum*. A proof-of-concept trial following the training period showed that the dog could rapidly and reliably identify *P. ramorum* and distinguish it from *Pythium* as the non-target scent in a range of substrates including water, coir, potting soil and Rhododendron leaves, with a 100% success rate. Current work is focused on training the dog to distinguish *P. ramorum* from *P. cinnamomi*, *P. gonapodyides* and *P. pseudosyringae* in water and soil, with promising results so far. We will be reporting the results from the final set of trials as well as the planned next steps. We propose potential use cases including import inspections at ports, and as part of statutory plant nursery inspections where the speed and accuracy of assessment is important. Currently, plant health inspectors rely on the observation of visual symptoms followed by laboratory testing of samples. This is a time-consuming and labour-intensive process. Using dogs to target symptomatic and asymptomatic plants would enable quicker assessments and guide the selection of samples for confirmation if required. There is also interest from government inspectors in the use of dogs in wider environment outbreaks for example in public parks and gardens. Detection dogs have the potential to provide an additional layer of biosecurity for preventing spread through international trade and through transmission from nurseries to the wider environment.

10.15 – 10.45 Conference close

10.30 – 11.00 Morning Tea

11.00 Post conference Transport and tour departs

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Wednesday 11th September field trip schedule

7.30 am Depart Copthorne Motel

Travel 40 minutes

8.10 – 9.15 am Manginangina Kauri Walk

Tiakina Kauri – response and operational management with key partners

Travel 1 hour 45 minutes

Bus 1 to go directly to Tane Mahuta.
*Bus 2 to stop in Omapere for 30 minutes to stagger the group **

11.00 – 12.00 pm / 11.30 – 12.30 pm* Tane Mahuta and lunch

Tāne Mahuta – The Lord of the forest - Kōrero tuku iho (stories of the past)

- The kauri ambassadors program
  Tāne Mahuta Kauri Management unit

Travel 10 minutes

12.10 – 1.15 pm / 12.40 – 1.45 pm* Kauri Walks

Holistic Forest Management

- Rākau Rangatira Kauri Management Unit
  Working with Researchers
- Tech transfer
- Allied work and data informing forest health
- Large scale pest control
  Remote sensing

Travel 45 minutes

2.00 – 3.15 pm / 2.30 – 3.45 pm* Trounson Park

The impacts of *Phytophthora agathidicida*

- Alternative hosts
- Phosphite treatment trials

Travel 2 hours 30 minutes

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Post Conference Tour

Friday 13th September

11.00 am Depart Copthorne Motel, Paihia (Packed lunch)

5.10 pm Coach arrives Auckland airport

7.30 pm Evening meal – Good George Hamilton

9.10 pm Arrive Sudima, Rotorua

Saturday 14th September

9.30 am Coach departs for field visit

9.45 am Scion

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Posters

Phytophthora diversity in watercourses of the highly urbanized Swiss Plateau

Phytophthora diversity in watercourses of the highly urbanized Swiss Plateau

Ruffner B, Schoebel CN, Rigling D, Prospero S

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BACKGROUND

- *Phytophthora* species can cause severe damage in agriculture, forestry, and natural ecosystems worldwide.
- Since water plays a crucial role in their dispersal, stream and river baiting is commonly used to survey risk areas for quarantine *Phytophthora* species.
- Here we assessed the **presence** and **diversity** of ***Phytophthora* species** in Swiss watercourses, with a focus on the highly urbanized Swiss Plateau.

A total of 32 watercourses, including the major rivers Aare, Rhine and Rhone; Sampling at 52 sites using rhododendron leaves; *Phytophthora* isolated and identified by sequencing part of the ITS.

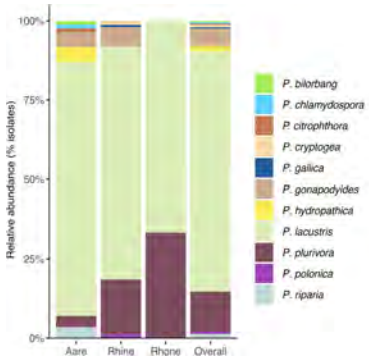
RESULTS AND DISCUSSION

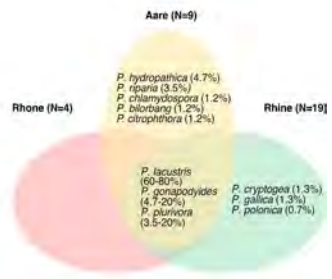
- Phytophthoras were detected **in all watercourses**, with a total of 241 isolates (1-42 per watercourse).
- **Eleven *Phytophthora* species** from five different ITS clades were identified: **Clade 6** was the most represented with 5 species.
- Most frequent species were ***P. lacustris*** (75.5% isolates; 30 watercourses), ***P. plurivora*** (12.4% isolates, 11 watercourses), and ***P. gonapodyides*** (5.8% isolates, 9 watercourses).
- Up to 8 species were detected in each of the **river basins** Aare, Rhine and Rhone.

1) To date, a total of 30 *Phytophthora* species are confirmed in Switzerland → In 1996 ("historical") only 15 species.

2) Species are not evenly distributed across environments → for diversity and distribution studies, need to sample different types of environments.

3) In this study, no invasive pathogenic species were detected → Stream baiting may not be the best method for detecting obligate biotrophs with a still limited distribution in the environment.





***Phytophthora* species found in Switzerland (as of 2024)**

clade	*Phytophthora* species	historical	watercourses	forests	nurseries
1	*P. cactorum*	●		●	●
	P. infestans	●			
	P. nicotianae	●			
	P. x serendipita			●	●
2	*P. citrophthora*	●	●		●
	P. multivora				●
	P. plurivora	●	●	●	●
3	*P. pseudosyringae*			●	
6	*P. bilorbang*		●		
	P. chlamydospora		●		●
	P. gonapodyides		●	●	
	P. lacustris		●		
	P. megasperma	●			
	P. riparia		●		
7	*P. x alni*			●	
	P. x cambivora	●		●	
	P. cinnamomi	●		●	●
	P. europaea			●	
	P. fragariae	●			
	P. rubi	●			
8	*P. cryptogea*	●	●	●	
	P. drechsleri	●			
	P. erythroseptica	●			
	P. porri	●			
	P. ramorum				●
	P. syringae	●		●	
9	*P. hydropathica*		●		
	P. polonica		●		
10	*P. gallica*		●		
12	*P. tubulina*			●	
	number of species	15	11	12	8

Schöbel CN, Prospero S, Rigling D, Ruffner B. 2024. Fishing for *Phytophthora* in watercourses of the highly urbanized Swiss Plateau. Mycological Progress 23: 17.

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Survey of oomycetes and pathogenicity of *Phytophthora cinnamomi* associated with root rot disease of western white pine (*Pinus monticola*) mortality

Natural Resources Canada | Ressources naturelles Canada

UNCLASSIFIED - NON CLASSIFIÉ

Survey of oomycetes and pathogenicity of *Phytophthora cinnamomi* associated with root rot disease of western white pine (*Pinus monticola*) mortality

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11th IUFRO Phytophthora in Forests & Natural Ecosystems Meeting – Paihia, New Zealand. September 8 – 13, 2024
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Introduction

Western White Pine (wwp) with genetic tolerance to blister rust disease caused by *Cronartium ribicola* is an invaluable resource for disease management. Over the last few years, chlorotic needles and root rot symptoms typical of a water-mold agent, led to sudden mortality in seed orchards on Vancouver Island. Variations in temperature and precipitation associated with changing climates in B.C., may influence the prevalence and distribution of oomycetes.

