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Refined systematics (taxonomy, nomenclature and phylogenetics) of *Phytophthora* for more accurate morphological-molecular identification: The importance of types and authenticated specimens.

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Phytophthora with 98 species, is an ecologically important genus that has been well-studied. Although considerable advances in molecular systematics have been made there is still confusion in recognizing new species and difficulty in identifying described species. This is due in part to the number of sequences in GenBank that are from misidentified cultures or that are poorly annotated. Species complexes for *P. capsici*, *P. citricola*, *P. cryptogea*, *P. dreschleri*, *P. megasperma*, and others have been named due to the difficulties to identify the sensu stricto or extype clusters. The position of the extypes suggests the presence of few to several distinct species in each complex. Establishing a database of sequences from accurately identified material is vital so that others can use that tool to make correct molecular identifications. Ex types define the species so sequences from those isolates should be the primary reference in the database. The World Phytophthora Genetic Resource Collection (WPC) is a valuable biological resource containing many ex types and has been extensively used in the Phytophthora Database (PD) which is a web-based source of genotypic and phenotypic data for the international community. The USDA PPQ Molecular Diagnostic Laboratory (MDL) with the WPC and PD are using the morphology and sequences of extypes and authenticated representative specimens to develop an innovative, interactive morphological-phylogenetic identification key. Some *Phytophthora* species of concern to the USA, such as *P. boehmeriae*-like, *P. quercina*-like have been reported from other countries but may have been misidentified and clarification is essential for regulatory purposes. The clear status of emerging pathogens and aspects of population genetics is important at this time of rapidly increasing international trade.

Phytophthora cinnamomi in the Mexican oak forest

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Ink disease, caused by *Phytophthora cinnamomi*, was reported for the first time in Mexico in 2000 affecting more than 800 ha of oak forest at El Arrayanal, Colima. Three years later, it was detected in oaks of the protected area called Reserva de la Biosfera of Manantlan, Jalisco State. In 2005, the pathogen was found causing massive oak mortality in Tecoanapa, Guerrero affecting approximately 1 500 ha. The main symptoms of ink disease include foliar necrosis, wilting, dieback and sapwood bleeding causing a black stain on the bark. To isolate the pathogen, plating of canker tissues in the field and the use of trap discs of the *Azalea* plant, available in Mexican nurseries, have been very useful. Molecular and morphological characterizations have been used to identify the pathogen.

The control strategies thus far implemented by CONAFOR, a national institution, are based on *Trichoderma* as compost incorporated in the soil and as liquid sprayed on the trunk base. Results have been limited showing that in the first case, more than one application is necessary and in the second case, it is possible to kill the cankers, however, new ones will appear the next season.

Experimental silvicultural activities including thinning, sanitation cuts, pruning, and the use of controlled fire have been under evaluation since 2002. Preliminary results showed that in plots where sanitation cuts were done, natural regeneration with resistant species has been successful. Pruned trees are responding well. In the case of thinning, infected trees died but the remaining trees look healthy.

Confirm Detection of *Phytophthora ramorum* and *P. palmivora* from positive ImmunoStrips® components using PCR

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This study was conducted to determine if ImmunoStrips® developed in Agdia to detect *Phytophthora* species can be used as the DNA source to confirm the detection of *Phytophthora ramorum* and *P. palmivora* by PCR. Samples flow vertically in the ImmunoStrips® from a sample pad to a conjugate pad, to a nitrocellulose membrane and then an absorbent pad. The test is negative if only the control line is visible and positive if two lines, control and test line; are observed. DNA was extracted from test line and sections of sample pads of ImmunoStrips® testing positive with extracts of rhododendron leaves infected with *P. ramorum* and extracts from palm trees infected with *P. palmivora.* PCR using the primer sets PHY-OO.F/R, specific to *Phytophthora*; amplified ca 750-800 bp. The sequence of the PCR indicated that *P. ramorum* was amplified in 100% of the DNA samples from positive ImmunoStrips® to *P. ramorum*. *P. palmivora* was amplified in 87% of DNA extracted from sample pads and 70% from the test line. These results suggested that positive ImmunoStrips® can be used to confirm the detection of *P. ramorum* and *P. palmivora* by PCR. Sample pads were more accurate to confirm the detection of *P. palmivora* but either the sample pad or the test line can be used to confirm the detection of *P. ramorum*. The ImmunoStrips® can be recommended as field tests and the pad as a source of DNA to confirm the detection *P. ramorum and P. palmivora* on the original field samples.

Pathogenicity of *Phytophthora* taxon Agathis (PTA)

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Kauri (*Agathis australis*, Araucariaceae) is a dominant tree of lowland native forests in northern New Zealand.
Phytophthora taxon Agathis (PTA) was first recorded from this host (as *P. heveae*) in 1974. Phylogenetic analysis indicate it is closely related to *P. castaneae* (syn *P. katsurae*), but it differs from this species morphologically in its rugose, as distinct from bullate, oogonia (Beever et al 2009). In the field, it associated with a collar rot, causing large bleeding lesions near the ground, yellowing foliage and frequent tree death. Quantitative permanent plot data indicate trees of a wide size range are affected in natural ecosystems. Pathogenicity tests in a greenhouse using small saplings and agar plug inoculation showed PTA is highly virulent to kauri. However, they indicate it is not virulent, or only slightly so, to species of *Araucaria* from Brazil (*A. angustifolia*) and Australasia (*A. bidwillii*, *A columnaris*, *A. cunninghamii*) and to a range of other woody species that occur in kauri ecosystems.

Beever, R. E., Waipara, N. W., Ramsfield, T. D., Dick, M.A., & Horner, I.J. 2009. Kauri (*Agathis australis*) under threat from *Phytophthora*? In E. M. Goheen & S.J. Frankel (Ed.) *Proceedings of the fourth meeting of the International Union of Forest Research Organizations (IUFRO) Working Party S07.02.09: Phytophthoras in forests and natural ecosystems. Gen. Tech. Rep. PSW-GTR-221* (pp. 74-85). Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station.

Comparative efficacy of hygiene treatments used for disinfestation of *Phytophthora* Taxon *Agathis* (PTA) inoculum contained in soil

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A series of *in vitro* and soil-bioassay based experiments examined the comparative efficacy of TriGene II Advance™ (a halogenated tertiary amine) to suppress PTA inoculum (i.e. mycelium, motile zoospores and thick-walled oospores) against alternative hygiene agents. Three other generic classes of active ingredient were compared; quaternary ammonium (e.g. Phytoclean™), dipotassium peroxodisulphate (e.g. Virkon® S) and sodium hypochlorite (i.e. household bleach, Janola®). Of the agents tested, TriGene, and Phytoclean completely suppressed growth of PTA mycelium at all *in vitro* concentrations tested. Zoospores placed into TriGene (2%), Phytoclean (10%), Virkon (1%) and Janola (5%), did not survive, and could not initiate any colony formation after treatment. Virkon (0.2% a.i.), and Janola (0.05% a.i.) had the most significant impact on oospore viability. To test the efficacy of the treatments against PTA in soil, 1,500 oospores of PTA / gram were added to a soil sampled from the Huia Dam-site. Soaking the PTA infested soil in TriGene (2%) and Phytoclean (10%) completely suppressed PTA. Soaking the PTA infested soil in Virkon (1%) and Janola (5%) suppressed PTA, but not all soil fungi and bacteria. Results of the spray treatments showed a similar trend with all four treatments significantly reducing the infective potential of PTA.

Ectomycorrhizal community structure in a healthy and a Phytophthora-infected chestnut (*Castanea sativa* Mill.) stand in central Italy

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Ink disease caused by *Phytophthora cambivora* is a major disease of sweet chestnut (*Castanea sativa*). In two *C. sativa* stands in central Italy, one (Montesanti) that is infected with *P. cambivora* and the trees showing symptoms of ink disease and another healthy stand (Puzzella), the ectomycorrhizal (ECM) community structure was investigated. On the roots of the surviving trees of the diseased stand, 29 different ECM species were determined compared to 23 in the healthy stand. Eleven ECM species were common to both stands; however, a number of species were unique to one of the stands. *Cenococcum geophilum* was dominant at both sites, but the percentage colonisation was much higher at Montesanti (40.8%) compared to Puzzella (27.2%). There was a switch in species from *Russula vesca*, *Russula lepida* and *Russula azurea* at Puzzella to *Russula nigricans*, *R. lepida* and *Russula delica* at Montesanti. Both *R. vesca* and *R. azurea* were found only at the Puzzella site. At the diseased site, the ECMs formed had a smaller root tip diameter, and the ECM at the healthy site had more abundant extramatrical hyphae.

FP801 From Nursery to Forest: The Increasing Threat to Trees and Forest Ecosystems from the Genus *Phytophthora*

Working Group 3: Diagnostics

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In Diagnostics for *Phytophthora* different aspects has to be handled with.
We identified the following aspects which should be addressed in the topic of Diagnostics for *Phytophthora* and raised questions to be answered for the different fields:

- Visual symptoms: Can we make a correct diagnosis on visual symptoms? Do we have clear symptoms?
- Sampling: What is the correct sampling strategy for symptomless material?
- Baiting: Is there a Golden Bait?
- Morphology: Can we identify correctly *Phytophthora* based upon morphology and which structures do we need for that?
- Serology: Is there enough specificity for *Phytophthora* species identification using antibodies available?
- Molecular Biology: Do we have enough sequence information for species identification? Which genes? What kind of techniques/methods do we have for molecular identification/detection?
- Identification: What kind of techniques/methods do we have for *Phytophthora* identification?
- Detection: What kind of techniques/methods do we have for *Phytophthora* detection?
- Databases: Make an inventory of the databases useful for *Phytophthora*
- Links: Useful links for *Phytophthora* Diagnostics?

WG3 of COST action FP801 (www.abdn.ac.uk/woodland-threats/) has at the moment 50 members from 20 countries. An inventory has been made on the existing expertise in the different participating countries in the different topic fields mentioned above. Within WG3 a web portal has been set up in which all information will be gathered and distributed to the WG3 members concerning the different fields addressed above.

Interactions between tanoak and *Phytophthora ramorum* on a microscopic and molecular scale

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Physiological interactions between the pathogen, *Phytophthora ramorum,* and one of its major hosts, *Lithocarpus densiflorus*, tanoak, are not well understood. The study of this interaction is important because tanoak is a species whose survival is threatened by this pathogen, and that plays a large role in facilitating the continued spread of the pathogen. The goals of our research are to determine which tissues and cell types of tanoak bark *P. ramorum* colonizes, identify the host defense responses that occur in infected tanoak tissue, and determine where elicitins are produced and localized in bark tissue. Elicitins are unique small proteins, produced by the pathogen with some implications for pathogenicity. The elicitin was traced in tanoak tissue through the use of a specific fluorescent antibody.

In results to date, hyphae have been observed entering bark and colonizing various bark tissues. Histological stains reveal production of phenolics and other host defense responses in conjunction with infection by *P. ramorum.* *P. ramorum* elicitin is produced in infected tanoak phloem tissues, illuminating hyphal call walls. Of the hyphae observable with this method, hyphal tips are seen in particular abundance. Hyphae have only been observed by this method in tissues that had been inoculated only two weeks before, and that were not yet heavily damaged.

New tree hosts and new disease etiologies beneath larch (*Larix*) infected by *Phytophthora ramorum* in the UK

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Prior to 2009 records of invasive *Phytophthora ramorum* on woodland trees in the UK were limited. Most consisted of bleeding stem lesions on beech (*Fagus*), *Nothofagus* and *Quercus* spp. and foliar lesions on *Castanea* and *Q. ilex* in western Cornwall. In August – October 2009, however, stands of mature Japanese larch, *Larix kaempferi* in south-west England developed extensive dieback and both foliage and bark were found to be infected with *P. ramorum* (see Webber *et al.* abstract). Many other trees and shrubs beneath the larch canopy were also infected. Some represent new host records and some new disease etiologies. Others represent more frequent or more severe expression of etiologies observed previously. For example young hemlock (*Tsuga*) was found for the first time with dieback and resinous stem lesions. Birch (*Betula*) was also found with bleeding stem lesions, associated with slash wounds. Sweet chestnut (*Castanea*) often exhibit heavy foliar and shoot infection, and defoliation; some have also developed large stem lesions but without bleeding. Beech and *Nothofagus* often show bleeding stem lesions at 5-9m above ground level; and the pathogen has been isolated from beech foliage for the first time. Understory rhododendron also appears to be infected from the larch. These new developments apparently result from (i) very heavy inoculum pressure from the larch canopy; (ii) exposure of new hosts to *P. ramorum* inoculum; (iii) three high rainfall summers in succession. They emphasise that much remains to be learned about the threat *P. ramorum* poses to trees on both sides of the Atlantic.

Tracking populations and new infections of *Phytophthora ramorum* in southern Oregon forests with DNA fingerprinting

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How *Phytophthora ramorum* infects and spreads through individual trees and entire forests is a topic of great interest. *P. ramorum* can infect plants through leaves, stems, or bark and persists in streams and soils. To better understand pathogen dispersal and infection in tanoak forests, we intensively sampled and genotyped isolates at several spatial and temporal scales. We collected isolates from infected plants and from soils, streams, and rainwater in southern Oregon forests. From 2001 to 2008, we identified 68 novel multilocus genotypes (MGs) with 10 to 35 MGs found in each year. While the majority of MGs were present in very low numbers (< 1%), one MG was dominant in all years representing 35 to 65% of isolates. We also genotyped isolates from multiple lesions within single trees. In 50% of the trees sampled more then one MG was recovered, demonstrating more than one individual isolate can infect a single host. Our data shows a likely introduction of *P. ramorum* into Oregon forests by one or few MGs followed by dispersal through wind and splash giving rise to new subpopulations in Southern Oregon with no new introductions from California forests or Oregon nurseries.

Molecular characterisation of a Phytophthora hybrid swarm in native ecosystems and waterways in Western Australia

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Studies in native forests, woodlands and waterways in Western Australia (WA) have recovered several hundred *Phytophthora* isolates belonging to ITS clade 6. With the exception of *P. inundata* and two isolates of *P. megasperma*, none of the isolates recovered correspond to any described species. In a phylogeny of ITS clade 6 the majority of isolates cluster together within sub-clade II with *P. gonapodyides* as the basal species. Two new species, currently designated at P.sp.3 and P.sp.11, have been identified within the cluster. However, most other isolates contained obvious single base pair polymorphisms (between 2 and 20). The ambiguous positions are not random but are always among the 40 variable positions that can be found within the WA cluster. In addition, there were many isolates for which only partial sequence could be obtained. After cloning of the ITS region of several isolates, arrays of between 2 and 8 alleles have been found for each isolate, some containing indels (up to 7 bp throughout the ITS sequence) as well as single base pair polymorphisms. Subsequently the *cox*1 region has been cloned, and while some isolates which are polymorphic in the ITS region are monomorphic in the *cox*1 region, other isolates are polymorphic in both regions. These data provide evidence for extensive and common hybridisation and supports both sexual and somatic hybridisation events. The hybrids appear to be stable, as there is evidence in the ITS region of ongoing homogenisation through crossing over. These isolates belong to a hybrid swarm, the importance of which in the natural environment is unclear. The implications of these findings will be discussed.

Finding the last stands of healthy Banksias protectable from *Phytophthora cinnamomi*

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Of the 78 described Banksia species, 61 species are endemic to Western Australia and over 40 of these occur within the high (600mm+) rainfall zone of the South West of the State. Site characteristics where Banksia species flourish are similar to those that favour the establishment of the introduced and invasive *Phytophthora cinnamomi.* Soils are nutritionally poor, often with impeded drainage and receive more than an average of 400mm of rainfall.

The distributions of all Banksia species known to occur in the south west of WA were overlain on the State's strategic dieback mapping, developed by Project Dieback a natural resource management initiative. The main source of the Banksia data was the Banksia Atlas which contains species distribution information collected with community input in the 1980's. This has informed a cross regional risk analysis addressing the goal of finding the most protectable areas of uninfested native vegetation.

The main conclusions from the study:

- Many plant species are seriously threatened with few uninfested populations remaining.
- There is an urgent need to focus broad scale management activities to protect WA's uninfested landscapes.

  Many coastal heath and woodland communities require assessment and nomination as threatened ecological communities which would help convey a sense of urgency with regard to their protection.

The use of compost to control *Phytophthora cinnamomi* in cork and holm oak seedlings

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Cork and holm oaks are evergreen Mediterranean oaks that have a very important ecological and economic impact in the Iberian Peninsula. The decline caused by *Phytophthora cinnamomi* is the main disease that in the last decades leading a widespread mortality of *Quercus suber* and *Q. ilex* in this area.

In order to study the effectiveness of compost for control of *P. cinnamomi* field and greenhouse assays were set. Field experiments were also designed to screen the potential tolerance based on genetic background of selected trees. Acorns of sixty four cork oaks and sixty holm oaks located in distinct regions of Portugal and Spain were sown in naturally heavily infested sites in Algarve (South of Portugal). For each species, half of acorns were sown in area amended with compost and, the other half in an area not amended. The percentage of acorn germination, mortality and height of the seedlings was evaluated.

In the greenhouse experiments were used 9 months-old plants from six cork oak families and four holm oak families in a peat-sand based subtract amended and not amended with compost. The plants were inoculated with *P. cinnamomi* and subjected to waterlogging 2 days per week. After 3 months all plants were removed and the severity of their areal parts and root system evaluated according to a scale 0-4 (0 = aerial part or root without symptoms, 4 = aerial part or dead root).

The results will be presented and discussed.

Genotypes of *Phytophthora ramorum* in Waterways in Washington State

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Since its initial detection in a nursery in 2003, *Phytophthora ramorum* has been found in a number of additional nurseries and spread into two waterways in western Washington. The first detection of this pathogen in a stream occurred in 2006 when it was detected in a seasonal Pierce County stream associated with a positive nursery. Subsequent baiting detected the pathogen downstream from the original nursery site in 2007, 2008, and 2009. Using eight microsatellite markers, genotyping isolates or DNA samples from this stream identified six genotypes of the NA1 lineage of *P. ramorum*. The second positive waterway is located in King County. *Phytophthora ramorum* was initially detected in a retention pond outside of a positive nursery and the river into which the pond flows in 2007. The 2007 river-positive bait site was positive again on two separate occasions in 2008. Extensive baiting of the river and retention pond during 2009 resulted in multiple positives at several bait sites at both locations. The presence of the European (EU1) and North American (NA1 and NA2) lineages of *P. ramorum* in the river, but only the NA1 lineage in the positive nursery, suggests that more than one source of inoculum may have contaminated this river. The spread of all three known lineages of *P. ramorum* into these waterways increases the risk that one or more lineages of this pathogen will spread onto plants in the landscape.

Susceptibility of 14 *Abies* spp. to Phytophthora Root Rot

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Phytophthora root rot is a common disease in Christmas tree plantations. A replicated field trial was established in 2004 to determine the susceptibility of 79 sources of 14 *Abies* spp. to this disease. The trial consists of 100 3mX3m cells laid out in a checkerboard design. A single seedling from each source was planted in each cell. To encourage disease development, individual cells were flooded for 2 to 3 days during the growing season in 2007, 2008, and 2009. Initial symptoms, wilting and branch flagging, were evident on some trees by early summer 2007. Since then, the incidence of symptomatic trees has increased each year. To obtain preliminary data on the susceptibility of each of the different species and sources of trees in this experiment, all of the trees in 26 of the cells were excavated during late June to early August 2009. Symptoms were recorded for each tree and the root systems were washed and examined for evidence of root rot. Isolations were done from symptomatic roots and stems to determine the *Phytophthora* species associated with root rot. Over 70% of the Shasta fir and 60% of the noble fir were killed by root rot. White fir (30%) and Fraser fir (23%) were the next most susceptible species. Less than 5% of the Turkish, Nordmann, grand, Nikko, and Canaan fir had evidence of root rot. Isolations indicated that the root rot in this trial was caused by *P. cambivora, P. cryptogea, P. citricola*, and *P. gonapodyides*.

Evaluation of algaecides to eliminate propagules of *Phytophthora* spp. in naturally-infested streams in South Carolina, USA

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Many algaecides are registered for use in potable water, irrigation ponds, and swimming pools. Because Oomycetes, including species of *Phytophthora*, are closely related to brown algae, algaecides may be effective at eliminating propagules of *Phytophthora* spp. in water. In laboratory studies, we have shown that two algaecides with copper hydroxide and copper carbonate as active ingredients were lethal to zoospores of seven species of *Phytophthora (P. cactorum, P. citricola, P. citrophthora, P. cryptogea, P. nicotianae, P. palmivora, P. ramorum)* at 1 h after treatment; sporangia of three of these species were eliminated at 4 h and chlamydospores of *P. ramorum* were eliminated at 8 h after treatment. Another algaecide with hydrogen dioxide as the active ingredient was less effective. In 15-L aliquots of water collected from six, naturally-infested streams in South Carolina, copper hydroxide and copper carbonate were lethal to propagules of *Phytophthora* spp. 1 h after treatment whereas hydrogen dioxide again was less effective. Recently, we have investigated the effects of season and temperature on the efficacy of a copper-hydroxide algaecide on natural populations of *Phytophthora* spp. in two streams. Aliquots of 10-L were collected in February, April, June, and August and were maintained at 5, 10, and 22°C during treatment; propagules occasionally were detected at 2 h after treatment but were not detected at 4 h after treatment. Copper algaecides appear to be effective throughout the year and at a range of temperatures; therefore, they may provide an effective management strategy for species of *Phytophthora* in some water systems.

Phytophthora Disease Management during the Relocation of a Bauxite Mine in the Jarrah Forest of Western Australia.

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Alcoa mines bauxite in the jarrah forest of Western Australia where the pathogen *Phytophthora cinnamomi* is widespread in the soil. A critical environmental objective of the Company is to mine and restore the forest with minimal spread of the pathogen. The current mine at Huntly clears and restores about 420 ha a year in forest where the pathogen is predominantly absent. Intensive disease management procedures are in place – these include constructing Phytophthora-free road surfaces and cleaning large earthmoving equipment in the field. In 2013 the mine relocates to an adjacent region of the forest where the majority of the forest is infested with *P. cinnamomi*. During the transition period both mines will be operating with the same fleet of trucks and earthmoving machinery. The relocation has major implications to the disease management program with the transition phase causing particular challenges. A process has been implemented to undertake an environmental risk assessment and a financial assessment of a range of management options. The assessment process will be discussed and the final disease management plan will be presented.

A new approach to changing the behaviours of users of remnant woodlands in the Perth region.

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Community-based social marketing is based upon research in the social sciences that demonstrates that behaviour change is most effectively achieved through initiatives delivered at the community level. The focus is on removing or minimising the barriers that prevent people from changing their behaviour while also making it easier or more attractive to do the right thing. The Dieback Community-based Social Marketing Strategy explores the behaviour characteristics towards Phytophthora dieback awareness and containment in five woodland and forest reserves located in five local government areas in and around Perth.

The project incorporated (i) preliminary research, (ii) development of a pre-campaign and post-campaign survey, (iii) feedback from focus groups of people living near the reserves and (iv) the implementation of communication tools. The collation of data from focus group discussions and telephone surveys data was required at the commencement and completion of the behaviour change project so we could use statistics to test for the significance of changes. The focus groups and survey outcomes provided the basis for development of the marketing and communication tools that were implemented on the reserves (e.g. signage and soil brush down stations).

The data gathered through the survey and focus groups provided a rich insight and depth into the understanding of local attitudes towards dieback, the reserves and their management. The limited but significant change to attitudes will be discussed.

Best Practice Management Framework for Phytophthora Dieback in southwest Western Australia

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The introduced plant pathogen *Phytophthora cinnamomi* (cause of Phytophthora dieback disease) is a major threat to the biodiversity of the southwest of Western Australia. Effective disease management procedures have been developed by state government departments and mining companies. These procedures are now known to other managers of natural ecosystems such as local governments (LGAs) but they are not used consistently and routinely. We recognized that there was a need for a system to cement these procedures in place at all relevant LGAs so that dieback management is not the responsibility of one person, but instead, is a standard work procedure similar to safety management procedures. LGAs manage many natural ecosystems with high conservation values so implementation of effective dieback management procedures is critical to the long term sustainability of these valuable areas.

The aim of this project is to increase the capacity, skills and knowledge of LGAs, community-based environmental groups and private landholders. We believe that this will lead to more effective management of Phytophthora dieback. The Phytophthora Dieback Best Practice Management Framework is a set of guidelines by which land managers can assess their compliance to best management practices thereby providing continuous quality development and leadership. The Framework and its implementation will be discussed.

Comparing *Phytophthora citricola and P. pseudosyringae* interaction with beech (*Fagus sylvatica*) regarding pathogen growth and physiological host responses

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Phytophthora citricola and *P. pseudosyringae* are well known soil-born root pathogens of European beech. Experiments were carried out in order to characterise their potential impact on beech physiology. *P. citricola* and *P. pseudosyringae* host-pathogen interactions were performed using thermal boxes specially designed to guarantee root temperature at 14 and 20^{o}C, respectively. We proved that both pathogens exhibited comparable growth and sporulation potentials at these temperatures.

Five month old beech seedlings were inoculated with 10^6 zoospores/ml. Photosynthesis, water uptake and biomass were analyzed for eight days. At the end of the experiment root infection was quantified from both pathogens using qPCR in combination with specific primers.

P. citricola root infection significantly decreased CO_2-uptake three days post infection compared to controls. These values diminished to almost zero at the end of the experiment. However, *P. pseudosyringae* did not impair photosynthesis throughout the whole experiment, with even slightly higher values being measured. Water uptake was also not affected. Conversely, seedlings infected with *P. citricola* showed a total water uptake blockade after 5 dpi. Due to severe wilting, total biomass of *P. citricola* infected seedlings was approximately reduced by 50%, whereas the effect of *P. pseudosyringae* was only marginal. Quantitative PCR of roots clearly proved that both species had colonized the root system by the same amount. The data suggest a susceptible beech-*P. citricola* interaction, as well as a resistant beech-*P. pseudosyringae* interaction. Experiments are still ongoing to characterize both interaction at the biochemical and molecular level to explore mechanisms concerning resistance and susceptibility.

How do *Phytophthora* spp. kill trees?

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Phytophthora spp. are being increasingly recognized as pathogens that cause tree death, without necessarily having any clear understanding of how this happens. Suggested mechanisms include:

- extensive fine root necrosis especially on wet or drought prone sites, leading to reduced water uptake, crown decline and death, e.g. *P. quercina* infection of European oaks
- root and stem cankers resulting from phloem invasion and cambial death, leading to death of basal buds and carbon starvation of the root system, e.g. *Phytophthora* hybrid infection of alders
- xylem invasion, leading to reduced conduction, hydraulic failure and death, e.g. *P. ramorum* infection of tanoaks
- hormonal imbalance and/or damage from toxins, e.g. *P. cinnamomi* infection of eucalypts

Trees are great survivors, having large water and carbon reserves within the branches, trunk and main roots. They have passive defenses against damage from physical and biological agents, together with active responses that compartmentalize wounds and infections. Their size and extensive root systems makes them difficult and expensive to investigate, and the timing of death is problematic. The trunk may have been drying out for several months before the crown dies, and many changes can occur during this time.

This IUFRO meeting provides an opportunity to pool our observations of dying trees and develop a clearer understanding of how infection can result in death.

Diversity of *Phytophthora* species in UK gardens

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Abstract

Phytophthora is a common cause of death of a range of herbaceous and woody plants in gardens. Information is lacking on the number of species and their host range occurring in UK gardens. Every year about 300 plants received through the RHS advisory service are tested for the presence of *Phytophthora* using apple baiting and PCR-based methods. Identification through the sequencing of the ITS region showed that there are at least 30 species or complex of species associated with root rot, stem cankers and leaf blight symptoms on a wide range of ornamentals. The most common species are *P. infestans*, *P. cinnamomi*, *P. cryptogea* and species of the *P. citricola* complex. The *citricola* complex has been recently re-evaluated and some isolates can now be reassigned to *P. plurivora* and *P. multivora* based on ITS sequence alone. Both species are present in gardens and *P. plurivora* is the commonest species identified in the complex. Through the survey new species never recorded in the UK previously were also identified including P. taxon 'niederhauserii', *P. tropicalis* and *P. austrocedrae*. Less than 2% of the species identified so far were *P. ramorum*. New host plants have been recorded for most *Phytophthora* species notably *Exochorda* x *macrantha*, a new host for *P. ramorum*.