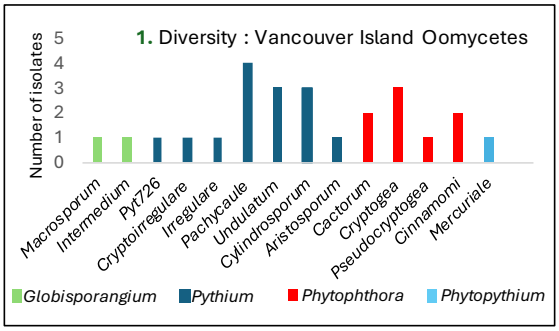
Objectives

- Detect the diversity of oomycetes associated with declining and symptomatic wwp.
- Assess pathogenicity of isolated *Phytophthora* species.
- Report the first record of *P. cinnamomi* on wwp in British Columbia, Canada.
- Molecular typing of *P. cinnamomi* using Oxford Nanopore MinION™

Materials and Methods

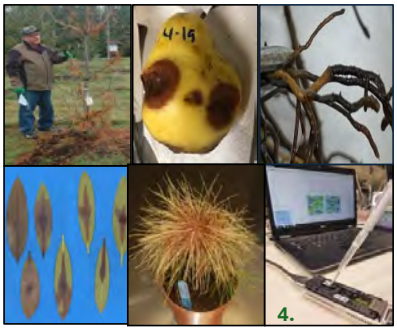
- Pears and rhododendron leaves were used as baits for soil and root samples. Necrotic tissues from the baits, along with host roots, were plated onto CMA – PARPH.
- Identification: Extracted DNA was PCR amplified using 3 primer sets (ITS 4 & 6, β-tubulin, and TEF1), products were Sanger sequenced at CHUL in Quebec.
- Pathogenicity testing with root or stem inoculations on seedlings and rhododendron leaves.
- Mating Type: *P. cinnamomi's* mating type and origin group determined using Oxford Nanopore (Minion™) sequencing.

Results



2. Isolate	Leaf lesion area (%)
P.cinnamomi-Pear	64%
P.cinnamomi-Root	59%
P. cryptogea	39%
P. pseudocryptogea	34%
P.cactorum	28%
Control	<1%

3. Species	Temp (°C)	Days until stage 4
P. cinnamomi	*15*	65 - 75
P. cactorum	*15*	80 - 100
P. cryptogea	*15*	70 - 95
P. pseudocryptogea	*15*	70 - 95
P. cinnamomi	*25*	29 - 46
P. cactorum	*25*	50 - 63
P. cryptogea	*25*	50 - 73
P. pseudocryptogea	*25*	50 - 73



1. Baiting and plating on selective media yielded a variety of oomycetes including four distinct phytophthora species.
2. Lesion assessment relative to rhodo leaf size, agar plug inoculation and kept in moisture chambers at 20°C for 7 days. Measurements done with Assess 2.0.
3. Pathogenicity assessment on wwp seedlings at two temperature regimes, with 14hr light and 10hr dark cycle. All inoculations led to disease, reported here is stage 4. (>50% canopy/needles as browned/dead). Other oomycete spp. omitted from table as none induced any observable disease. Koch's Postulates were satisfied by re – isolating the disease agents from diseased seedlings.
4. Generating whole genome sequences using MinION™, the *P. cinnamomi* genome was assigned to genetic group 3 (PcG2-A2 lineage) with >90% confidence.

Conclusion

Various oomycetes are associated with *Pinus monticola* but only the isolated *Phytophthora* species contributed to disease, decline, and eventual mortality in wwp seedlings. During stem inoculations, symptoms appeared within the first two weeks and mortality followed a few months later. All *phytophthora* isolates induce necrosis on rhododendron leaves, with *cinnamomi* as most aggressive based on lesion size. Minion™ sequencing assigned our *P. cinnamomi* isolates to PcG2-A2 lineage (Shakya et *al.*, 2021), the A2 lineage is the main driver behind the global *P. cinnamomi* epidemic. See our published paper in the reference section for the full report!

References

Shakya, S.K., *et al.* (2021). Phylogeography of the wide-host range panglobal plant pathogen *Phytophthora cinnamomi*. *Mol. Ecol.* 30:5164-5178.

Shamoun, S.F., *et al.* (2024). Survey of oomycetes and pathogenicity of *Phytophthora cinnamomi* associated with root rot disease of western white pine (*Pinus monticola*). *Can. J. Plant Pathol.* 1 – 13.

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Development of ecofriendly agents to sequentially target *Phytophthora* life stages

Development of ecofriendly agents to sequentially target *Phytophthora* life stages

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INTRODUCTION

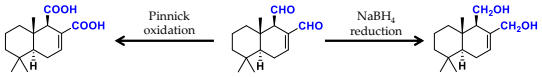
- *Phytophthora* - notorious pathogens threatening natural, agricultural and forest ecosystems
- In Aotearoa,
  - *Phytophthora agathidicida* - kauri dieback disease
  - *Phytophthora pluvialis* - red needle cast of radiata pine trees
  - *Phytophthora cinnamomi* - avocado root rot
- *Phytophthora* species have a complicated life cycle with multiple life stages
  - Mycelia - vegetative growth stage
  - Zoospores - inoculation of new hosts
  - Oospores/chlamydospores - long-term survival
- Current treatments: phosphite (phosphorous acid), fosetyl-Al, inorganic copper salts etc.
- Limitations: phytotoxicity, risk of resistance development, not active against multiple life stages
- Our work: Evaluating ecofriendly compounds to combat different life stages of *Phytophthora* species[1]

Ecofriendly Anti-*Phytophthora* compounds

1. Polygodial and its derivatives:

Pressurised Hot Water Extraction (PHWE) and chromatography-free isolation[2]:

- Polygodial – sesquiterpene dialdehyde, known for antifungal and antifeedant activities
- Extracted from horopito plant leaves (endemic shrub of New Zealand)
- Synthetic derivatives of polygodial:

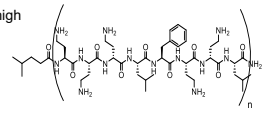


Oxidised form MIC = >100 µg/mL | **Polygodial** MIC = 5-10 µg/mL | **Reduced form** MIC = >100 µg/mL

Modifications of the dialdehyde resulted in the loss of activity → Further derivatization retaining the dialdehyde is under progress

2. Peptide-based compounds:

- Peptides: short chains of amino acids; have high potency against microbial pathogens
- Lipopeptide: Lipid + peptide, secondary metabolites of bacteria and fungi
- Battacin - cyclic lipopeptide, broad spectrum antibacterial activity (*in vitro* and *in vivo*)
- Linear analogues of battacin (monomer, dimer and trimer) synthesized[3] and tested



n = 1; inactive
2; inactive
3; active against zoospores & oospores

3. Natural bioactives:

Plant bioactive molecules show excellent anti-*Phytophthora* activity:

- Compound 1: Extremely active against *P. pluvialis*
- Compound 2: Selectively active against *P. agathidicida*

RESULTS

***In vitro* anti-mycelial activity**

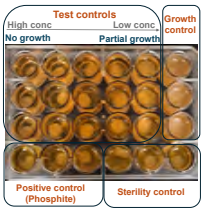


Fig. 1 Antimycelial assay of active compounds against *P. pluvialis* mycelia

Mycelial agar plugs from freshly grown *Phytophthora* species (*P. agathidicida*, *P. cinnamomi*, *P. multivora*, *P. infestans* and *P. pluvialis*) agar plates were transferred to the wells containing the appropriate compound in clarified liquid V8 medium.

Compound	Minimum Inhibitory Concentrations* (µg/mL)
Polygodial	5-10; *P. agathidicida* and *P. pluvialis* 10-50; *P. multivora* and *P. cinnamomi* ≥ 50-200; other *spp.*
Lipopeptide	≥ 50-200; other *spp.*
Natural bioactive 1	1.5-2.5; *P. pluvialis* 10-25; *P. agathidicida* ≥ 50-200; other *spp.*
Natural bioactive 2	25-50; *P. agathidicida*

*Inhibitory activity of the compound is defined as the minimum inhibitory concentration at which no growth was observed after 7 days of incubation.

SEM images of *P. agathidicida* mycelia after treatment

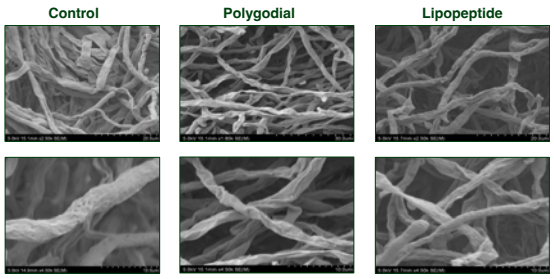


Fig. 2 SEM images of *P. agathidicida* mycelia in the absence (control) and presence of test compounds at 2 x MIC. Top panel images: lower magnification (1800-2500 x); bottom panel images: higher magnification (4500 x).