Morphological Characteristics *of Phytophthora* Species Isolated from Nurseries in Mexico

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Different *Phytophthora* species have been isolated from ornamental nurseries in several countries nevertheless, studies in Mexico are missing. The objective of this research was to morphologically characterize isolates of *Phytophthora* obtained from ornamental plants with wilting symptoms in nurseries located in Michoacán, Mexico, and identify the species. Six *Phytophthora* isolates were obtained and characterized. They were isolated from the following hosts: *Capsicum* (ornamental pepper), *Buxus*, *Petunia, Gardenia*, *Cestrum*, *Helianthus* and *Gerbera*. The isolates showed diversity in their morphological characteristics: the isolate from *Capsicum* presented papillated (1 or 2 papillae), caducous sporangia; the isolate from *Petunia* showed papillated and persistent sporangia, chlamydospores and hyphal swellings; the isolate from *Gardenia* presented papillated and caducous sporangia, chlamydospores; the isolate from *Cestrum* showed papillated *sporangia,* plerotic oospores and amphyginous antheridia; the isolate from *Helianthus* presented non papilllated sporangia and hyphal swellings; and the isolate from *Gerbera* showed non papillated and caducous sporangia. Five isolates were heterothallic and one was homothallic. Pathogenicity of isolates from *Capsicum* was confirmed. The isolate from *Buxus* showed similar characteristics to those of *P. cinnamomi*, including hyphal swellings, chlamydospores and non papillated sporangia. To confirm the identity of this isolate, sequences of the ITS were obtained and compared to the ones deposited in GenBank. The compatibility type was A2. The results obtained indicate that several species of *Phytophthora* are present in ornamental nurseries in Mexico. Research is underway to identify the rest of the isolates. Management strategies should consider that *Phytophthora* is one the pathogens causing losses in nurseries in Mexico.

Phytophthora kernoviae in New Zealand - research update

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Phytophthora kernoviae has been recorded in New Zealand since the 1950's but has only recently (2002) been found acting as a pathogen to the introduced cherimoya (*Annona cherimola*). In a 2008 study undertaken at a site where *P. kernoviae* was first recorded anecdotally over 50 years ago it was recovered from the soil during all but four months which were the warmest and driest of the year, and most readily during mid-winter. The pathogen was not recovered from any of the understorey plants, a mix of natives and exotics, surveyed within the stand of trees. Rhododendron cv 'Cunningham White' trap plants placed in the stand however developed dieback from which *P. kernoviae* was isolated.

Most locations in New Zealand from which *P. kernoviae* has been recorded are highly modified sites with a flora of primarily northern hemisphere shrubs, grasses and forbs. Although it has been postulated that *P. kernoviae* may be native to Australasia its origin is not known and hence its potential pathogenicity to indigenous plants is of interest. To assess pathogenicity of *P. kernoviae* on New Zealand native plants, mycelial inoculations to stem wounds, and zoospore inoculations of detached foliage were carried out. No high level of susceptibility of indigenous species to *P. kernoviae* was recorded.

Stem application of phosphite controls *Phytophthora cinnamomi* in native plant communities from south-west Western Australia

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The South-West Botanical Province of Western Australia (WA) is an internationally recognised biodiversity hotspot. *Phytophthora cinnamomi* is a major threat to the conservation of native plant communities within the province and invasion by the pathogen results in reduced floristic diversity and a decline in biomass.

Application of phosphite (phosphorous acid) has proven effective in controlling *P. cinnamomi* infection in a range of native plant communities from south-west WA. Phosphite increases the survival of susceptible species, reduces rates of disease centre expansion and maintains crucial ecosystem processes within infested sites. Phosphite is either applied by aeroplane or via on-ground deliver that combines low-volume, low concentration spraying and trunk injection. These different application methods provide different degrees of control and longevity of effectiveness.

In this experiment the efficacy of phosphite was determined when applied as a high concentration (30% active ingredient) spray to the stems of susceptible overstorey species. The experiment was conducted in three different native plant communities at Gull Rock, Stirling Range and the Fitzgerald River National Parks from the south coast of WA. It was hypothesized that a strategic application of phosphite along a disease front would reduce disease centre expansion and effectively act as a chemical barrier to invasion by the pathogen.

Stem application of phosphite significantly increased survival of susceptible species, affected the epidemiology of the pathogen and reduced disease centre expansion. Although significant phytotoxicity was observed in the understorey species, most affected species had recovered 12 months after application.

Containment and eradication of *Phytophthora cinnamomi* in the Fitzgerald River National Park in Western Australia

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Fitzgerald River National Park on the south coast of Western Australia (WA) is one of the most diverse botanical regions in the world, reflected in its designation as an International Biosphere Reserve. Around 2000 species of native flowering plants are found in the park, representing nearly 20 per cent of the total number of plant species in WA. Included in this are over 62 endemic plant species with a further 48 plant species more or less confined to the park. This diverse flora supports a number of threatened and critically endangered animals

Although the introduced pathogen, *Phytophthora cinnamomi,* is widespread across the south coast of WA, the Fitzgerald River National Park is largely free the disease. Unfortunately, the park is also home to a 217-hectare Phytophthora dieback infestation introduced during the construction of an illegal access track in 1971.

Faced with the challenge of containing the infestation within its current micro-catchment, and with no known cure for the disease, the Department of Environment & Conservation in collaboration with South Coast Natural Resource Management Inc. is implementing a $3 million Phytophthora dieback management plan.

The project is utilizing a wide range of innovative management techniques including: a comprehensive hygiene plan; controlled access; a fire management plan; perimeter fence to prevent animal vectoring; root impermeable membranes; fungicide (phosphite) treatment; fumigants; revegetation of the infested area; and engineering works to alter the sites hydrology. The project has also included a substantial amount of scientific based research including an epidemiological study of *P. cinnamomi* on the site, trialing of remote sensing techniques and hydrological modeling of climate scenarios on future spread of the pathogen. The details of the project and successes to date will be discussed.

Progress in developing containment and eradication methods for *Phytophthora cinnamomi* in natural ecosystems

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Since the 2007 meeting where we presented research findings on developing eradication methods for *Phytophthora cinnamomi* at two sites, (Western Australia and Tasmania), we have started to apply the methods as a management tool. We are now working with industry and government agencies to contain and eradicate *P. cinnamomi* from areas of high conservation value, and from spot infestations which if not contained will result in large areas of highly susceptible plant communities becoming infested. Such infestations will result in significant changes in ecosystem health and function. We will describe how we have developed and applied containment and eradication methods to four strategically significant infestations, from a management perspective and the processes that need to be addressed to ensure the activities are successful and maintained in the long term.

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Evidence that *P. pinifolia* is an introduced pathogen in Chile

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Phytophthora pinifolia is the causal agent of the needle disease of *Pinus radiata* in Chile, referred to as "DFP". The sudden occurrence of this disease and its impact in *P. radiata* plantations raises questions concerning the origin of this pathogen. In order to address this question we isolated *P. pinifolia* from *P. radiata* at several localities in Chile to assess the level of genotypic diversity in the pathogen population based on amplified fragment length polymorphisms (AFLP) using four primer combinations. Results of these analyses show that all (eighty eight) isolates belong to a single genotype. The fact that a single clonal genotype dominates the population of *P. pinifolia* in Chile is evidence that this pathogen has been recently introduced into pine plantations in Chile. The pathogen only infects certain species of pine suggesting the presence of a high level of host species specificity. This strongly negates the likelihood that it represents a host shift from some other species in Chile that previously showed no pathogenicity towards pines, a genus which is not naturally represented in Chile. Understanding that *P. pinifolia* is most likely an introduced pathogen into Chile's pine plantations will facilitate management approaches for the disease. The unexpected and sudden appearance of *P. pinifolia* has implications for other *P. radiata*-growing countries, especially because its origin and pathway of entry is unknown. Future efforts to determine the origin of *P. pinifolia* should greatly aid in efforts to prevent additional introductions or introductions of similar pathogens into new environments.

Phytophthora species associated with declining *Eucalyptus rudis* (Flooded Gum) in Western Australia

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Tree decline is a serious problem facing remnant Eucalypt forests and woodland ecosystems all over Australia (Keane *et al*, 2001). The causes of decline in some Western Australian *Eucalyptus* species have been intensively studied (e.g. *Phytophthora cinnamomi* on *Eucalyptus marginata*) while the causes of decline in other species are barely understood. *Eucalyptus rudis* (Flooded Gum) is a species typically found along watercourses, on floodplains and around wetlands. *Eucalyptus rudis* is declining across its range in the South West of Western Australia, and the symptoms are typical of those associated with *Phytophthora,* i.e. sparse small-sized foliage, production of epicormic shoots, crown dieback, extensive losses of fine roots, dieback of tap roots and necrotic lesions on small woody roots. Preliminary isolation tests have recovered *Phytophthora* species from declining *E. rudis*. This study reports on *Phytophthora* species associated with declining *E. rudis* from across a range of water bodies, soils, and vegetation types. Discussion will also cover the response of trees to phosphite and the results of preliminary pathogenicity studies.

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Companion plant effects on susceptibility to Phytophthora dieback

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Plant responses to a particular pathogen may be altered by the presence of other plants. In a glasshouse experiment, we are testing if the effects of *Phytophthora cinnamomi* (Pc) on the highly susceptible *Xanthorrhoea semiplana* (Xs) are modified by the presence of companion plants (acacias) with different susceptibility to phytophthora dieback. Seeds of *Acacia pycnatha* (reportedly tolerant to Pc) or *A. myrtifolia* (reportedly susceptible to Pc) were sown in pots containing 1-year-old plants of *X. semiplana*. Controls comprised pots containing single plants of each of the species. Half of the pots were inoculated with Pc when acacias were 3 months old. We are currently monitoring plant growth and mortality, and symptom development. Preliminary data suggest that more inoculated Xs plants have died in pots where an acacia plant is present, irrespective of species, than in pots with a single inoculated Xs. We will report and discuss the data from the final assessment.

Influence of elevated CO_2 on European beech infected with *Phytophthora citricola*

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In Germany European beech (*Fagus sylvatica*) is frequently infected by *Phytophthora citricola*. In two soil infestation experiments, we studied the influence of elevated CO_2 on beech saplings infected with this pathogen. In order to adapt plants to different environmental conditions beech were grown for one whole year under ambient and elevated CO_2.

Elevated CO_2 increased susceptibility of beech saplings towards *P. citricola* proved by visual root rot estimation and quantitative PCR. Mortality of beech was about eight times higher under elevated as compared to ambient CO_2. Three weeks after inoculation, net photosynthesis rates of infected plants grown under elevated CO_2 were transiently reduced up to 60% as compared to controls. However, all the surviving plants recovered regarding photosynthesis in the second year. In parallel, these plants were characterized by an increased shoot to root ratio as well as by increased specific root tip densities.

In a second experiment we studied the influence of elevated CO_2 on the allocation and partitioning of C and N metabolites of beech infected with *P. citricola* using stable isotope labeling techniques. The root infection increased the partitioning of new formed assimilates in leaves at the expense of the root system. In contrast, partitioning of N-metabolites was increased in roots and decreased in above ground biomass, mainly in leaves. However, elevated CO_2 had no effect on the patterns of C and N partitioning.

In conclusion, the measured enhanced susceptibility of beech towards *P. citricola* under elevated CO_2 cannot be the result of an altered resource partitioning.

Fate of Phosphite in Citrus Trees and Control of Root Rot and Brown Rot of Fruit: Model for Control of Phytophthoras on Forest Trees

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Phosphite ($H_2PO_3^-$) is well characterized for its ability to rapidly deliver P to plants and to induce host-mediated resistance to Phytophthora in a wide range of plants. Hence, phosphite is one of the principle controls for Phytophthora root rot and brown rot of citrus. The advantage of phosphite over other modes of action is rapid uptake and highly systemic movement in phloem enabling spray applications of phosphite on the tree canopy to move to fruit and provide protection against brown rot caused *Phytophthora palmivora* for up to 164 days post application. Foliar-applied phosphite also readily moves to the roots for control of root rot caused by *P. nicotianae* for several weeks after application. Soil application of phosphite is more effective for control of root rot than foliar applications due to higher concentrations of phosphite in roots, but soil-applied phosphite may be quickly oxidized to phosphate by soil bacteria before root uptake. Field evaluation of phosphite movement in 5-yr-old Valencia orange and 8-yr-old grapefruit trees after foliar spray application showed a linear decrease of phosphite in leaves and fruit and a concomitant increase of phosphite in fibrous roots. Because phosphite moves readily to metabolically active root, shoot and reproductive tissues, foliar applications may be highly effective for long term protection of forest trees against Phytophthora infection and reproduction.

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Progress in understanding *Phytophthora* diseases of trees in Australasia 2008-2010

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Since the 2007 meeting a number of new *Phytophthora* species have been described. A number of these appear to be associated with woodland and forest tree declines, but may have been misidentified in earlier work. These will be reviewed and updates provided. At least two 'Fishing for *Phytophthora'* activities have been undertaken and new *Phytophthora* species together with hybrid species are evident. Significant community driven projects are being undertaken in Western Australia and elsewhere relating to mapping, prioritisation of areas for hygiene and phosphite activities, effective communication to the wider public and initiatives to obtain on-going funding to Phytophthora management. Eradication and containment activities are now being put into practice in wild land communities in Western Australia. At the research level there are a number of applied and basic research studies on understanding, *Phytophthora* in nurseries and urban parklands and reserves, *Phytophthora* and fire, host resistance, the action of phosphite at a molecular and biochemical level, and how phosphite induces a phosphate starvation response in plants and possible implications. These research activities and more will be reviewed.

Plant costs for a root pathogen

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How expensive is it for a plant to host a root pathogen? Carbon is essential in plants for metabolic processes, maintenance, growth and storage. Plants balance their carbon between source and sink organs depending on the need for growth and development. An additional carbon sink arises by keeping symbiotic ecto/endomycetes like mycorrhiza alive. This plant investment is paid back in terms of mineral nutrients such as phosphate. But what about pathogenic interactions? In general pathogens don't get any support by their host but avail themselves of existing carbon pools to meet their own demands. Pathogenic activities additionally will cause damages in root tissues resulting in loss of function and die-back of infected roots. Thus the plant has three extra costs: (1) carbon loss by pathogen uptake, (2) repair and re-growth of roots and (3) defence reactions. Here we present our carbon sink model developed for the host-pathogen-interaction between European beech (*Fagus sylvatica*) and *Phytophthora citricola*. In invitro assays we observed differential mycelial growth depending on the carbohydrate source. Long and almost unbranched hyphae were observed for low carbohydrate supply whereas fluffy mycel was found at high carbohydrate concentrations. These results will be used to create the *Phytophthora* growth-model which will be implemented into the generic plants model "Platho" in order to simulate the amount of growth of *P. citricola* in roots and the degree of destruction. Finally plant costs to host the pathogen will be expressed as rates of growth depression.

Phytophthora Species Associated with Kawakawa Tree Decline in New Zealand

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Kawakawa, *Macropiper excelsum*, is a small tree common in the North Island and endemic to New Zealand. During 2008-9, a large number of kawakawa trees displaying symptoms of leaf yellowing, branch wilt, and sudden collapse of trees were observed in Auckland and Whangarei. Kawakawa trees in Oratia, Auckland initially showing symptoms of decline have subsequently all died. Declining trees were found in poorly drained soil, at least temporarily water logged during the winter, while those growing on well-drained sites were generally healthy. Leaf, stem, root, and soil samples were collected for isolation of plant pathogens. *Phytophthora* isolates were recovered from root and soil samples using a cedar needle baiting assay. The *Phytophthora* species were identified as *P. citrophthora*, *P. cryptogea*, and two distinct *Phytophthora* isolates in the *P. citricola* complex based on morphology and molecular analysis. This is the first report of *Phytophthora* species associated with kawakawa. Koch's postulate is required to determine the pathogenicity of these *Phytophthora* species. Development of disease management strategies is needed to establish an effective disease control on declining kawakawa trees.

Phytophthora citricola and Related Taxa in New Zealand

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Phytophthora citricola was first recorded in New Zealand in 1938 on hops (*Humulus lupulus*) with black root-rot (Brien, 1938). Since then it has been recorded on a wide range of hosts in New Zealand, including many economically important and ornamental plants: *Actinidia*, *Annona*, *Asparagus*, *Buxus*, *Callistemon*, *Chamaecyparis*, *Citrus*, *Corynocarpus*, *Cucumis*, *Cucurbita*, *Cyphomandra*, *Dianthus*, *Diospyros*, *Eucalyptus*, *Fagus*, *Ficus*, *Humulus*, *Lupinus*, *Lythrum*, *Malus*, *Olea*, *Olearia*, *Persea*, *Phaseolus*, *Pinus*, *Plagianthus*, *Populus*, *Prunus*, *Pyrus*, *Ribes*, *Rosa*, and *Vitis* (Gadgil, 2005; Anon., 2009a, b). With the revision on the species delineation of the *Phytophthora citricola* complex in New Zealand, the host range of these species needs to be revisited.

Phytophthora citricola is known as a species complex, comprising isolates that are morphologically different (e.g. Waterhouse, 1957). The advancement in molecular tools has lead to new species being described or recognised (Jung & Burgess, 2009; Scott *et al*., 2009). Morphology and molecular analyses on selected isolates of *Phytophthora citricola* from an in-house collection and the International Collection of Microorganisms from Plants (ICMP) have resulted in several new records for New Zealand.

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Phytophthora Records from Passive Surveillance Programme in New Zealand

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The MAF Plant Health and Environment Laboratory is responsible for the identification and validation of all suspected exotic, new, and emerging pest and diseases affecting plants and the environment in New Zealand. Since 2001, over twenty species of *Phytophthora* were detected from plant and soil samples submitted by members of the public, regional councils, crop consultants, growers, and scientists. *Phytophthora* speices were isolated by root and/or a soil baiting assay using cedar needles, and were identified by morphology and/or molecular analysis.

The most common species recovered were *Phytophthora citricola*, *P. cryptogea*, and *P. cinnamomi*, contributing over half of the isolates. These three *Phytophthora* species were found on a wide range of hosts, including native plants (e.g. *Agathus* and *Dacrydium*), ornamental plants (e.g. *Begonia*, *Buxus*, *Camellia*, and *Cycas*), horticultural plants (e.g. *Olea*, *Persea*, *Prunus*, and *Solanum*), and forestry hosts (e.g. *Fagus*, *Pinus*, *Quercus*, and *Sequoia*). Around a quarter of the *Phytophthora* species detected were recently described (post 2000). This paper reports several new host and new to New Zealand records.

Fishing for *Phytophthora* across Western Australia's Waterways

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The outbreak of *P. ramorum* in California and Europe, where early detection of an infested area is important to the success of containment and eradication efforts, has popularised the stream surveys in native ecosystems. In Australia, it has recently been used to detect *Phytophthora* spp. in Victoria resulting in several species being isolated (2). Our aim was to catalogue the *Phytophthora* spp. present in Western Australia's (WA) waterways using the stream baiting technique.

Seventy-seven waterways were sampled during October to December 2008. Bait bags with leaves were deployed, retrieved and returned after ~10 days in the water. Leaves were plated onto NARPH agar and checked periodically for *Phytophthora* colonies during incubation at 20°C for 2 weeks. Colonies were isolated into pure culture and grouped into morpho-types. One representative morpho-type from each site per sampling was identified using the sequence of the ITS region of the rDNA, conducting a BLAST search on Genbank and a phylogenetic analysis (1).

A total of nine *Phytophthora* species were isolated of which 6 belonged to ITS clade 6. Only two are currently described (*P. inundata* and *P. cinnamomi* var. *pavispora*), while the remaining seven are possibly new species. These undescribed species were assigned taxa numbers as described in (1); P.sp. 12-15 are potential new taxa. The most frequently isolated species in the southwest were *P. inundata*, P.sp.12 and P.sp.13. That *P. inundata* is widespread in WA's southwest is of concern as it has been found previously in soil and roots from dead native vegetation (3). Little is known about P.sp.12 and P.sp.13.

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Recovery of *Phytophthora* species from drainage points and tributaries in two forest stream networks

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A stream network consists of a main stream and its tributaries. If the occurrence of species of *Phytophthora* at the drainage point of the main stream represents the overall population within the upstream network, a stream network could be surveyed effectively at the drainage point without sampling the tributaries. Two stream networks, Davidson River and Cathey's Creek, in western North Carolina were studied to test this hypothesis. A 1-liter water sample was collected from the drainage point and tributaries in each stream network and filtered through polycarbonate membrane filters with 3-µm pores. The filters were incubated on PARPH-V8 medium for 72 h, and colonies of *Phytophthora* species were counted and then subcultured for identification. Six species of *Phytophthora*—*P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. gonapodyides*, *P. heveae*, and *P. pseudosyringae*—and four distinct undescribed groups of isolates were detected in the two stream networks, which were each sampled twice. Of these ten taxa, nine were detected in Davidson River tributaries and five were detected at the drainage point. In the Cathey's Creek network, seven taxa were found in tributaries and five of them were detected at the drainage point. Even though all the taxa found in tributaries were not detected at the drainage points, all of the taxa in a network representing at least 10% of the total population were detected at the drainage point. More intensive sampling throughout a stream network may be necessary to detect a species with a low population density.

Australian Native Plant Susceptibility to *Phytophthora ramorum*

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Phytophthora ramorum causes considerable and widespread damage in nurseries, gardens and natural woodland ecosystems of the USA (where it causes Sudden Oak Death) and Europe, and is classified as a Category 1 plant pest in Australia. It is of particular interest to Australian plant biosecurity as, like *P. cinnamomi*, it has the potential to become a major economic and ecological threat in areas with susceptible hosts and conducive climates. Research was undertaken in California to assess the pathogenicity of *P. ramorum* on Australian native plants. Sixty-nine plant species within 24 families were sourced from established gardens and arboretums, and selected based upon provenance from areas of climatic suitability for *P. ramorum* as well as ecological and economical importance. Foliar, branch and log susceptibility were tested using detached leaf, branch and log inoculations. Sporulation potential and chlamydospore production was also tested on detached foliage of a select mid to upper storey species. All species demonstrated some level of foliar susceptibility, and some asymptomatic infection was recorded. Disease incidence and severity were greater during the summer, and when the leaves were wounded. Branch inoculations indicated some species may be affected by branch dieback. However, only juvenile branches of *Eucalyptus leucoxylon* displayed symptoms more severe than the positive control, *Rhododendron* 'Colonel Cohen'. Sporulation was recorded for a few species, particularly on juvenile foliage, while putative bole canker hosts in the *Eucalyptus* have been identified. Results of the studies will be discussed in relation to their implications for disease entry, spread and development of an epiphytotic within an Australian biosecurity framework.

Progress in understanding *Phytophthora* diseases of trees in Europe 2008-2010

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Much new information produced on *Phytophthora* diseases of trees in Europe since the 2007 IUFRO meeting will be reviewed. The presentation will include updates on *P. alni* induced dieback of alders, including first reports of the pathogen from devastated riparian stands in Spain and evidence for the selection of frost resistant strains in *P. alni* ssp. *alni* in alpine and continental parts of Germany; advances in understanding deciduous and mediterranean oak declines; dieback of European beech and ink disease of chestnut, including a possibly climatically driven northward migration of *P. cinnamomi;* and serious new developments in the spread of *P. ramorum* and *P. kernoviae* in Europe. Further evidence will be presented for the importance of (i) the international nursery trade as an intercontinental pathway for alien invasive *Phytophthora* species; (ii) the planting of infested nursery stock as a primary pathway into forests and natural ecosystems. Also covered will be the monitoring of *Phytophthora* populations in streams; the development of new molecular-based identification tools; the unravelling of the *P. citricola* complex; and the current status of the remarkable array of new *Phytophthor*a taxa recovered from forest and natural ecosystems in Europe over the past 15 years.

COST Action (FP0801): Established and Emerging Phytophthora: Increasing Threats to Woodland and Forest Ecosystems in Europe

Working Group 4

Thomas Jung[1,2], Anna Maria Vettraino[3], WG4 members

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COST Action FP0801 Working Group 4 focusses on the management and control of Phytophthora diseases and comprises 63 experts from 19 European countries, Tunisia, Australia, New Zealand and the USA. The main targets of WG4 are: 1. Collate informations on established and novel control and management methods and assess their efficacy and practicability for nurseries, individual trees and forests. 2. Explore the feasibility of environmentally friendly control methods, including biological control (antagonists, suppressive plants), low-impact chemical methods (fungistatic products, surfactants, nutrition and soil ameloration), physical methods (host removal, solarisation, water management, water filtration), and host resistance. 3. Discuss the concepts and efficacies of integrated management protocols and the reasons for their success or failure. WG4 members will be reviewing their national literature, collect and assess information and experiences from their countries and submit these data and data from their own trials to a WG4 database which will be accessible for the scientific community, decision makers, practitioners and the general public through the COST Action website. At the end of this COST Action the state-of-the-art and future perspectives on control and management methods for *Phytophthora* diseases will be published as a book comprising key papers written by leading experts with large-scale and longterm experiences and articles on certain aspects written by qualified WG4 members.

Aerial application of AgriFos in Oregon Tanoak Forests.

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Phosphonate (AgriFos) is effective in limiting bole cankers of Sudden Oak Death (SOD) when sprayed directly onto or injected into individual trees boles. There have been no tests, however, of aerial application and uptake under forest conditions. Governmental registration and permission for use of phosphonate in the forest requires demonstration of efficacy. We are conducting trials in Oregon to demonstrate the feasibility of foliar application from helicopter, the uptake and translocation of chemical within treated trees, and the duration of response. We compared three doses: 0, 3, and 6 gallons Agrifos per acre.

Two methods of application of AgriFos are being compared: helicopter application to mature tanoak forest trees, and bole injection of mature tanoak trees. Three different biological assays are being used to measure Agrifos uptake: shoot dip in zoospore suspension; bole inoculation with *P. gonapodyides* in the field; and laboratory inoculation of log bolts with *P. ramorum*. *Phytophthora gonapodyides* was used in field inoculations instead of *P. ramorum* due to quarantine regulations.

Helicopter spray treatments were applied November 2007 and May 2008. In a third treatment, trees were sprayed twice, first in December 2008 and again in May 2009. At each time, we sprayed three blocks, each consisting of three 10-acre treatment plots. All assays (shoot dip and bole inoculation) indicated presence of AgriFos in trees sprayed at both spray concentrations 6 months after treatment. *P. gonapodyides* was a useful surrogate for *P. ramorum* in challenge inoculations. Persistence after 18 months will be assayed November 2009.

Early Detection and Eradication *of Phytophthora ramorum* from Oregon Forests, 2001-2009

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Sudden Oak Death (SOD), caused by *Phytophthora ramorum*, was first discovered in Oregon forests in July 2001. Since then an interagency team has been attempting to eradicate the pathogen though an evolving program of early detection (aerial and ground surveys, stream baiting), destruction of infected and nearby host plants, post- treatment monitoring, and follow-up treatments.

Eradication treatments (herbicide injection followed by cutting and burning host plants) have eliminated the pathogen and the disease from most (but not all) sites, and have slowed spread of the disease compared to similar areas in California. During the early years of the program (2001-2005) we detected an average of 20 new infested sites per year, despite our eradication efforts. Since 2005, we detected an average of more than 50 new infested sites per year. Although the number of infested sites and the area affected remains small, the extent of the disease has expanded, leading to increases in the quarantine area from 9 mi^2 in 2001 to 162 mi^2 in 2009. We attribute the continued spread of the disease to latency of the pathogen, which hinders early detection, and delays in funding, which affect all aspects of the program, especially rapid response and treatments.

The Spread of *Phytophthora cinnamomi* by Feral Pigs

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Feral pigs have long been implicated as vectors in the spread of the plant pathogen *Phytophthora cinnamomi*. The wallowing and rooting activities of feral pigs predispose them to the transport of infested soil. These activities not only disturb the soil structure but also reduce leaf litter and vegetation cover which can lead to an increased susceptibility of an area to colonisation with *P. cinnamomi*. The non-fastidious diet and use of rub and tusk trees by feral pigs may also represent an avenue of pathogen spread. However, there is very little evidence to support these ideas.

Replicated feeding trials using *P. cinnamomi* inoculated millet seed, fine roots and pine have shown that the pathogen can survive passage through the pig gut, in some instances for up to seven days before being passed. The study is also investigating the home range of pigs within the forest and potential distances over which spread is likely to occur. The controlled experiments and field work from this research will be presented and the implications for the management of feral pig populations as an important component of *P. cinnamomi* management will be discussed.