Anti-zoospore activity

Compound	Minimal inhibitory concentrations* (µg/mL) Zoospore motility (left) and germination (right)		
	P. cinnamomi	*P. multivora*	*P. agathidicida*
Polygodial	12.5-25; 50-100	12.5-25; 25-50	10-20; 25-50
Lipopeptide	4-8; 25-50	5-10; 12.5-25	4-8; 12.5-25

*Inhibitory activity of the compound is defined as the minimum inhibitory concentration required for all the zoospores to become immobile within the first five-minutes of the assay.

Anti-oospore activity

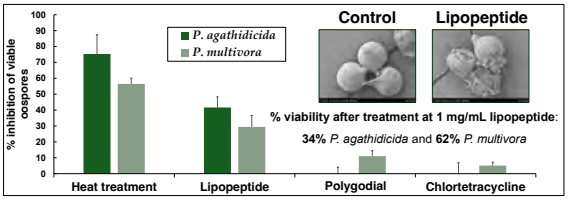
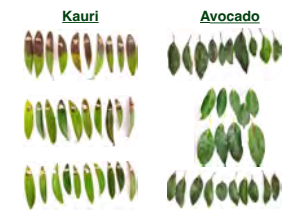


Fig. 3 (Left): Bar graph showing % inhibition of viable oospores of *P. agathidicida* and *P. multivora* under different conditions. (Right): Scanning electron microscopy images of *P. agathidicida* oospores.

Phytotoxicity studies



Surface coverage by *P. agathidicida* after treatment	
Water control 51%	Lipopeptide, polygodial 0 - 10%

Lipopeptide and polygodial: No noticeable necrosis or lesions

NOT PHYTOTOXIC

Fig. 4 Leaves infected with *P. agathidicida* (kauri), and *P. cinnamomi* (avocado) and treated with the inhibitory compounds

CONCLUSIONS AND FUTURE WORK

- Several natural products have been identified with promising activity against *Phytophthora* mycelia. Further derivatization to build a library of anti-*Phytophthora* compounds active against multiple life stages, is under progress.
- Synthetic lipopeptide showed moderate activity against zoospores (motility and germination) and oospores (viability).

ACKNOWLEDGEMENTS

We acknowledge previous funding from George Mason Center for Natural Environment (GMCNE), Freemasons Foundation and current funding from Ministry of Business, Innovation and Employment (MBIE). Earlier discussions with Michael Steadman (Kaiaratiaki) were key to the start of some of the horopito-based investigations undertaken in this manuscript. We acknowledge Forest Herbs for supplying horopito leaves for this research.

REFERENCES

1. De Zoysa, Gayan H., et al. *Plant Pathology* 72.5 (**2023**): 912-923.
2. Just, Jeremy, et al. *Organic & biomolecular chemistry* 13.46 (**2015**): 11200-11207.
3. De Zoysa, Gayan H., et al. *European Journal of Medicinal Chemistry* 146 (**2018**): 344-353.

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Investigating the impact of foliar phosphite (Foschek®) and Du-Wett® application on the water relations of kauri (*Agathis australis*)

SPTS No. 26170_CB0-3661

Matthew Arnet

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Investigating the impact of foliar phosphite (Foschek®) and Du-Wett® application on the water relations of kauri (*Agathis australis*)

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Introduction

- Kauri (*Agathis australis*) is a large foundation tree species endemic to New Zealand and is highly susceptible to root infection by *Phytophthora agathidicida*[1].
- Phosphite injection and foliar application have been used to treat trees infected with phytophthora[2,3,4] in a range of forest systems worldwide.
- While phosphite is an effective treatment for phytophthora infection, foliar phosphite (Foschek® 400) + Du-Wett® application has been shown to initially reduce stomatal conductance, assimilation and water use in kauri saplings[5].
- We aimed to elucidate which chemical is responsible for the reduction in water relations and investigate the mechanism behind the changes in a glasshouse trial.

Method

Five-year-old kauri seedlings of similar size were arranged in a randomised complete block design.

Trees were treated with foliar 7.5 g/L Foschek, 7.5 g/L Foschek + 0.2% Du-Wett, 0.2% Du-Wett, or distilled water to drip.

Plastic skirts secured around the base of the trees prevented sprays from entering the soil. Once sprayed, trees were allowed to dry completely before the skirts were removed.

Symptom development was observed, and stomatal conductance and assimilation measurements taken weekly, 1 week before foliar application, and for 4 weeks post treatment.

Stomatal apertures were measured on the day before and 1 week following spray application. Phytotoxicity of spray application was recorded after 4 weeks.

Results

Phytotoxicity was significantly higher in the phosphite-only treatment while all others remained low or had no phytotoxicity (Figure 1).

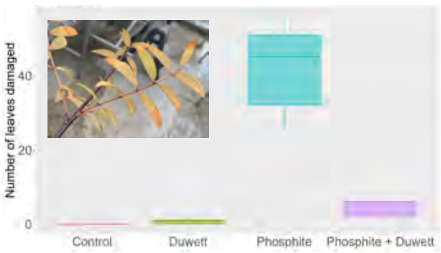

Figure 1: Visible phytotoxicity on kauri (*Agathis australis*) leaves 4 weeks following foliar spray treatments of Du-Wett® (■), 7.5 g/L phosphite (■), 7.5 g/L phosphite and Du-Wett (■) and untreated control (■).

Stomatal conductance values were significantly lower the week following spray application but had recovered completely after 2 weeks (Figure 2).

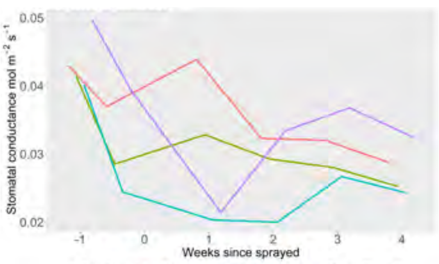

Figure 2: Stomatal conductance (LI-COR 6800) of kauri 1 week before and 4 weeks following spray application of Du-Wett (■), phosphite (■), phosphite and Du-Wett (■) and untreated control (■).

Assimilation (A) values (a measure of photosynthesis) of kauri were significantly reduced 1 week following application of phosphite while other treatments were unaffected. After 2 weeks, none of the spray treatments differed from the untreated control.

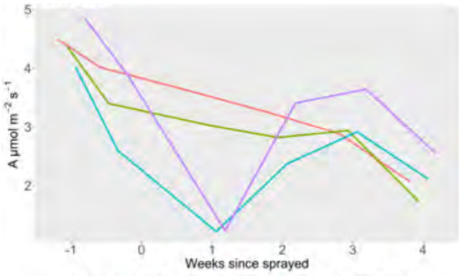

Figure 3: Assimilation (A) (LI-COR 6800) values of kauri 1 week before and 4 weeks following spray application of Du-Wett (■), phosphite (■), phosphite and Du-Wett® (■) and untreated control (■).

Stomatal aperture showed some reduction in the Du-Wett® treatments but was not significant, while phosphite and control treatments remained stable.

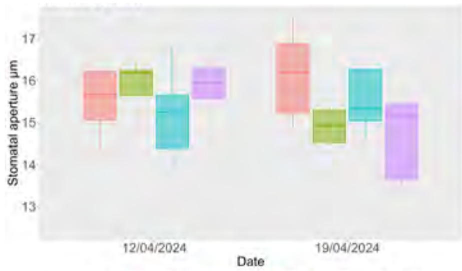

Figure 4: Stomatal aperture 1 day before and 1 week following spray application of Du-Wett (■), phosphite (■), phosphite and Du-Wett (■) and untreated control (■).

Conclusions

- If phosphite is applied, it should be applied with a wetting agent to avoid phytotoxicity.
- Phosphite caused significant reductions in water relations, but plants recovered quickly.
- Stomatal aperture was not responsible for the reduction water relations.

References

1. Waipara N, et al. 2013. N Z Plant Prot 66: 235-241.
2. Shearer B, et al. 2007. Australas Plant Pathol 36(4): 358-368.
3. Shearer B, et al. 2007. Australas Plant Pathol 36(1): 78-86.
4. Solla A, et al. 2021. Forest Ecol Manage 485.
5. Arnet M, et al. 11th IUFRO WP Conference, Paihia, New Zealand.