Phytophthora cinnamomi in native vegetation: A South Australian perspective

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In spite of the threat that *Phytophthora cinnamomi* (Pc) represents for South Australia (SA), basic information about the effects of the disease on native threatened plant species is lacking. The aim of this project is to increase understanding of the susceptibility of threatened and key plant species and ecological changes in plant communities due to phytophthora dieback in SA. Nineteen plant species are being tested for susceptibility to Pc in pot experiments in a greenhouse. Disease symptoms and mortality are being monitored and Koch's postulates confirmed. So far, some species appear to be susceptible for example, *Prostanthera euryboides* and *Allocasuarina meulleriana.* The rate and pattern of spread of Pc are being studied at two field sites comprising native vegetation in the Mount Lofty Ranges, SA. Permanent quadrats have been established and the following parameters measured and data collected in 2008 and 2009: soil and fine root samples baited for Pc; numbers and health of key indicator species, e.g. *Xanthorrhoea semiplana* and other vascular plants; percentage cover by vascular plants, leaf litter and bare ground. Data such as soil moisture and temperature, rainfall and soil chemistry are also being collected. To date, the data indicate that the spread of the disease and pathogen is patchy at both sites and does not follow a clear 'front of advance' pattern as reported for some other areas in Australian affected by the disease. Results from susceptibility experiments and information regarding disease dynamics in the field will be presented.

Distribution and detection of *Phytophthora cinnamomi* in soils of mixed hardwood-pine forests of the southeastern USA

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Inconsistent detection of *Phytophthora cinnamomi* (Pcin) in forest soils has been documented in climates with seasonally wet and dry periods. Detection may be complicated further by the physical location of Pcin in soil. Our objectives were to investigate storage conditions required to detect Pcin in dry soil and determine the spatial distribution of Pcin in forest soil. Infested soil from a forest site in western South Carolina was collected, air-dried, and remoistened. Aliquots were sealed in plastic bags with and without wounded rhododendron leaves and stored at 5, 15, and 25°C; sub-samples were assayed every two weeks for up to 12 weeks. A baiting bioassay was used to detect Pcin. Detection from re-moistened soil was rare (1/90 samples); additional studies are needed to better understand factors affecting persistence of Pcin in soil. Horizontal distribution was determined in three square grids (210 cm per side) at each of three forest sites; soil samples were collected at 30-cm intervals in each grid. Pcin was found in seven grids, and detection in those grids ranged from 14 to 97% of the samples. Vertical distribution was studied in 13 soil cores (5 cm diameter, 50 to 74 cm deep) collected from the three forest sites; subsamples from standard depths in each core were assayed. Pcin was present in 85% of vertical cores, was detected up to 74 cm below the surface, and often was not contiguous in a core. The distribution of Pcin in soil is variable and may play a role in survival during seasonally dry periods.

Monitoring the Effectiveness of *Phytophthora ramorum* Eradication Treatments in Southwest Oregon Tanoak Forests

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Phytophthora ramorum, the cause of sudden oak death was first discovered in Oregon forests in July 2001, and has since been the focus of an aggressive eradication effort. Eradication treatments consist of cutting and burning infected and exposed host plants, and where possible, injecting herbicide into tanoaks to prevent sprouting. The effort has slowed, but not stopped, long-distance dispersal of the pathogen. To monitor the effectiveness of eradication treatments we are revisiting treated sites and sampling soil and vegetation in fixed plots centered on stumps of known infected trees. All samples are assayed for *P. ramorum* at Oregon State University and Oregon Department of Agriculture laboratories. To date we have collected samples from 119 SOD-infested sites that had received treatments between 2001 and 2007. Time since treatment for the sampled sites ranged from one to five years. *Phytophthora ramorum* was not recovered from 70 (59%) of the 119 sites sampled. Thirty-seven (31 percent) plots yielded *P. ramorum* from soils only, the pathogen was present in soils and vegetation on eight plots (7 percent), and on four (3 percent) plots, *P. ramorum* was recovered only from vegetation. All positive vegetation samples were tanoak; most of the diseased material was collected from tanoak basal sprouts. Most of the *P. ramorum*-positive plots were associated with more recent treatment years (2006-2007). Herbicide application to prevent sprouting appears to play a positive role in *P. ramorum* eradication. Sampling continues and will provide a quantitative basis to infer persistence of *P. ramorum* on treated sites.

Effects of vegetal compost and mycorrhizae fungi on *Phytophthora cinnamomi* infection and disease on cork and holm oak plants

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Phytophthora cinnamomi is a soil borne plant pathogen involved in oak decline that has caused a high mortality in cork oak (*Quercus suber*) and holm oak (*Quercus rotundifolia*) trees during the last two decades in Portugal.
We evaluated the development of cork and holm oak seedlings from different origins submitted to different treatments under shelter conditions in presence of *P. cinnamomi*. Acorns were collected from trees located in 12 regions of Portugal and Spain. Under controlled conditions, in greenhouse, pre-germinated acorns were sown in pots filled with two different substrates: (A) a mixture of a soil naturally infested with *P. cinnamomi*, collected from a field with high disease incidence, plus vermiculite (2:1 v/v); (B) the same previous mixture with the addition of a chemically and microbiologically characterized vegetal compost (2:1 v/v); (C) soil like in (A) but inoculated with ectomycorrhizae fungi (*Pisolithus tinctorius* and *Scleroderma verrucosum*) collected from cork oak stands, located in the Algarve and Alentejo, two months after sowing cork and holm oak pre-germinated seeds. All the plants were evaluated 18 months after the sowing through different parameters, such as, dry weight root biomass, shoot height and disease severity index. In soil (B) amended with compost, the plants height (50% for both species) and root biomass (38% for cork oak and 75% for holm oak) increased significantly ($P< 0.05$). On the contrary, disease severity (45% for cork oak and 37,5% for holm oak) was significantly reduced ($P< 0.05$). However, *P. cinnamomi* was present in 100% of the soil sampled from the pots, showing that the pathogen suppressiveness was not achieved with this soil treatment as it was expected. Moreover, for cork oak, significative differences were observed within and among each family ($P<0.001$). These results suggest that the use of the vegetal compost tested is not effective for *P. cinnamomi* control by itself but it can contribute to the reduction of the *P. cinnamomi* infection in cork and holm oak stands preferentially if used in combination with other disease control practices. Mycorrhizal treatment had no effect on the plants development probably because roots of cork and holm oak plants were infected with *P. cinnamomi* before soil inoculation was carried out with these ECM fungi. This treatment must be repeated in the pre-germinated acorns before sowing or in the seedlings before plantation, in order to elucidate this question.

The *Phytophthora* Dieback threat to natural resources in Western Australia: communications and community engagement

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"Project Dieback" is a Natural Resource Management initiative to tackle realistically the unrelenting invasion of vulnerable ecosystems of the South West of Western Australia by *Phytophthora cinnamomi* Rands. The Project team has put considerable effort into communications and the development of images to convey key messages. An overriding goal has been to engage community, industry and government and foster partnerships for effective on ground management. The steps were logical – build a team, find the enemy, anticipate its movements, recognise its targets, prioritise the protectable, establish management procedures, communicate to stakeholders, build capacity and implement. Project Dieback focused on two major communication themes - The Problem and The Solution. A major issue is the majority of the communities' ignorance of the scale of the disaster. A range of mediums were used to communicate set messages to different audiences provoking an awareness of the magnitude and threat of dieback and unified management to protect threatened valuable areas. Products to date include a dynamic web site (www.dieback.net.au), an award winning DVD and a cross tenure standard dieback signage system for all land managers to use. A range of visual maps, emotive posters, powerful presentations, training programmes, and interpretive pamphlets has also been produced. Economic and effective hygiene stations for vehicles and footwear are also being designed. Community engagement and involvement in the management of Phytophthora dieback is essential for success as many key area to protect have high public visitation.

Phytophthora in Africa: with special reference to the Southern African region

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Relatively little is known regarding *Phytophthora* spp. in Africa. Most species recorded from this continent are those that are well known pathogens of agronomic crops and many of these also have relatively wide distributions globally. The best-known species is *P. infestans* that occurs in every African country that produces potatoes. The cacao industry in Africa is also severely hampered by black pod disease, caused by *P. palmivora* and *P. megakarya*, of which the latter is most aggressive. Another prominent species in Africa is *P. cinnamomi* and this is largely due to its wide distribution and host range. In this regard, *P. cinnamomi* is an aggressive pathogen of many agronomic and forestry crops in Africa. Studies on *P. cinnamomi* in South Africa have shown that it is an introduced pathogen and it is the only known alien *Phytophthora* sp. to have become invasive in native ecosystems on the continent. Recent studies in South Africa have focussed on undisturbed forestry environments have shown that there are many species of *Phytophthora* in the region that have yet to be described. This is almost certainly true for the rest of the continent and studies regarding the diversity and relative importance of these potentially important organisms should be encouraged.

Phytophthora ramorum Found in Streams During the National Early Detection Survey of US Forests

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The National *Phytophthora ramorum* Early Detection Survey of US Forests was initiated in 2003 using a terrestrial host plant survey protocol. Trials of leaf baiting in streams were conducted in 2006 after similar methods were used successfully to monitor *P. ramorum* in infested forests in CA and OR. This approach replaced terrestrial surveys in 2007. Two mesh bags, each containing four non-wounded leaves of *Rhododendron* spp., were deployed in streams for 1-3 weeks during cooler months of the growing season. Leaves were retrieved, sorted by symptom severity, and divided into two replicate subsamples which were sent to separate laboratories for diagnosis. One replicate was assayed by isolation on selective medium, and the other was assayed by PCR. Over the past four years, more than 300 unique streams in 28 states have been monitored. The pathogen was detected in 13 previously non-infested streams in seven states. Of these, six streams drained watersheds in CA and OR near areas where *P. ramorum* was known to occur. The other streams drained watersheds where currently or previously infested ornamental plant nurseries were located. In streams associated with infested nurseries, *P. ramorum* has been detected each year after initial detection—even after completion of regulatory requirements for pathogen eradication, and surveys of streamside plants in which the pathogen was not detected. These results demonstrate that *P. ramorum* inoculum is leaving infested nurseries in runoff water despite regulatory measures. Either infested plants have been re-introduced, or eradication efforts have been unsuccessful at these sites.

Phytophthora species from stream water in South Africa

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Very little is known regarding the diversity of *Phytophthora* spp. in South Africa. Most species recorded from the country are those associated with agronomic or forestry crops and thus those that have a relatively wide global distribution. We have recently initiated surveys to gain a broader understanding of the diversity of these important organisms in the country. In this study, stream water in the Walter Sisulu National Botanical Garden draining a native woody ecosystem and Hennops river in Gauteng, were sampled for *Phytophthora* species. Stream water was baited using rhododendron and citrus leaves. Three putative new *Phytophthora* species were identified based on morphological features and comparisons of DNA sequences for the ITS1-5.8S-ITS2 gene regions. *Phytohphthora* sp.1. was heterothallic or sterile. It produced pyriform clavate to limoniform, non-papillate, and non-caducous sporangia (37.5 × 54.8µm) with 13µm apical pores. This species resides in Clade 9 and is closely related to *P. parsiana*. *Phytophthora* sp.2. was also heterothallic or sterile and formed the same shaped sporangia (36.5 × 58.6µm) with a 11µm apical pores. It is closely related to *P. gonapodyides* and *P. megasperma* in Clade 6. *Phytophthora* sp.3. was homothallic, and had oogonia (41µm in diameter) with smooth walls and paragynous antheridia. Oospores were plerotic and 36 µm in diameter with 1.4µm thick walls. It produced non-papillate and non-caducous sporangia, pyriform clavate (37.6 × 50.9µm) or obpyriform (37.2 × 67.7µm) in shape with 13.7µm apical pores. It resided in Clade 9 and is closely related to *P. insolita*. A growth study showed that the optimal temperature for growth of all the *Phytophthora* species described here was 25-30°C on CMA. The species found in this study were obtained from a very small area of South Africa and this emphasises the fact that there are many *Phytophthora* that remain to be discovered in the country.

Phytophthora cinnamomi associated with disease of *Quercus cerris* in the Western Cape Province of South Africa

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Quercus cerris (Turkey Oak) is native to the Orient and South east Europe and is a well known component of native forests of Turkey. *Phytophthora* spp. have been recognized as being involved in decline of *Quercus* spp., including *Q. cerris,* in eastern and north-central U.S., Europe, and the Iberian Peninsula. Those species isolated from soil associated with declining *Q. cerris* include *P. cryptogea* and *P. quercina*, while *P. syringae*, *P. ramorum* and *P. cinnamomi* have been isolated directly from sapwood of symptomatic trees showing bleeding canker in Europe. Recently, bleeding cankers very typical of *Phytophthor*a infection were found on *Q. cerris* trees growing on the Vergelegen Estate near Somerset West in the South Western Cape Province of South Africa. Isolations were made from diseased tissue and a *Phytophthora* sp. was recovered from all diseased trees sampled. Isolates were identified based on morphology and comparisons of sequence data for the ITS gene regions as being *P. cinnamomi.* Four isolates were tested for mating type by pairing with known *P. cinnamomi* mating tester strains. One isolates was of the A1 and the remaining three of the A2 mating type. *Phytophthora cinnamomi* is a well-known pathogen of native and introduced plants in the South Western Cape Province and it has been shown to have been introduced into the country. This study represents the first report of *P. cinnamomi* on *Q. cerris* in South Africa and it is most likely also the cause of the disease on these trees.

Histological, physiological and molecular investigations of woody plants infected with different Phytophthoras

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Many Phytophthoras penetrate from fine into coarse roots destroying phloem and cambium tissue. Ho and Hansen (2007) showed that hyphae of *P. lateralis* grew more slowly in cortical cells of resistant Port-Orford-cedar and were not observed in vascular tissue. However, Brown and Brasier (2007) and Parke et al (2007) proved that Phytophthoras occupied xylem tissue and inhibited sap flow. Portz et al (2010) showed that *P. citricola* was able to grow from infected roots into hypocotyl and epicotyl tissue of beech seedlings. Hyphae were mainly growing intracellular in parenchyma and xylem tissue. Transmission electron microscopy displayed disintegration of xylem vessels and of parenchyma cells. Water uptake of infected beech seedlings was inhibited up to 80% with 125000 zoospores/ml corresponding to 90 ng *P. citricola* DNA per root dry weight 8 days post infection.