Acknowledgements

Scion and Te Roroa for supplying the kauri seedlings.

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Composting of biowaste infected by *Phytophthora cinnamomi*: study of pathogen survival comparing different diagnostic techniques

Composting of biowaste infected by *Phytophthora cinnamomi*: study of pathogen survival comparing different diagnostic techniques.

Guidoni L., Morales-Rodríguez C., Drais M.I., Riggi S., Vannini A.
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INTRODUCTION

The use of bulking agent, such as woodchips, is fundamental to provide the optimal conditions for bio-waste composting. This study aimed to investigate the use of chipped chestnut plants infected by *P. cinnamomi* as bulking agent for bio-waste composting, comparing different diagnostic methods to obtain both qualitative and quantitative data on the pathogen's presence throughout the composting process.

METHODOLOGY

Chestnut plants infected with *P. cinnamomi* and biowaste were composted in two replicate composters over a period of 60 days. Temperature was monitored daily for the first 30 days, baiting was conducted at the beginning and at the end of the processe while metabarcoding and qPCR sampling followed temperature changes on a 10 °C range, resulting in 28 total samples. Laboratory incubation tests were carried out on *P. cinnamomi* grown on millet seeds to assess the effect of temperature on pathogen eradication.

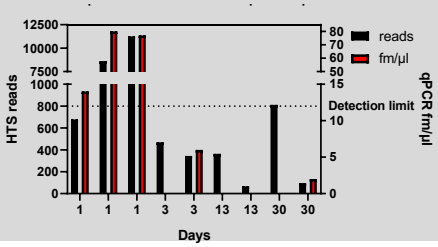
Starting date	18.07.23
U.M.	kg
Woodchips	3
Infected woodchips	3
Bio-waste	20
Total	26

Weights of each component used in the composting process. Values refer to a single composter of 90l. U.M.= unit of measure

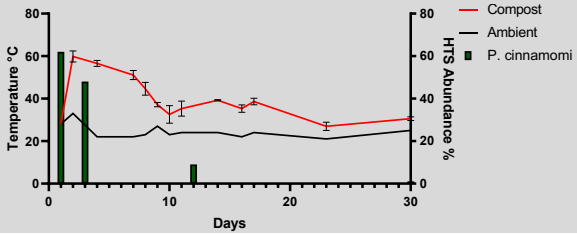
RESULTS

During the thermophilic phase, temperatures stayed above 50°C for five consecutive days. No *P. cinnamomi* growth resulted on PARPNH media from incubation tests on inoculated millet seeds following the same temperature regime of thermophilic phase. All diagnostic techniques detected *P. cinnamomi* on the first day. Only three samples from day 1 were above the qPCR detection limit of 12 fm/µl and considered positive, while metabarcoding detected *P. cinnamomi* in nine samples from day 1 to day 30. Graph 1 shows the comparison between both methodologies for each sample. Metabarcoding was the only technique showing a gradual decrease in the pathogen presence for the first 30 days (Graph 2). By the end of the maturation phase on day 60, no *P. cinnamomi* was detected by any diagnostic test.

Metabarcoding/QPCR comparison



Temperature and pathogen abundance during the active phase



DISCUSSION

Temperatures recorded during the thermophilic phase were sufficient to eradicate *P. cinnamomi*, as confirmed by incubation tests. Frequent turning of the compost is crucial to ensure *P. cinnamomi* is exposed to these lethal temperatures and to prevent its survival at the edges of the pile. Other factors, such as microbial antagonism, feedstock inhibition, and the presence of toxic compounds, may also contribute to the eradication of *P. cinnamomi*, but temperature remains the most reliable and measurable variable. qPCR was less sensitive than metabarcoding in detecting *P. cinnamomi*, especially when it was present in low abundance. Metabarcoding, moreover, allowed to measure the gradual decline of *P. cinnamomi* throughout the composting thanks to its capacity to be quantitative within a single OTU.

CONCLUSIONS

Temperatures recorded during the thermophilic phase can be established as a minimum standard for *P. cinnamomi* eradication in biowaste composting. Metabarcoding proved to be the most reliable technology for pathogen detection compared to qPCR, moreover, it gives the possibility to study entire microbial community than just a single target-organism.

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Global warming and *Phytophthora cinnamomi* invading Fagaceae ecosystems along an altitudinal gradient in the Mediterranean basin

Global warming and *Phytophthora cinnamomi* invading Fagaceae ecosystems along an altitudinal gradient in the Mediterranean basin

Guidoni L., Caccia R., Vannini A. , Sen M., Morales-Rodríguez C.,
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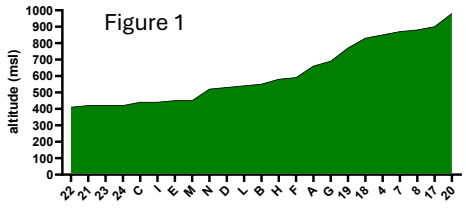
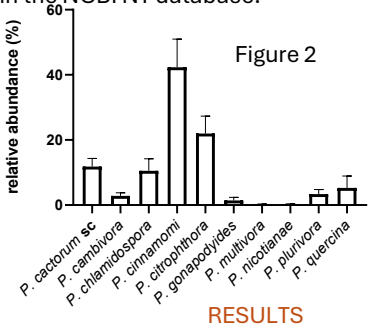
INTRODUCTION

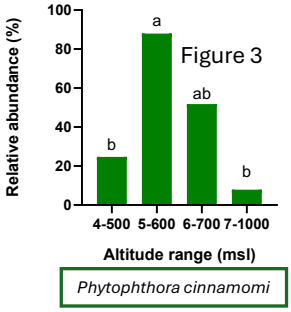
The Monti Cimini forest area is characterized by a succession of Fagaceae. Deciduous oaks dominate at lower altitudes being substituted by sweet chestnut in an altitude range between 550 and 950 m and European beech at the highest altitudes. Ink disease driven by *Phytophthora cinnamomi* represents nowadays the limiting factors for chestnut sustainability in the area. The hypothesis is that global warming is favoring the establishment of *P. cinnamomi* vs the less aggressive *P. xcambivora* at low and medium altitudes. In this work, we studied the changes in soilborne *Phytophthora* community along an altitudinal gradient in Fagaceae ecosystems of the Monti Cimini area using metabarcoding.

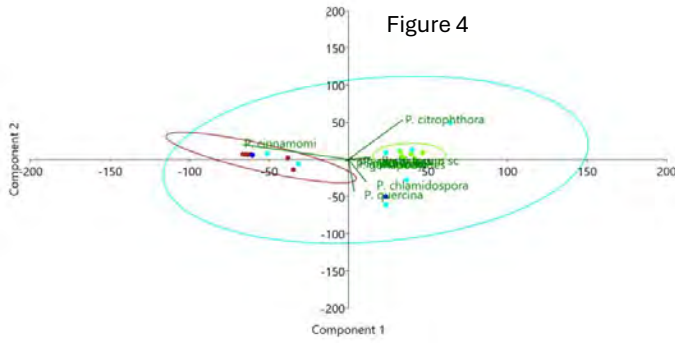
METHODOLOGY

Total DNA was extracted from soil samples (24) collected along an altitudinal gradient from 400 to 1000 msl (Figure 1) in sweet chestnut orchards in the Monti Cimini area in Central Italy. HTS analysis with Illumina MiSeq was run on ITS oomycetes libraries (ITS6/7). Once files with representative sequences, AVS frequency per sample, and taxonomy were created, taxonomy was assigned to ASVs using the Bayes taxonomy classifier in the Feature Classification plugin in the NCBI NT database.









RESULTS

Ten *Phytophthora* species were identified whose relative abundance is shown in Figure 2. *Phytophthora cinnamomi* was the most abundant specifically between 500 and 600 msl, reducing its presence at higher altitudes (Figure 3). *Phytophthora* community was distinct between the medium (5-600 msl, dark red dots) and high (700 and up, light green dots) altitudes, while at lower altitudes the community did not clearly cluster (light blue dots) (Figure 4). *Phytophthora cinnamomi* was characterizing the community at medium altitude (Figure 4).

DISCUSSION and CONCLUSIONS

Surveys carried out in mid '90 up to early 2000 evidenced that *Phytophthora x cambivora* was the main driver of ink disease in the area (Vettraino et al., 2001, 2005; Vannini et al., 2013). The present study revealed a wider distribution of *P. cinnamomi* vs the less aggressive *P. x cambivora* specifically in an altitude range between 500 and 600 msl probably busted by an increase of minimum soil temperature of 1 °C in the last decade (Guidoni, 2019 Bachelor dissertation). Based on the present study *P. cinnamomi* is not favoured at high altitudes but the risk of altitudinal invasion might be high following an additional increase in minimum soil temperature. To be noticed, *P. cinnamomi* seems to be not favoured at low altitudes where long drought periods and high summer temperatures might restrict its spread.

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Exploring Phytophthora community associated with severe decline of cork oak forests in Tunisia: distribution and potential impact

11th Meeting of the IUFRO Working Party 7.02.09: *Phytophthora* in Forests and Natural Ecosystems
8-13 September, 2024
Paihia, New Zealand

Exploring *Phytophthora* community associated with severe decline of cork oak forests in Tunisia: distribution and potential impact

Islem Yangui[1,2] , Carmen Morales-Rodriguez [3], Bruno Scanu [4] , Andrea Brandano [4], Antonio Deidda [4], Salvatore Seddaiu [5] , Luca Sarais [5] and Andrea Vannini[3]

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4 University of Sassari (Italy)
5 AGRIS Sardegna (Italy)

Introduction

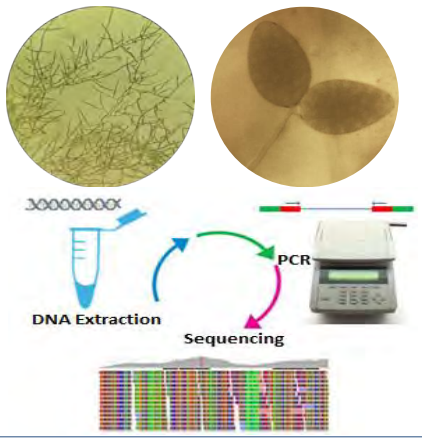
The alarming decline of Tunisian cork oak forests since 2020 represents a complex ecological crisis. While multiple factors likely contribute, a deeper investigation into the distribution and potential impact of *Phytophthora* species as a key driver is urgently needed.

Methodology

Survey and soil sampling

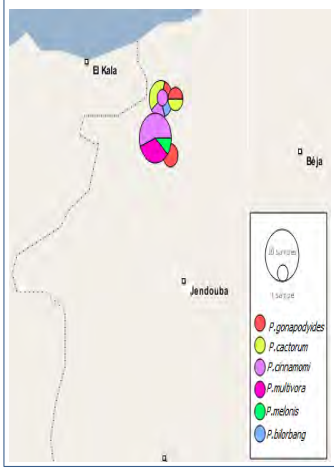
Baiting and isolation

Morphological and molecular identification



Results

Distribution of *Phytophthora*



Out of the nine prospected sites, five yielded positive isolations of *Phytophthora*. The dominant species was *P. cinnamomi*, followed by *P. cactorum* sc and *P. multivora*.

Other unusual *Phytophthora* species for oaks were also detected but with lower frequency like *P.melonis* and *P. bilorbang.*

Ongoing study and conclusion

Pathogenicity test

Conclusion

This is the first comprehensive survey of *Phytophthora* diversity in Tunisian cork oak forests, revealing a concerning prevalence of these pathogens mainly *P. cinnamomi*.
The pathogenicity tests will further elucidate the risk these pathogens pose, enabling the development of suitable conservation strategies.

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Monitoring ink disease epidemics in chestnut and cork oak forests in central Italy with remote sensing data

Monitoring ink disease epidemics in chestnut and cork oak forests in central Italy with remote sensing data

Alessandro Sebastiani [1], Matteo Bertozzi, Andrea Vannini [2], Carmen Morales-Rodriguez [2], Gaia Vaglio Laurin [1,2]

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2 Department for Innovation in Biological, Agro-food, and Forestry Systems, Tuscia University, Viterbo, Italy

Co-funded by the European Union

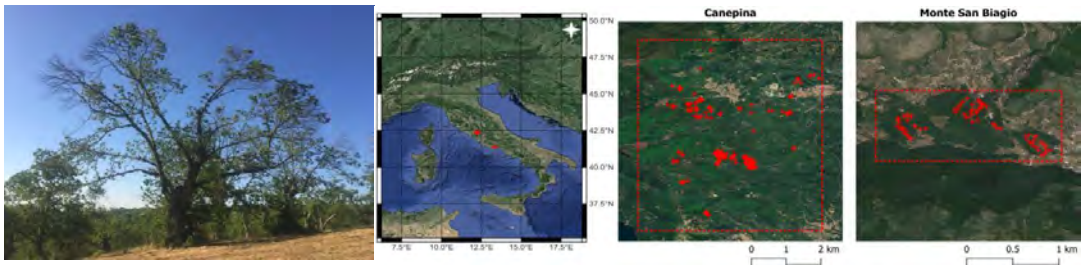
Context and aim

Remote sensing is currently used to monitor forest health including the impact of root rot pathogens such as *Phytophthora* spp. (Vannini et al., 2005, 2009, 2021; Martins et al., 2007). Most of these studies employed high-definition images acquired by aircraft with high operational costs. However, the potential use of free-share or low-cost satellite imageries with medium-resolution for *Phytophthora* impact monitoring in forests is still to be demonstrated

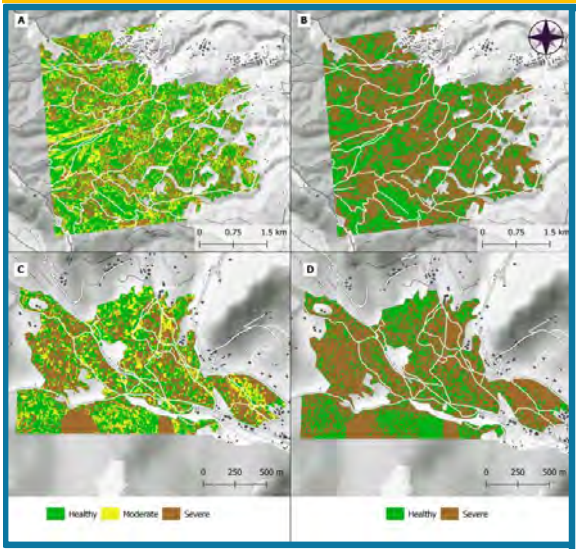
This study aimed to test single and joined **multispectral and SAR data** for detecting the presence and intensity of **ink disease d**riven by ***Phytophthora cinnamomi*** in pure sweet chestnut and cork oak forests in Central Italy in the Municipalities of Canepina and Monte San Biagio respectively

Methods

Since 2015 ground surveys, aimed at collecting information on the impact caused by *Phytophthora* spp., were repeatedly carried out providing detailed maps of infection foci that were used as ground truth data



Multispectral Sentinel-2 (S2) PlanetScope (PS), and SAR Sentinel-1 (S1) imagery covering the period 2018-2022 were used. Several indices computed over healthy, moderately and severely diseased areas at different times were tested to predict the presence and severity of the disease. Remote sensing inputs were selected using the J-M separability test and then used as input for several **Random Forest classifications**.



Main results and conclusive remarks

Sentinel-2 has a better discrimination capability, thanks to the red-edge REIP index.

The majority of the selected indices came from spring and summer, when the reduction of the photosynthetic capacity driven by the action of pathogens is more evident.

Best accuracies for the 2 damage classes classifications were 74% and 68% for Monte San Biagio and Canepina. Monte San Biagio's classification uses S1+S2+PS as inputs; Canepina's uses S2.

Higher **spectral capabilities** are more important than the spatial resolution for large canopies. Instead, the **spatial resolution** (e.g. PS imagery is at 3 m) becomes more relevant with small crowns.