Net CO_2-uptake rates and stomatal conductance of infected plants often decreased as compared to controls (Maurel et al. 2004; Robin et al. 2001; Brummer et al. 2002). For beech saplings Portz et al. (2010) showed that invertases were involved locally and systemically in the conversion of sucrose of *P. citricola* infected roots. During the growth of the pathogen in roots, a transient expression of the 1-aminocyclopropane-1-carboxylic acid (ACC)-oxidase gene was quantified in leaves. This gene expression was detected in parallel with the first peak of a biphasic ethylene outburst. Recently Manter et al. (2007) showed that *P. ramorum* elicitins caused similar reactions in different hosts. The role of Phytophthora effector molecules in host-pathogen- interactions will be discussed.

COST Action (FP0801): Established and Emerging Phytophthora: Increasing Threats to Woodland and Forest Ecosystems in Europe

Working Group 2: Host-Pathogen Interactions

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The COST Action FP0801 started in November 2008 and will run four years. FP0801 provides a platform for an enhanced collaboration between European research groups dealing with *Phytophthora* problems on woody plants. There are significant gaps in our understanding of the mechanisms responsible for the success of these aggressive and often invasive pathogens. Within the action, working group 2 scientists from 20 European countries are working together to increase understanding why Phytophthora infections result in such devastating consequences for infected trees. In order to achieve this goal WG2 members focus on the following objectives: (1) Determine how Phytophthora species kill woody plants, (2) identify the significance of Phytophthora toxins, elicitins and other effectors for infection and disease development, (3) elucidate possible host resistance mechanisms, (4) develop genomic tools to study host-pathogen interactions and (5) explore the influence of global change factors and scenarios on disease incidence and development. Working group members will review, discus and collate current knowledge based on results of already completed and still ongoing nationally, as well as internationally-funded research projects during the yearly working group meetings. The outcome of this work can be used for resistance breeding programs. In addition short-term scientific missions (STMS) will enable early stage researchers within the consortium laboratories to gain experience in state-of-the-art methods for their investigations, and to gain wider experience in scientific methods in Europe. Knowledge acquired in this group will be transferred through the Action web site, through the production of information leaflets and during the final Action conference.

Stimulation of Chlamydospore Germination and Potential for Acquisition and Transmission of *Phytophthora ramorum* after passage through the Pacific banana slug *Ariolimax columbianus*

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We investigated, under controlled conditions, the potential for acquisition of *P. ramorum* from infected plant material and transmission of disease by the Pacific banana slug, *Ariolimax columbianus.* We also determined the effect of slug passage on chlamydospore germination.

Slugs fed V8 agar cultures of *P. ramorum* produced feces that contained viable hyphae, sporangia, and chlamydospores. Chlamydospores separated from hyphae and sporangia were then tested to determine if passage through the slug gut affects the frequency of germination. Chlamydospore germination after passage through slugs (23%) was significantly greater than for chlamydospores not passed through slugs (13%).

Feces from banana slugs allowed to graze on artificially infested "litter" consisting of inoculated bay, rhododendron, and tanoak leaves contained viable *P. ramorum*. Tanoak and rhododendron leaves that were inoculated with slug feces or exposed to slugs that had grazed on artificial "litter" developed *P. ramorum* lesions. Three of nine tanoak logs placed in containers and exposed to banana slugs that had ingested *P. ramorum* also developed typical *P. ramorum* lesions. This work indicates the potential for slugs to disperse inoculum of *Phytophthora ramorum*. The possibility that slugs serve as vectors of *P. ramorum* under natural conditions was not tested.

The Global Forest *Phytophthora* Website

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Plant pathogens in the genus *Phytophthora* threaten the biodiversity and sustainability of forest ecosystems worldwide. Information on forest Phytophthoras is currently scattered and it is difficult and time consuming for professionals to keep up with the scientific literature, regulatory programs, and management activities, and to develop or access existing educational materials.

The overall aim of the website is to provide science-based information to aid in the understanding and management of the world's forest *Phytophthora* species. While newly emerging, damaging species are the highest priority, information on other Phytophthoras will also be included in an attempt to gradually elucidate Phytophthoras' diverse roles in terrestrial and aquatic ecosystems. The website will contain reference pages for all published species of forest Phytophthoras. Species descriptions will include photos and information on host range, distribution, ecology, disease symptoms, links to DNA sequence data and key references. The website will feature a fully searchable database of *Phytophthora* species, with a user-friendly map interface, enabling searches by geographic location, host, or habitat. Resources for forest managers and outreach specialists will include risk assessments, management options, pest alerts and fact sheets.

The Global Forest Phytophthora website, to be launched in March 2010, will also serve as the website for the IUFRO Forest Phytophthoras work group and provide an archive for meeting proceedings and web pages.

Genotypic and phenotypic characterization of Spanish *Phytophthora ramorum* isolates

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Since 2002, official plant health surveys have detected *Phytophthora ramorum* on *Rhododendron*, *Viburnum* and *Camellia* in ornamental nurseries in northern Spain. *P. ramorum* was isolated from symptomatic plants at different geographical locations and identified based on morphology and sequence of the rDNA ITS regions. All isolates belonged to the A1 mating type; oogonia production could only be induced by *P. cryptogea* A2 tester strains. The aim of this study was to generate information about the genetic variation and population structure of the (95) Spanish *P. ramorum* isolates. A previous study, which assessed genetic diversity based on Inter Simple Sequence Repeat (ISSR) using four dinucleotide and six trinucleotide ISSR primers, did not identify any polymorphisms. In contrast, this study used a new approach with four existing and three recently developed microsatellite markers that are polymorphic within the European population of *P. ramorum.* All isolates belonged to the EU1 lineage of *P. ramorum*. Three out of the seven markers used were polymorphic in the Spanish population of *P. ramorum* and we identified 12 intra-lineage genotypes, five of which have not yet been detected in Europe. Fifty-four isolates (57%) were of genotype EU1MG1, which is also the dominant genotype in other European countries. The other previously described genotypes were EU1MG29 (12 isolates), EU1MG22 (eight isolates), EU1MG13 (five isolates), EU1MG2 (two isolates), EU1MG18 (two isolates), and EU1MG26 (two isolates). We identified five genotypes that are so far unique to Spain, namely EU1MG38 (four isolates), EU1MG41 (three isolates), EU1MG37 (one isolate), EU1MG39 (one isolate), and EU1MG40 (one isolate). Genotypes EU1MG37, EU1MG39 and EU1MG40 were isolated from *Rhododendron* from one region, and EU1MG38 and EU1MG41 were isolated from *Camellia* from two different regions. Isolates of genotype EU1MG38 were resistant to metalaxyl. We will discuss genetic diversity as a function of geographic origin and isolation year.

Physiological effects of *Phythophthora alni* on *Alnus glutinosa*

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The new hybrid *Phytophthora alni* sp. nov. is spreading in Germany. Along rivers and creeks, surrounding bogs and lakes *Alnus glutinosa* is showing the classic symptoms of leaf drying and crown defoliation as well as stem cankers and bleeding. Many of the infected trees die within the following years. To study the ecophysiological effects of *P. alni* on *A. glutinosa* experiments with intact twigs, intact detached leaves and seedlings were performed in the field as well as in the laboratory. It was shown that infected trees have less and smaller leaves with a clearly reduced chlorophyll content. Photosynthetic capacity, measured either as electron transport capacity (ETR) via Chl-fluorescence or by CO_2 gas exchange was clearly reduced in infected trees. Also corticular photosynthesis was affected around artificial inoculations of stems. Following anatomical changes around the inoculation, also the chlorophyll content of the inner bark was drastically reduced. ETR measurements underlined this fact. Field observations showed a dramatic spreading of the stem infection within months. Absorptivity measurements showed clear changes in light absorption of infected and uninfected tissues of the stems. When seedlings or detached twigs were fed with extracts from infected trees, leaf wilting was observed.

Phytophthora diversity on *Viburnum* and *Rhododendron* species in Swiss nurseries

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Phytophthora ramorum was first detected in Switzerland in 2003 on a *Viburnum bodnantense* in a nursery. Since then, nurseries that are subjected to the European plant passporting system are controlled once a year by trained inspectors. During the inspections, symptomatic plant material is collected from potential hosts of *P. ramorum* and sent to the WSL institute for *Phytophthora* isolation and identification.

So far, about 200 samples coming from 54 nurseries and from 13 parks/gardens were processed. Symptomatic plants included especially *Rhododendron* (50%) and *Viburnum* (44%) species, together with a few other *P. ramorum* host species. Using selective CARP medium, *Phytophthora* could be isolated from 30% of the samples, all recovered from *Rhododendron* and *Viburnum* species.

Morphological and PCR-based identification showed that the 58 *Phytophthora* samples belonged to at least six different species. The most common species was *P. ramorum*, followed by *P. citricola*, *P. cactorum*, *P. citrophthora*, *P. cinnamomi*, and the recently described *P. multivora*. From symptomatic Rhododendrons, all six *Phytophthora* species could be isolated, with *P. citricola* being the most frequent. On *Viburnum* species, *Phytophthora* diversity was lower as only *P. ramorum*, *P. citricola* and *P. cactorum* were identified. The highest incidence of *P. ramorum* was observed on different varieties of *Viburnum bodnantense*.

Microsatellite genotyping showed that the Swiss *P. ramorum* population is characterized by a very low genetic diversity.

This study provides the first information on the *Phytophthora* species that could potentially be introduced into the environment by planting *P. ramorum* host plants from Swiss nurseries.

Phytophthora leaf blight – a new disease of California wax-myrtle (*Morella californica*) in Oregon, USA caused by *Phytophthora syringae*

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Morella californica (Cham. & Schlecht.) Wilbur (syn. *Myrica californica*) is a native woody evergreen shrub or small tree found along the California and Oregon coast and in southwest Washington, USA. Commonly referred to as Pacific bayberry or California wax-myrtle, it occurs in mesic to moist lowland areas, and in coastal dunes. It is also widely used as a landscape plant for its tolerance to salt air.

In Spring, 2009, the OSU Plant Clinic received reports of severe defoliation of wax-myrtle plants in the area of Lincoln City, on the north-central coast of Oregon. Symptoms included black leaf lesions, beginning at tips or margins, which spread to encompass the entire lamina and petiole, resulting in casting of affected leaves. Isolations were made from symptomatic tissues onto a corn-meal based medium and incubated at 16ºC. Hyphal tip derived colonies of a putative *Phytophthora* species were used for further analyses. Total DNA was extracted from four colonies and used as template in PCR reactions using primers DC6 and ITS4. The PCR products were sequenced and isolates from wax-myrtle gave ≥99.7% identity to *P. syringae* over 753-865 nucleotides, with one or two nucleotide mismatches. Inoculations of two isolates from wax-myrtle to healthy plants resulted in black leaf lesions similar to those originally observed. Although *P. syringae* has been present in Oregon for decades, this is the first report of it causing disease in *M. californica* in the landscape. Phytophthora leaf blight of wax-myrtle is widespread in the central coast of Oregon.

Fishing for *Phytophthora* in the Waitakere Ranges, Auckland, New Zealand

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Increasing acknowledgement of the threats to New Zealand forest systems from species of *Phytophthora* has lead to a demand for surveillance and monitoring tools. Monitoring within streams for the presence of *Phytophthora* is proving a useful method to detect species such as *P. ramorum* in America and Europe. This method, as well as detecting known threats at a catchment scale, enhances our understanding of the diversity of *Phytophthora* species within these ecosystems and their community structure.

Phytophthora taxon Agathis (PTA) has recently been identified as a serious threat to kauri (*Agathis australis*) which is a dominant tree of forest ecosystems in northern New Zealand. It causes a collar rot leading to ring-barking and tree death. The current methods for determining the presence of PTA involves surveying for symptoms (including gummosis) and sampling for the presence of PTA in tree lesions and by soil baiting. A small scale trial of stream baiting was undertaken in the Waitakere Ranges, in the summer of 2008. This trial demonstrated that *Phytophthora* species could be detected via stream baiting in this system, although PTA itself was was not detected.

The present study aims to further develop stream-based sampling protocols to document the *Phytophthora* species present in streams in kauri forest. This approach will be trialed in six selected sub-catchments within the Waitakere Ranges, Auckland, New Zealand, from October 2009 to October 2010. These data will provide the first examination of how *Phytophthora* species present in these streams vary spatially and through different seasons. If it proves feasible to monitor PTA in this way, the protocol will be refined over this period as a passive detection methodology for PTA. This will enable prioritising of catchments that are likely to contain PTA-infected trees for better targeted management.

Three undescribed pathogenic *Phytophthora* taxa from the south-west of Western Australia.

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The *Phytophthora* culture collection of the Vegetation Health Service of the Department of Environment and Conservation of Western Australia (WA) has been re-evaluated using DNA sequencing (Burgess et al., 2009). This has revealed many undescribed taxa previously classified as known morpho-species, one of which has recently been described as *P. multivora* (Scott et al., 2009).

The aim of this study was to describe three of these taxa, all of which occur in WA native ecosystems. They were compared with both the morphological species to which they are most similar and their closest phylogenetic relatives. In addition, the pathogenicity of these taxa was assessed in glasshouse trials.

P. taxon lateriticola has been isolated from revegetated bauxite mine-pits and undisturbed sites in the jarrah (*Eucalyptus marginata*) forest. The distribution of this taxon appears to be restricted to this ecosystem. It belongs to ITS clade 2 of Cooke et al. (2000) and is most closely related to the nursery pathogens *P. bisheria*, *P. frigida*, and *P. mutlivesiculata*. This pathogen of *Eucalyptus* and *Banksia* appears to have been introduced to WA, as indicated by the *cox*1 sequence data.

P. taxon kwonganina and P. taxon arenaria occur in the Kwongan vegetation of the sandplains to the north and south of Perth. They reside in clades 9 and 4 (Cooke et al., 2000), respectively. Both taxa are pathogens of proteaceous plant species, particularly Banksias.

All three taxa are homothallic with paragynous antheridia, and have a large oospore wall index possibly indicative of adaptation to the seasonal extreme heat experienced in the ecosystems in which they occur.

This study highlights the utility of DNA sequencing as a tool for delineating species where morphological identification is ambiguous, and indicates that both introduced and endemic *Phytophthora* species may be present in different ecosystems in the south-west of WA.

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Using Rain Bucket Spore Traps to Monitor Spore Release During SOD Eradication Treatments in Oregon Tanoak Forests

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The complete Oregon eradication treatment calls for early detection and: 1. prompt killing of infected trees plus a buffer of visibly healthy trees using herbicide to prevent re-sprouting; 2. falling the killed trees and cutting other host plants, and; 3. burning all slash. The goal is to halt sporulation and dispersal of sporangia as quickly as possible. These treatments are expensive, slow, and resisted by some landowners. There has been no rigorous comparison of the effectiveness of these tree killing methods on inoculum production, however. We will report on trials to test these treatment variables, using baited rain traps to monitor production of sporangia under the different eradication treatment conditions.

Rain traps baited with rhododendron (*Rhododendron macrophyllum*) leaves were placed beneath tanoak trees in areas distant from known infection, in known infested stands before eradication treatments began, and in infested stands during and after eradication treatments. Traps were moved as the treatments progressed, and traps were placed in new areas as *P. ramorum* was confirmed.

Conclusions:

1. Inoculum is available during rain events throughout the year.
2. In infested stands, *P. ramorum* was recovered at a high frequency, apparently correlated with rainfall.
3. Inoculum continued to be released from infected trees killed by herbicide treatments until the crowns had dried out.
4. The pathogen was seldom recovered from traps placed beyond the perimeter of treatment areas or from within treatment areas after treatment was complete.

Phytophthora species in soil, water, canopy drip, and vegetation in southwestern Oregon.

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Soils, streams, vegetation and canopy drip were sampled for *Phytophthora* as part of a larger detection and management effort aimed at limiting the spread of *Phytophthora ramorum* in a quarantine zone located in native forest in southwestern Oregon. Environmental samples were baited with green pear, or rhododendron (*Rhododendron* spp.) and tanoak (*Lithocarpus densiflorus*) leaves. Vegetation samples included tanoak with stem cankers, and leaves and twigs of other plant species collected during soil sampling or landscape surveys. Baits and vegetation samples were plated on media semi-selective for *Phytophthora* spp. After incidence of *P. ramorum* was recorded, other *Phytophthora* species were subcultured for identification. Over a 5 yr period, isolations from over 1000 tanoak stem cankers detected *P. ramorum* in 34% of samples and other *Phytophthora* species in 11% of samples. *Phytophthora ramorum* was detected in ca. 10 % of stream samples and other *Phytophthora* species were detected in ca. 86 % of stream samples over a 3 yr period. Of 173 soil plots sampled over an 8 yr. period, *P. ramorum* was detected in 73 plots and other *Phytophthora* species were detected in 23 plots. Vegetation associated with soil plots yielded *Phytophthora* only rarely. Of 2552 canopy drip samples collected over a 2 yr period, *P. ramorum* was detected in 512 samples and other *Phytophthora* species were detected in 109 samples. DNA sequencing is being used to identify the unknown *Phytophthora* species.

Sequencing the *Phytophthora cinnamomi* genome - update

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Many species of Phytophthora are significant pathogens of plants of agricultural, forestry and native plant systems throughout the world. Of these, Phytophthora cinnamomi is of particular significance due to its broad host range and worldwide distribution.

CPSM's successful bid to have the P. cinnamomi genome sequenced through the Joint Genome Institute's Community Sequencing Program gives many Phytophthora researchers the opportunity to collaborate and unlock some of the central conundrums of this pathogen including, but not limited to its extraordinarily wide host range, response to control measures and diagnostics.

The proposed approach and expected outcomes, along with opportunities for collaboration will be presented and discussed.

rRNA sandwich hybridization assay (SHA) a high throughput method for the diagnosis of *Phytophthora* species

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Phytophthora species are a worldwide threat to natural ecosystems and the nursery industry. To prevent the spread of these pathogens with (latently) infected plants, easy to handle diagnostic systems with high sensitivity and high throughput are demanded. Furthermore such methods should give results within a short time. Analysis of a large quantity of phytopathological samples is still time consuming and expensive. Even cutting-edge diagnostic methods based on Real Time PCR still need a labour-intensive extraction and purification of DNA. Also a differentiation between live and dead pathogens is not possible with analysis methods based on DNA.
Here we present the development of an rRNA based high throughput diagnosis method for *Phytophthora* species. This method is based on the HybriScan® test system for the identification and quantification of microorganisms and pathogens. This technique only detects living cells, is easy to handle and uses standard laboratory equipment and cost-efficient read-out devices. Sample preparation and RNA extraction for the SHA can be done in a very fast single step procedure. This SHA detection system can be used for high throughput analysis (96 microwell pates) as well as for single samples.
The SHA has been developed within a three year research project (German Federal Agency for Agriculture and Food – BLE) and will be adapted to plant samples from commercial nurseries, forest trees etc..

In vitro screening of systemic fungicides against *Phytophthora* causing abnormal leaf fall disease of rubber (*Hevea brasiliensis*) trees

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Abnormal leaf fall (ALF) disease caused by *Phytophthora* spp. is an annually recurring destructive disease of rubber trees in India causing significant loss to rubber production. Copper oxychloride (COC) in mineral oil (as low volume spraying) and 1% Bordeaux mixture (as high volume spraying) are extensively used for the management of this disease. With an aim of devising possible better strategies for controlling *Phytophthora*, some preliminary studies using systemic fungicides were undertaken.
Effectiveness of five systemic fungicides [Aliette (Fosetyl-Al), Ridomil (Mancozeb + Metalaxyl), KPHITE 7LP (Mono and di-potassium salts of Phosphorous acid), Calixin (Tridemorph) and Tilt (Propiconazole)] along with one contact fungicide Fytran (COC) was tested in the laboratory for their efficacy against *Phytophthora* following standard techniques. Ridomil, KPHITE 7LP and Aliette inhibited hyphal growth in fungicide amended media (poisoned food technique) at 20, 400 and 1250 ppm respectively. These three fungicides also inhibited sporangia formation and zoospore germination at 100 ppm and thus appeared to be effective in curtailing growth of *Phytophthora in vitro.* Leaf disc assay performed *in vitro* by inoculating leaf discs with *Phytophthora* and floating them in test fungicide solutions revealed Ridomil and KPHITE 7LP were most effective in inhibiting *Phytophthora* while COC was less effective. A***cropetal*** transport of the systemic ***fungicide***s was noted with Aliette, Ridomil and KPHITE 7LP in an assay using excised shoots from two clones: RRII 105 (relatively tolerant clone) and RRIM 600 (susceptible clone), dipped in the fungicide solution and inoculated with *Phytophthora* as no necrosis was observed. Studies to ascertain the protective and curative role of these fungicides revealed KPHITE 7LP, Aliette and Ridomil were effective as protective treatment, whereas KPHITE 7LP alone was efficient as curative treatment. Since *Phytophthora* survives in the soil and litter present in the floor of rubber plantation during unfavourable climatic conditions, an experiment was designed by providing artificial inoculum in known quantity of sterilized soil and drenching the soil with these fungicides. Growth of *Phytophthora* was completely inhibited when the soil was drenched with KPHITE 7LP and not with any of the other test fungicides.
The results obtained from the present preliminary studies indicate that the systemic fungicides KPHITE 7LP, Ridomil and Aliette are equally effective against *Phytophthora* causing leaf disease in rubber. However, this study is being extended to the glass house condition followed by field-testing to arrive at a conclusion about the possibility of using these systemic fungicides in the control of *Phytophthora* on rubber trees.

Regeneration of high hazard sites in Victoria, Australia, severely impacted by eucalypt dieback caused by *Phytophthora cinnamomi*

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Phytophthora cinnamomi has been the cause of significant dieback of susceptible eucalypts in native forests in Victoria since the early 1950's. After the identification of the pathogen as the cause of the dieback in 1969, a significant research program was initiated to determine host susceptibility and the parameters influencing disease development and expression. Initial studies showed that many of the monocalyptus species of eucalypts were highly susceptible to disease development, particularly on poorly drained (high hazard) sites under a forest management regime that included selective harvesting. The initial planting trials on these high hazard sites in the 1970's, resulted in over 95% mortality, although the 5%survival suggested that there was a degree of intraspecific resistance in the population. Based on this, a regeneration program was initiated in an attempt to restore these sites. Several 'graveyard sites' in East and South Gippsland were subjected to a management regime that included clearfelling, burning and sowing with a mixture of seed containing susceptible and tolerant eucalypt species at sowing rates of up to 200,000 viable seeds per hectare. While following germination of the seed there was a significant mortality of susceptible species, after 5 years the sites were considered to be adequately stocked with all species, and for which most have survived even after 25 years. The poster describes this research and the implications for forest management.

The Impact of *Phytophthora ramorum* Isolates Expressing Differing Levels of Elicitin on Tanoak Physiology and Hydraulic Conductivity

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Two mechanisms have been proposed for pathogenesis by *Phytophthora ramorum* on tanoak *(LIthocarpus densiflorus)*. The first mechanism is water stress that results from reduced stem hydraulic conductivity and reduced sap flow. The second mechanism involves elicitins, which have been proposed to cause photosynthetic decline through a hypersensitive-like response.
To determine whether one or both mechanisms contribute to disease, an eight-week-long growth chamber experiment was conducted. Stems of 60 tanoak (*Lithocarpus densiflorus*) saplings were wound-inoculated with one of three treatments including a high elicitin expressing *P. ramorum* isolate (PR-07-058), a low elicitin-expressing *P. ramorum* isolate (4353), or a noninoculated wounded control. Physiological parameters including photosynthesis and water usage were measured weekly. Sets of trees from each treatment were sampled destructively twice monthly to measure the conductive properties of stem xylem tissue and to examine for the presence of tyloses.
Preliminary data indicates that there was a large difference between the high and low elicitin treatments in stem hydraulic conductivity as early as week two of the experiment. Significant treatment differences were also observed in tree mortality. Trees inoculated with the high elicitin expressing isolate died sooner and at a higher rate than trees inoculated with the low elicitin expressing isolate. Further analysis will determine if any differences in photosynthetic rate exist between treatments.

Characterizing the Assemblage of *Phytophthora* species in forest streams in Oregon and Alaska

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Little is known about indigenous *Phytophthora* species, especially in wild ecosystems. Previous work suggested that *Phytophthora* species are relatively abundant in natural streams in healthy forests, but the species present are poorly characterized, and their ecology is essentially unknown. The primary aim of this work was to compare methods for the collection, identification, and enumeration of *Phytophthora* spp. in forest streams and to apply those methods to the analysis of the *Phytophthora* taxocene in forest streams in western North America. Three methods of isolate collection from streams were compared (various leaf baits, pear fruit baits, and filtration), and species identification was carried out using morphological and growth characters, and by DNA sequencing two regions of the genome (ITS rDNA and mitochondrial COX spacer region). Stream sampling was conducted in forest streams in western Oregon near Corvallis OR, in southwestern Oregon, and in south central Alaska.

Isolates were collected by baiting and filtration from 113 streams, and identified by morphological and molecular methods. A total of 18 species were recognized among the approximately 1300 isolates identified; 11 of these are named species, four are previously described but not yet named, and three are undescribed new species. Species from ITS Clade 6 dominated the *Phytophthora* assemblage in all three regions. *P. gonadodyides* was the most abundant species overall, and in two of the three regions. One species common in Oregon streams was not identified in Alaska, and two abundant species in Alaska were not found in the Oregon streams.

COST Action (FP0801): Established and Emerging Phytophthora: Increasing Threats to Woodland and Forest Ecosystems in Europe

Working Group 1: Invasive Potential and Ecology of Phytophthoras

Sabine Werres[1], Joan Webber[2], WG1 members

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Working Group (WG) 1 of the COST Action will focus on the invasive potential and ecology of *Phytophthora* species including nursery-nursery and nursery-forest pathways for distribution of these plant pathogens. This WG will examine *Phytophthora* spread at intercontinental, continental, regional and local scales and will explore possibilities for studying potentially invasive *Phytophthora* spp. in centres of origin. Key targets for WG1 include a collection of data on the current distribution of *Phytophthora* species threaten European trees and forest ecosystems but also in nurseries, a description of the major routes for dissemination of *Phytophthora* spp. at various levels within and into Europe and the development of detailed modelling for biological, ecological and impact of *Phytophthora* spp. in different European ecotypes. Basic data on the severeness of *Phytophthora*/host interactions will be collected via a photo gallery of *Phytophthora* disease symptoms and via all available data on single outbreaks and disease development.
The outcome of this WG can be used for risk analysis and for further ecological studies. Knowledge acquired in this group will be transferred through the Action web site, through the production of information leaflets and during the final Action conference.
Members of WG1 see http://www.abdn.ac.uk/~wmb137/uploads/files/GGTSPU-styx2.bba.de-11542-785217-DAT/COST-FP0801-WG1-expertise-Oct2009.pdf

Application of remote sensing techniques to Ink disease of chestnut in central Italy

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Forest diseases monitoring using remote sensing integrated with geographic information systems (GIS), is getting more and more popular. Remote sensing offers an important landscape and regional perspective on vegetation phytosanitary conditions. This study introduces the utilisation of multi-spectral high resolution images, acquired with digital sensor mounted on an aircraft, to map the chestnut areas affected by Ink disease. The study was conducted over a total area of about 5200 hectares in the Central Italy. The images definition allows to distinguish diseased plants and foci on the base of crown reflectance signature. We used a supervised method to classify images with maximum likelihood classifier. Training data for the supervised method were chosen for four information classes: bare soils, healthy, disease and dead crowns. Transformed Divergence statistical method showed insufficient separability of spectral signature between bare soils and dead crowns. A stratified method of separating dead tree crowns from bare soils, associated with a maximum likelihood classifier is proposed. Accuracy assessment is the procedure used to quantify product quality. It was found that the Ink disease of chestnut gave an accuracy of 81.60%, while the discrimination between asymptomatic and decaying plants has given a classification accuracy of 98.12%. The delivered product was a distribution map of Ink disease of chestnut.