The present study is under final revision for publication on Journal of **Remote Sensing Applications: Society and Environment**

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Citizen science and outreach: *Phytophthora ramorum* education in southern Oregon

Oregon State University – Forestry and Natural Resources Extension

CITIZEN SCIENCE AND OUTREACH

Phytophthora ramorum education in southern Oregon

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SUMMARY

Sudden oak death (SOD), caused by a non-native pathogen *Phytophthora ramorum* has killed hundreds of thousands of tanoak (*Notholithocarpus densiflorus*) trees in Curry County Oregon since it was first detected in 2001. Despite efforts to slow the spread, the pathogen that causes SOD continues to spread in the moist and windy conditions of the southern Oregon coast. New detections of the European lineage (EU1) in 2015 and the North American lineage (NA2) in 2021 has increased the need for outreach education of local landowners about SOD and state quarantine regulations.

Since 2018, Oregon State University Extension has collaborated with Oregon's SOD program and the OSU LeBoldus Lab on a coordinated citizen science and outreach program to teach local residents about disease recognition, early detection methods, and effective treatment options.

CITIZEN SCIENCE PROJECT IMPLEMENTATION

Citizen scientists deploy buckets baits (winter) and stream baits (summer) at the leading edge of the disease and at control sites located within the generally infested area. Bait leaves consist of 2 rhododendron leaves and 2 tanoak leaves (provided to the volunteers).

Bucket baits: Citizen scientist select and sample 2 tanoak trees per site with 2 buckets placed under each tanoak.

Stream baits: Bait leaves are placed in a mesh bag secured by a cord to a stable object near the stream.

Sampling protocol: Bait leaves are mailed to the OSU LeBoldus Lab. Buckets and stream baits are re-set every two weeks for a 2-3 month period.

PROJECT OUTCOMES

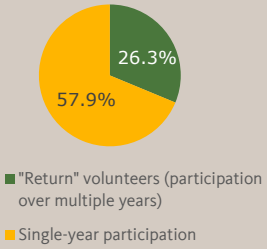
Outreach and education:

- In-person workshops
- Webinars
- Field site visits
- > 600 educational contacts.

Citizen science:

Sampling Results: 2019-2024	
# total samples (bucket and stream baits)	536
# samples positive: non-target *Phytophthora* species	39
# samples positive: *P. ramorum*	12

Percentage of "Return" Volunteers



Oregon State University

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Improved qPCR sensitivity for *Phytophthora pluvialis* detection using a mitochondrial target

SCION
FORESTS • PRODUCTS • INNOVATION

Improved qPCR sensitivity for *Phytophthora pluvialis* detection using a mitochondrial target

R. O'Neill[1]*, E. McLay[1], L. Nunes Leite[1], A. Pérez-Sierra[2], A. Eacock[2], J. M. Le Boldus[3], E. A. Stamm[3], S. Fraser[1], R. L. McDougal[1].
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Introduction

Current detection of *Phytophthora pluvialis*, forest pathogen and causal agent of red needle cast (RNC) in radiata pine (*Pinus radiata)*, relies on isolation and culture, baiting or real-time PCR (qPCR) using a single-copy gene target with limited sensitivity at low levels of infection.

This study aimed to design a qPCR assay targeting multiple-copy mitochondrial gene regions with higher specificity and sensitivity than the current single-copy nuclear gene target, ras-related GTP-binding protein 1 (*ypt1*)*.

Figure 1: *Foliar symptoms of RNC caused by* P. pluvialis *on radiata pine*

Materials and methods

Candidate qPCR assays were designed for four mitochondrial gene regions suitable for primer design (*cox1, cox2, nad9* and *rps10)*. Assays targeting mitochondrial cytochrome c oxidase subunit 2 (*cox2*) were developed the furthest based on success with *in vitro* testing for sensitivity followed by specificity testing using DNA from related and unrelated species. These assays were then tested on DNA collected from infected plant material and a final candidate assay was selected and validated for successful diagnostic application.

Controlled inoculations of radiata pine needles with *P. pluvialis* zoospores were used to assess the sensitivity of qPCR assay detection in a background of host tree DNA (data not shown). Assay performance in field conditions was tested on a robotic qPCR platform using needles expressing typical symptoms of RNC following natural field inoculation.

Results

The successful candidate assay cox2-581F/2R/2P targeting mitochondrial *cox2* region is a highly sensitive, suitably species-specific diagnostic qPCR assay. It has a detection limit of 12.8 fg mycelial DNA and detected *P. pluvialis* DNA on average 6.12 qPCR cycles before the *ypt1* assay; 93-fold more sensitive.

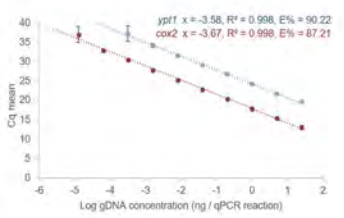

Figure 2: *Standard curves for cox2_581F/2R/2P and Ypap2F/2R/P* using a 5-fold serial dilution of* P. pluvialis *gDNA from 25 ng per reaction down to 2.56 fg, in six technical replicates. The data point showing the highest Cq value is considered to be the limit of detection (LOD) for that assay. (12.8 fg for cox2_581F/2R2P and 320 fg for Ypap2F/2R/P). Error bars represent the standard deviation (SD).*

In forest samples, the *cox2* assay consistently detected *P. pluvialis* similarly ahead of the *ypt1* target for all stages of needle disease symptoms from early lesion infection through to fully cast red needles.

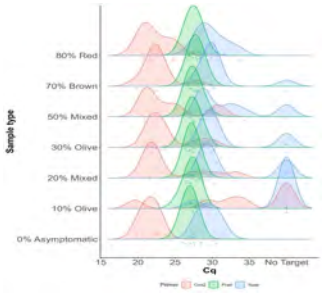

Figure 3: *The sensitivity of* cox2 *(red) and* ypt1 *(blue) qPCR assays as well as radiata pine internal reference control (CAD, green) on forest samples during a natural disease outbreak. Samples were categorised into a range of symptom severities from 0-80+ % severity. The Cq value of each sample is shown as a jittered point and a density curve for each assay shows trends in performance. Samples with no detection are set to a maximum value of 40.*

The cox2_581F/2R/2P assay was able to detect *P. pluvialis* in 100% of samples across all sample types except those with small olive lesions (~ 10% of the needle length), where it was detected in 57% of samples, potentially due to the difficulties with sample homogeneity from small needle fragments containing very little symptomatic material.

However, we also found that the increased sensitivity of this new assay allowed for the detection of asymptomatic infection of *P. pluvialis* in radiata pine forest samples which only began to develop visual symptoms of disease four weeks after positive detection by qPCR.

Conclusions

The new *cox2* target assay has:

- Greatly improved sensitivity (93-fold)
- Consistently detected *P. pluvialis* at lower Cq than the *ypt1* target for all stages of needle disease symptoms.
- Demonstrated use on high-throughput robotic diagnostic platforms
- Detected asymptomatic infection by *P. pluvialis* before the development of visual disease symptoms
- Useful application for informing biosecurity responses, treatment and management plans, further research across multiple forest host species.

Acknowledgements

This work was completed in the Resilient Forests Programme and funded by the Forest Growers Levy Trust and MBIE through Scion's Strategic Science Investment Funding. The authors wish to thank Preeti Panda, David Lane and Dale Corbett for their contributions to this work, and SlipStream Automation for qPCR services.

References

*McDougal RL, Cunningham L, Hunter S, Caird A, Flint H, Lewis A, Ganley RJ. (2021). Molecular detection of *Phytophthora pluvialis*, the causal agent of red needle cast in *Pinus radiata*. J Microbiol Methods. Oct;189:106299.

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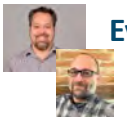
Development of a real-time PCR assay for sensitive detection of mitochondrial targets in *Phytophthora pluvialis*, foliar pathogen of forest trees



Evaluation of prescreening and monitoring methods using sequencing technologies for Phytophthora and oomycetes

Guillaume J. Bilodeau[1] and Hervé Van der Heyden[2]

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Introduction

Metabarcoding can provide insightful information on species diversity, for surveillance of introduced species, while isolation and bating could underestimate species diversity, high throughput sequencing can be used as new tools for early detection of oomycetes including *Phytophthora* species that can cause major diseases, and some are regulated and invasive alien species. Conventional qPCR-based monitoring approaches require a detailed knowledge of the target organisms and be limited to only few species. High throughput sequencing (HTS) technologies allow us to investigate different types of samples, process large numbers of samples and produce even greater volumes of genomic data. Metagenomics tools with different regions combining Ion Torrent sequencing and Oxford Minion nanopore and custom bioinformatic pipelines can be used to evaluate potential sampling methods for pathogens in forestry and agriculture and contribute to identify spreading pathways. Sampling methods exploiting eDNA isolated from air, soil, tissues, have revealed sources of oomycetes potentially cause problems. Few monitoring activities for *Phytophthora* were evaluated to understand the limitations of the technology in biosurveillance. We aim to provide a framework combining sampling tools with HTS-based methods, appropriate bioinformatic pipelines and qPCR assays for early detection of emerging and invasive alien species and determination of type of samples that can be use. Evaluation of those methods will help and improve early warning, promote public awareness, and support our regulatory activities.

Figure 1: Method diagram, from sample preparation to sequence analysis.

Methods

A- Oomycetes Communities Associated with Phytophthora Root Rot in Christmas tree plantations in southern Québec, Canada (https://doi.org/10.1002/edn3.529)

Sampling : Plantations reported with PRR-like symptoms were identified with the help of agronomists and crop specialists. Sampling was conducted in the fall of 2019 and 2020 at 30 sites.

PCR and for High throughput sequencing (HTS): For Ion Torrent sequencing, PCR amplification of the ITS1 region from oomycetes with fusion primers OOM-LO5.8S47 and OOM-UP18S67 were performed (For more information see Tremblay et al. (2019)).

Metadata analysis by a discovery pipeline for oomycetes: Data were analysed using Dr Marc-Olivier Duceppe's pipeline (available here: https://github.com/duceppemo/QIIME2_ITS) with a custom database and appropriate R packages (ivanderheyden/cimdec_phytophthora.github.com)).

B- Genomics-enhanced biovigilance to improve crop disease management

Sampling : Data were collected by Dr. Odile Carisse's (AAFC) team once a week in 2021, 2022 and 2023 in the Muck's region southwest of Montreal using a Cyclone sampler, for a total of ~800 samples (data from 2021 are presented in this poster).

PCR and for High throughput sequencing (HTS): DNA extractions were performed in Dr. Wen Chen's laboratory (AAFC) and sequenced on Illumina (Chen–oomycetes only), IonTorrent (Bilodeau–oomycetes only) and Nanopore (Van der Heyden–oomycetes and fungi).

Metadata analysis by a discovery pipeline for oomycetes: For IonTorrent and Illumina data, the analyses were performed as described in A, while Nanopore data were anlaysed using a custom script based on Minimap2. For all the analyses, we used a database modified from UNITE v9.0 (2023-07-18), which includes the oomycetes DB used in A.

C- Nanopore sequencing for species identification

ITS and rps10 region from *Phytophthora* species obtained from CFIA and NRCAN collections were amplified, sequenced using the Nanopore technologie (V14 kit and R10.4 Flowcell) and identified as described above.

Acknowledgments

The authors would like to thank Jean-François Légaré, Éric Dussault, Christian Lacroix, Dominique Choquette, and Jacinthe Drouin for their contributions to fieldwork and sampling, Debbie Shearlaw, Miranda Newton, Andréanne Sauvageau, and Anne Piuze-Paquet for their technical assistance, and all the growers for giving us access to their plantations. Funding: This study was supported in part by a grant in the Cellule d'innovation des methodologies de diagnostic des ennemis de cultures from the Prime-Vert funding program of the Québec Ministry of Agriculture, Fisheries and Food (19-011-2.2-C-PHYTO). Wen Chen, Odile Carissa AAFC for sampling and DNA material for biovigilance project (Biovigilance pour la durabilité des sols organiques J-002302 (PJS #3304) AGR-16581; Projet 01730-12 de PEGR (SSP #2582) and Euphresco collaborators for DNA material N-000474.

References

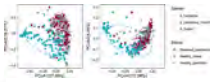
Van der Heyden, H., Duceppe, M.-O., Charron, G., Tanguay, P., & Bilodeau, G. J. (2024) Environmental DNA, 6, e529. https://doi.org/10.1002/edn3.529
Charron G, Yergeau J, Van der Heyden H, Bilodeau GJ, Beaulieu C, Tanguay P. (2024). Plant Dis. 108:6, 1445-1454 https://doi.org/10.1094/PDIS-12-23-2670-SR
Tremblay, ÉD., Duceppe, MO., Thurston, GB., Gagnon, MC., Côté, MJ., Bilodeau, GJ. (2019). Environmental DNA. 1:155–175 https://doi.org/10.1002/edn3.17

Metagenomics is the study of genomic content in a complex mixture of microorganisms. The field of metagenomics has also been referred to as environmental genomics, eco-genomics, and community genomics.

Pre-Screening is the sampling and early detection of potentially harmful organisms, enabling the refinement of sampling regimes and to identify hot spots. This is not a confirmation, and other methods are needed in support.

Results: A- Oomycetes Communities Associated with Phytophthora Root Rot in Christmas tree plantations in southern Québec, Canada.

Figure 2 : Left) Distribution of taxonomic groups across soils from forest samples, healthy plantations, and diseased plantations. The relative abundance of each species was partitioned for these three soil types (healthy forest, healthy plantation, and diseased plantation). Right) Principal coordinate analysis of weighted UniFrac distance between oomycete ASVs inferred from soil samples, showing plantation samples clustering together, while samples from natural forests also clustering together.



A total of 864 samples were processed by PCR, and of these, 219 soil samples, 125 baited samples, and 128 diseased root samples were oomycetes positive, for a total of 572 positive samples, and ~181 million reads.

143 oomycete species, clusters, or complexes were identified. Pythiales (81.51% of the reads), Saprolegniales (12.55% % of the reads), Peronosporales (5.66% of the reads), Lagenidiales (0.25% of the reads), Leptomitales (0.03% of the reads) Albuginales (0.001%).

In the soil, Pythiales (90.89%), Saprolegniales (7.37%), and Peronosporales (1.60%) were the most abundant orders; the others being below 0.01% of the reads obtained for soil.

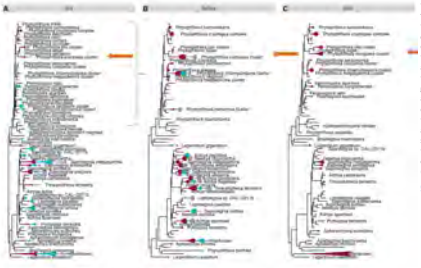


Figure 3 : Taxonomic trees of the clustered ASVs (without the Pythiaceae) inferred from soil (A), rhododendron leaf baiting (B), and roots (C) samples, Arrow represent *P. europea* cluster (*P. abietivora*)

Charron et al. 2024 confirmed species prevalence, https://doi.org/10.1094/PDIS-12-23-2670-SR:
The obtained isolates were identified using a multi-locus sequencing and phylogenetic approach. A total of 44 isolates were identified, including eight *P. chlamydospora*, eight *P. abietivora*, seven *P. gonapodyides*, three *P. gregata*, six *P. megasperma*, and two *P. kelmanii* isolates, plus 10 isolates belonging to a previously unknown taxon that is phylogenetically close to *P. chlamydospora* and *P. gonapodyides*. Among the known species, *P. abietivora* was the most prevalent isolated species associated with trees showing aboveground PRR-like symptoms.

Results: B- Genomics-enhanced biovigilance to improve crop disease management

Organic Soil, for 2021 data: Ion Torrent for oomycetes 112 samples were sequenced, for 24M seqs with Ion Torrent, Nanopore and Illumina also prepared. Different species as *P. pseudosyringae* identified and other oomycetes potentially important on the different farm. Results from the three sequencing technologies were compared.

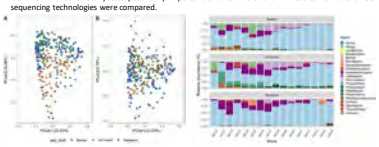


Figure 4 : a) PCoA showing , b) genus bar chart of relative abundance by technologies

Results: C- Nanopore sequencing for species identification

Table 1: Preliminary results of the comparative study between ITS and rps10 regions obtained by **Nanopore** sequencing (V14 kit, flow cell R10.4), on a subset of *Phytophthora* species.