Mapping temporal and spatial distribution of resident *Phytophthora* on ink diseased chestnut stands in central Italy

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In Italy ink disease of chestnut is caused by *Phytophthora cambivora* and, at a lesser incidence, by *P. cinnamomi*. Previous investigations demonstrated that additional Phytophthoras are associated to Italian chestnut stands. Although most of these species are pathogenic to young chestnuts, their role in the disease has been considered marginal. Furthermore most of these species have to be considered invasive in chestnut groves and then susceptible to further adaptation in a scenario of global climatic changes. Four areas, located in central Italy, were selected to monitor *Phytophthora* spp. distribution in ink diseased stands. Maps including plants, roads and natural creeks were generated using a GPS receiver (Leika GS20) and ArC Wiew 3.2 (E.S.R.I.). Samples were collected at the base of plants, from rural roads and from natural creeks. Nine species of *Phytophthora* were identified. *P. citricola, P. cactorum* and *P. cambivora* were the most abundant species isolated from plants in three areas while *P. gonapodyides* from water canals and roads. *P. pseudosyringae* and *P. nicotianae* were isolated for the first time in chestnut forest in Italy. *P.cambivora*, the main cause of ink disease in central Italy was present in soil around trees and along roads and creeks. *P. cinnamomi* was abundantly present in a area close to the Tyrrhenian coast . The last finding is of particular interest since it represent the first record of epidemic spread and overwintering of *P. cinnamomi* in chestnut stands in central Italy.

Phytophthora root rots on Frasier fir – Potential for biocontrol using spent mushroom compost

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Fraser fir [*Abies fraseri* (Pursh) Poir.] is one of the most important commercial tree species grown in the eastern United States. Because of its superior postharvest quality, Frasier fir is in high demand for Christmas trees and production acreage is increasing. However, the species is very susceptible to root rot diseases. Phytophthora root rot, primarily caused by *Phytophthora cinnamomi* Rands., is common on Frasier fir planted in poorly drained soils. The disease is best controlled through integrated pest management that includes use of healthy seedlings and proper site selection. Antagonist microorganisms found in soil and composted materials may also play an important role in controlling Phytophthora root rots. In this study, recycled mushroom compost mixed with pine bark soil is being evaluated as potting media for control of root and crown rots caused by *P. cactorum, P. cinnamomi, P. citrophthora, P. cryptogea* and *P. drechsleri*. Inoculated Fraser fir seedlings in potting media were flooded at alternating temperatures of 10 and 24°C for two days to promote infection, and subsequently moved to a greenhouse for disease assessment. Soil analysis, molecular methods, and traditional culturing techniques will be used to study possible mechanisms of disease suppression.

Integrated control protocol of ink disease of chestnut in central Italy.

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Ink disease caused by *Phytophthora cambivora* represents one of the major threat for sweet chestnut cultivation in central Italy. Ink disease management largely rely on pathogen epidemiology and knowledge of its biological cycle. An Integrated Control Protocol (ICP) including the management of superficial water flows, localised copper sulphate and metalaxyl applications and potassium phosphite trunk injections, has been applied in three ink diseased chestnut areas in central Italy varying for location, extension, management and geomorphology. The monitoring of *Phytophthora* populations during the protocol steps applications has been useful to identify the best combination and timing of the measures applied. One year application of ICP in larger areas resulted in a reduction of *Phytophthora* inoculum and in a decrease of disease descriptors as like as incidence and severity. The results reported in the present study, although to be considered preliminary, clearly evidence a positive effect of ICP application to control Ink disease in chestnut orchards in Italy.

Use of mRNA by as Viability Marker of *Phytophthora cambivora* inoculum

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The monitoring of viable *Phytophthora cambivora* inoculum in soil and chestnut tissues is of great importance in order to predict Ink disease spread and to evaluate the efficacy of applied control measures.. Diagnostic methods based on pathogen isolation in pure culture are time consuming, and with low sensibility. However diagnostic methods based on DNA do not provide information on inoculm viability. The inoculum viability in biological samples can be evaluate using RNA transcripts as molecular marker, quickly degraded in dead cells. In the present study *P. cambivora* inducible cytochrome oxidase II (*cox* II) and *β-tubulin* transcripts were studied for their use as inoculum viability markers Real time RT-PCR (qPCR) was performed in order to investigate changes in transcription of *cox* II following exposition of mycelium to different chemical and physical treatments. In these experiment *β-tubulin* was used as housekeeping. The transcription of *cox* II gene was induced by glucose. To test the efficacy of this inducer *in vivo*, roots of chestnut seedlings were inoculated with *P. cambivora* zoospores and treated with glucose before RNA extraction. *In vivo* qPCR analysis only detected the signal of *P. cambivora β-tubulin* .. This could be due to lack of efficacy of *cox* II transcripts induction on infected plant tissues, or to quality/quantity of extracted RNA. However, these results suggest the use of *β-tubulin* mRNA as a possible marker to diagnose viable *P. cambivora* inoculum. To evaluate the utilization of this marker, qPCR experiments will be performed on natural infected host tissues.

Variability in biology and pathogenicity of a *Phytophthora cinnamomi* population from southern Europe

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Phytophthora cinnamomi is a root and crown rot pathogen of worldwide impact. It affects wild and cultivated plants and represents a serious threat to commercial plant production, to private and public gardens and parks as well as to natural ecosystems. Such event may be increasing as world trade in plants intensifies. In southern Europe, this pathogen is rising its occurrence and severity in different environments ranging from commercial nurseries to fruit orchards and forests.
Sixty isolates of *P. cinnamomi* isolated from several hosts, such as walnut, oaks and ornamental plants, in Italy, France and Spain were characterized by mating type, growth rate at different temperatures, pathogenicity, sensitivity to metalaxyl and amplified fragment length polymorphisms (AFLP) analysis. All the investigated isolates were A2 mating type. Isolates were very sensitive to low temperature and only few grew at 4°C. Isolates that grew at 4°C were resistant to methalaxyl. One isolate the number AB69, obtained from red oak in France, showed to be the highest in virulence, growth rate and resistance to methalaxyl.
Isolates clustered according to hosts or geographic origin, and on the basis of phenotypic traits. Isolates exhibited relatively little genetic variation overall, which suggests that they may have come from a common origin. The possible implications of these variations are discussed.

Management of Kauri Dieback and *Phytophthora* taxon Agathis

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Kauri Dieback, has been identified as an increasing problem affecting kauri (*Agathis australis*) across the Auckland region. *Phytophthora* taxon Agathis (PTA), has been identified as a causal agent of Kauri Dieback at some locations, in the Waitakeres Ranges Regional Park and on Great Barrier Island. *Phytophthora cinnamomi* has also been linked with kauri tree ill-thrift. Both of these *Phytophthora* species are presently considered as exotic to New Zealand. PTA is associated with a collar rot causing large bleeding basal lesions, yellowing foliage and tree death. In 2008, Auckland Regional Council implemented a range of standard operational procedures to pro-actively manage this disease complex across the region. Surveys are underway to assess overall tree health in relation to various environmental stress factors as well as the distribution of *Phytophthora* spp. in Auckland's kauri forests. Survey sites were prioritised in areas with high conservation value, iconic trees, or high levels of soil disturbance, such as tracks intersecting kauri root zones. Site inspections where also undertaken on private land where landowners or the public had reported potential observations of Kauri Dieback. Risk-based management of the suspected pathways and primary vectors of *Phytophthora* spp., including people and feral pigs, is underway.

Spread of *Phytophthora ramorum* on to foliage and stems of mature plantation larch (*Larix*) in the UK

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Phytophthora ramorum (NA1 lineage) has caused heavy mortality of tanoak and true oaks in western USA over the past 15 years. It has also attacked many understorey species. Inoculum comes mainly from canopy infections on *Umbellularia* and *Lithocarpus.* In 2003, *P. ramorum* (EU1 lineage) was found spreading from nurseries and public gardens into woodlands mainly in western Cornwall, south-west England, causing (along with *P. kernoviae*) extensive dieback and mortality of understorey *Rhododendron ponticum.* Inoculum coming from rhodendron foliage has resulted in bleeding stem lesions on *Fagus* and *Quercus*; also foliar infections of *Castanea, Quercus* and other hosts. Tree damage at these sites has been comparatively limited to date, mostly because only a limited range of trees has been exposed to inoculum. However in August – October 2009 widespread dieback of both mature and young Japanese larch, *Larix kaempferi*, was reported from several locations in south-west England ~30-100 km to the east of previous tree-infected sites. Symptoms include needle blackening, shoot wilting, extensive resinosis on branches and stems and bark necrosis. *P. ramorum* has been isolated from shoots and needles in the larch canopy, from bark lesions and from larch needle litter on the forest floor. Observational evidence and preliminary experiments suggests *P. ramorum* has the potential to sporulate heavily on larch foliage and spread from there to the stems (cf. tanoak infection in the USA). This is the first record of *P. ramorum* on larch and the first serious development on a conifer host on either side of the Atlantic.

Occurrence of *Phytophthora* in irrigation water used in forest and woody ornamental nurseries

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67 water samples from commercial nurseries all over Germany were examined on the contamination with *Phytophthora* in July/August 2008. The samples originated from different sources like retention reservoirs where the surplus water from container stands is collected, ponds, rivers, streams, wells, etc.. Nearly 63% of the water samples were with sediment and nearly 37% without sediment. To detect *Phytophthora* a bait test with Rhododendron leaves was used. The Rhododendron leaves were examined by direct isolation and with PCR (different primers). To determine the *Phytophthora* species the pure cultures were sequenced.
41.8% of the samples were tested positive with *Phytophthora*. The percentage of samples with *Phytophthora* was much higher if sediment was included in the bait test. *Phytophthora* was detected mainly in samples from the retention reservoirs. All 10 samples from wells were free of *Phytophthora*.

Long-term survival of *Phytophthora kernoviae* oospores

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Phytophthora kernoviae is a pathogen recently found in the UK and New Zealand. To date, *P. kernoviae* is not known in the USA, but there is interest in studying this species and to prevent its entrance and establishment. *Phytophthora kernoviae*, not known to produce chlamydospores, is homothallic and produces abundant oospores. Therefore, the propagule most likely involved in long distance dispersal will be the oospore, making it important to study its biology. This study was conducted to examine long-term survival of oospores buried in sand at different temperatures. Oospores of one *P. kernoviae* isolate from the UK and one from New Zealand were imbedded separately onto 20 µm-mesh screens, buried in moist, autoclaved sand, and incubated at 4, 10, 20, or 30°C. Over time, four screens were removed from each replication. The pathogenicity potential was checked by placing three screens on Rhododendron leaf disks. The remaining screen was exposed to 1% MTT, staining the oospores for viability. After 1 year, viability of the oospores was 82, 81, 79, and 58% for the UK isolate and 86, 75, 82, and 78% for the New Zealand isolate for the 4, 10, 20, and 30°C treatments, respectively. No necrosis was observed on leaf disks exposed to oospores after 1 year at 30°C. However, necrosis was observed at the other temperatures after being buried for 1 year. This demonstrates that *P. kernoviae* oospores can persist in sand for long periods of time at different temperatures and could be significant in spread of this pathogen.

Assessing the survival, sporulation and pathogenicity of *Phytophthora cinnamomi* within water bodies on a mine site: a risk assessment based approach and implications for on-ground management

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It is often assumed that *Phytophthora* species accumulate in streams, lakes and manmade reservoirs from infestations within the water catchment. Based on the pathogen's biology, it has been assumed that any inoculum present in large water bodies poses a risk for the spread of the pathogen to non-infested areas. It is poorly understood how well *Phytophthora* species and in particular *P. cinnamomi* survive and sporulate in water bodies with different chemical profiles or at different times of the year. Furthermore, water baiting studies indicate that *P. cinnamomi* is rarely isolated directly from high volume water bodies draining directly from areas known to be infested with *P. cinnamomi*.

This study investigated the growth of *P. cinnamomi* in seven water bodies within a single mine site to asses the associated risks of spreading *P. cinnamomi* during operational activities. Each water body varied in terms of the organic particulates and dissolved chemicals due to different underlying soil types, water influx and water recycling regimes. Water quality was shown to have a significant impact on the sporulation, infection of available plant material and survival of *P. cinnamomi* in the water across the seven water bodies tested. The experimental findings and implications of this research for on ground management will be discussed.

COST Action FP0801 – Established and Emerging *Phytophthora*: Increasing Threats to Woodland and Forest Ecosystems in Europe.

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With the rapidly growing international trade in plants and ongoing impacts of climate change, activities of plant pathogens in the genus *Phytophthora* are increasing, threatening the biodiversity and sustainability of European forest ecosystems. COST Action FP0801 unites scientists and disease control experts working on *Phytophthora* in forest ecosystems with the overall aim of increasing understanding of the biology and ecology of *Phytophthora* species with potential to cause damage to European forestry; this knowledge will be used in the development of effective control and management protocols for the problems caused. Outcomes of the Action will be promoted in an effort to increase knowledge and awareness of the problem by disseminating information to end-users and authorities in the forestry sector, and to the general public. Four interrelated working groups have been established to examine (i) the ways in which *Phytophthora* species spread into and within Europe; (ii) determine how Phytophthoras kill woody plants and elucidate mechanisms for host resistance; (iii) disseminate state-of-the-art rapid molecular diagnostics techniques, and (iv) seek sustainable protocols for management and control of the diseases. Project outcomes will include understanding of threats to forest ecosystems by *Phytophthora*, increased abilities rapidly to detect the pathogens, and sustainable management solutions to the diseases caused by these destructive organisms.

Setting priorities for Phytophthora Dieback management in vulnerable Western Australian ecosystems

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[2] *South Coast NRM Inc., 39 Mercer Rd, Albany, 6330, WA, Australia*

The establishment of a set of priorities and targets for *Phytophthora cinnamomi* management across the conservation estate and remnant native vegetation in Western Australia has been a goal of *Project Dieback*, initiated in 2004. A strategic approach was adopted to attract funding and to foster community involvement in the protection of both regional and local priority protection areas.

The first task of *Project Dieback,* was to oversee and coordinate strategic dieback occurrence mapping for over 6 million hectares. Once the extent of infestations where mapped, drawing on all available information regardless of land tenure, the disease situation became evident for the five participating Natural Resource Management Regions of the State. The disease situation varies across the regions. In some areas of the South West infestations are now so extensive that there is an urgent need to both defend and protect areas that are still disease free as well as working to save species and communities most chronically threatened.

The collation of biodiversity data sets and their analysis in relation to the threat has assisted in regional priority setting but other values and community attachment have directed some management activity at the local scale. All the data gathered and analyzed has not only been used to inform management response strategies which are incorporated into regional dieback management plans, but also to convey the message that *Phytophthora cinnamomi* represents the greatest threat to biodiversity across the higher rainfall areas of the South West land division of Western Australia.