Species	ITS	rps10	Species	ITS	rps10	Species	ITS	rps10
P. asparagi			*P. sansomeana*			*P. sansomeana*		
P. brassicae			*P. syringae*			*P. europaea*		
P. cactorum			*P. trifolii*			*P. abietivora*		
P. cinnamomi			*P. irrigata*			*P. erythroseptica*		
P. drechsleri			*P. ramorum*			*P. porri*		
P. fallax			*P. gregata*			*P. primulae*		
P. foliorum			*P. boehmeriae*			*P. sojae*		
P. gallica			*P. quininea*			*P. alni subsp. alni*		
P. hibernalis			*P. richardiae*			*P. alni subsp. uniformis*		
P. insolita			*P. rubi*			*P. kelmanii*		
P. lateralis			*P. morindae*			*P. megasperma*		
P. nemorosa			*P. gonapodyides*			*P. cambivora*		
P. nicotianae			*P. chlamydospora*			*P. kernoviae*		
P. pinifolia			*P. cryptogea*			*P. bonnalii*		
P. quercina			*P. pseudosyringae*			*P. riparia*		

Species that can be directly identified
Species can't be resolved and need to be grouped in a cluster of species

Discussion/Conclusion

- Establish an oomycetes profile, allowing us to identify novel or emerging pathogens, including PRR, that may present a threat.
- We showed that the *P. cryptogea* cluster, *P. europaea* cluster, *P. sansomea*, and *P. chlamydospora* cluster were significantly more abundant in soils collected from plantations under diseased trees.
- We confirmed that the *P. europaea* cluster (which includes *P. abietivora*) was most frequently associated with trees showing *Phytophthora* root rot-like symptoms.
- We report that land use (anthropogenic activities) shapes oomycete diversity; plantations can act as a gateway for invading natural forests. The *P. europaea* cluster might already have crossed this boundary, and other species might follow, advocating the importance of improved surveillance of oomycete diversity in various environments.
- However, to enable the resolution of these *Phytophthora* clusters at the species, the presence and abundance of some *Phytophthora* species need to be confirmed either with direct isolation, species-specific molecular tools, or new metabarcodes, such as the rps10 gene (Foster et al., 2022).
- We are continuing the evaluation of the *Phytophthora* and oomycete airborne and soilborne communities with various sequencing methods and metabarcodes (ITS, rps10, whole genome) (preliminary analysis).

Challenges, limitations and perspectives

- May provide guidance for CFIA biosurveillance surveys (perspectives)
- Potential identification of hotspots and high-risk areas (perspectives)
- These tools have opened the door to design novel approaches for rapidly development of molecular methods (perspectives)
- Facilitate detection and identification of the pathobiome and improve the efficiency of our regulatory activities (perspective)
- Tsunami of data that they generated and their requirements in terms of standard and non-standard analysis and reference databases, which are more developed for some pathogens than others.
- Identification is sometimes impossible beyond the family or genus levels (limitation)
- NGS error rates can exceed the genetic differences between species (limitation)
- Consequences of the results in regulatory research, double check with other methods (challenge)
- Baseline of what is present or might be present, Be careful on resolution of some genomic regions, this is detection and not a first report, no Koch postulates (limitation)

Connections with other projects:

- Euphresco: Understanding and managing the impact of *Phytophthora* in horticulture.
- *Phytophthora* in public gardens: understanding pathways and mitigating risks (Phyto-gard).

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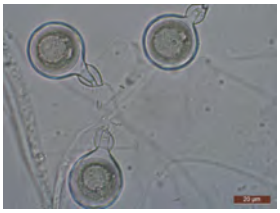
Use of high-throughput automated qPCR for rapid detection of *Phytophthora agathicida*



Use of high-throughput automated qPCR for rapid detection of *Phytophthora agathidicida*

Kiryn Dobbie[1], Suzanne Simpson[2], Renelle O'Neill[1], Katie Daley[1], Benjamin Franzmayr[2], Mike Cook[2], Rebecca McDougal[1]
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Introduction

Phytophthora agathidicida (PA), the causal agent of kauri dieback disease, is a soil-borne pathogen causing disease of kauri (*Agathis australis*) and results in yellowing of the foliage, bleeding cankers on the lower trunk, and eventual tree death (Figure 1). Currently, PA presence is confirmed via soil baiting. The protocol is a lengthy, labour-intensive, and costly. Several factors, including the presence of other oomycetes, in particular *P. cinnamomi* can affect the accuracy of this test. The aim of this project was to assess the possibility of using a high-throughput system as a viable, cost effective and efficient method for testing large numbers of samples for PA detection.

Materials and methods

Treatments included PA and *P. cinnamomi* in isolation and in combination and a negative control with no *Phytophthora*. Soil baits (*Cedrus deodara*) were plated onto PARPH after 5 days of baiting soils and assessed for the presence of PA and *P. cinnamomi*. Baits that were negative for PA were peeled from the agar plates and sent to SlipStream Automation where genomic DNA extractions and qPCR analysis was performed. Two qPCR assays were used for detection of PA including a multiple-copy gene target (internal transcribed spacer (ITS), Than et al., 2013) and a single-copy gene target (ras-related ypt protein (Ypt1), C. Probst, Unpublished, Manaaki Whenua).

Credit: © Ministry of Primary Industries

Figure 1: ***Mortality of kauri trees due to PA infection.***

Results

From the soil baiting, PA and *P. cinnamomi* were readily isolated from their respective treatments where only *P. cinnamomi* was isolated from the combination treatment. Figure 3 shows the results from SlipStream Automation for the ITS and Ypt assays. The ITS assay was more sensitive than the Ypt assay. The target DNA is amplified at a lower number of cycles in the positive control, PA and PA + *P. cinnamomi* treatments, than the negative control and *P. cinnamomi* treatments, which did not contain PA, however there was still amplification in these latter treatments.

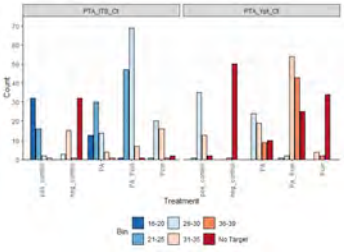

Figure 3: ***Results from the ITS and Ypt assays using negative peeled cedar baits.***.

Optimisation work

Further dilutions with PA and PA + cedar resulted in an abnormal curve using the ITS assay for the PA + cedar indicating that there is some host inhibition/interaction (Figure 4). *P. cinnamomi* and cedar DNA were tested using both assays in isolation. There was no amplification with *P. cinnamomi* DNA but there was amplification from cedar DNA using the ITS assay. We assessed a High-Resolution Melting Assay (HRM, unpublished) for the validation of our suspected false positives. This assay differentiates between PA and other *Phytophthora* species. Due to changes in equipment, the HRM was re-validated. Results were replicated between Scion and SlipStream and showed that PA and *P. cinnamomi* could be detected within cedar baits (Figure 5).

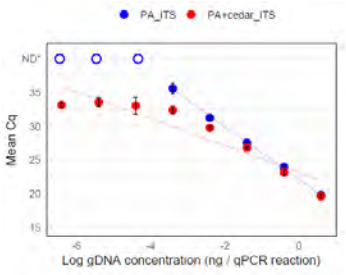

Figure 4: ***qPCR standard curve ITS. ND* = not detected***

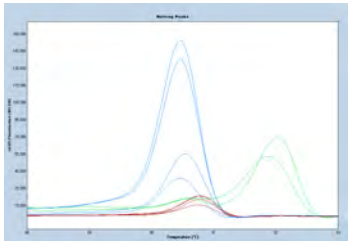

Figure 5: ***HRM, SlipStream Automation showing results with Cedar spiked with PA (blue) and P. cinnamomi (green).***

Conclusions

- The development of a high-throughput system for detecting PA in soil baits shows promise
- Further assessment of the potential interference of the cedar bait on the ITS and YPT1 assays needs to be conducted.
- Validation of the suspected false positives needs to be carried out using the HRM and subsequent sequencing where necessary.

Acknowledgements

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References

Than, D., Hughes, K., Boonhan, N., Tomlinson, J., Woodhall, J., & Bellgard, S. (2013). ATaq Man real-time PCR assay for the detection of Phytophthora 'taxon Agathis' in soil, pathogen of Kauri in New Zealand. *Forest Pathology, 43(4), 324-330.*

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