



ABSTRACTS - POSTER PRESENTATIONS

(Presented alphabetically by author's last name)

DETECTION AND DIAGNOSIS OF *PHYTOPHTHORA RAMORUM* WITH SPECIFIC HYBRIDIZATION REAL TIME PCR PROBES

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Sudden Oak Death, caused by *Phytophthora ramorum*, poses a serious threat to native American oaks, and is also present in Europe where it has been discovered in numerous European ornamental plant nurseries. Its proven aggressiveness against plants in the Fagaceae and Ericaceae and the damage it has caused in North America lead to assign it a quarantine pathogen. It has also been listed in Europe in the biosecurity group 3, usually mostly devoted to severe pathogens for humans and animals.

The timely and accurate detection of *P. ramorum* is a critical aid in the study of the epidemiology and biology of this pathogen. As a regulated organism, the availability of a sensitive and reliable assay is essential when attempting to achieve early detection of the pathogen. In this work, new specific hybridisation probes for a real time PCR amplification method were found to be rapid, robust, and labour-saving, and proved suitable for routine use in a molecular diagnostic laboratory.



OCCURRENCE OF NOVEL *PHYTOPHTHORA* TAXA IN HUNGARIAN ALDER FORESTS

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Numerous formally or informally designated new *Phytophthora* taxa have been recently described from natural and semi-natural ecosystems using the advantage of molecular techniques. Of the novel taxa, *P. alni* has been identified in Hungary. However, during surveys for the incidence of this pathogen, we also obtained three additional *Phytophthora* isolates from alder root and soil. They could not be identified accurately based solely on morphological characteristics. Therefore, our aim was to carry out a complex morphological, physiological and phylogenetic analysis to reveal their correct taxonomical and phylogenetic positions. The phylogenetic work included sequence analyses of the rDNA ITS, the nuclear α and β -tubulin, translation elongation factor 1 alpha and actin coding genes as well as the mitochondrial NADH dehydrogenase subunit 1 and cytochrome c oxidase 1 and 2 genes. The results indicated that the three studied isolates represented two different taxa, designated as *Phytophthora* sp. 1 and *Phytophthora* sp. 2, each belonging to Waterhouse's group V. Morphological differences between them were observed in the dimension of oogonia, oospores and sporangia as well as the colony pattern on carrot agar. Multigene sequence analysis revealed that *Phytophthora* sp. 1 possessed an internal transcribed spacer identical to that of the informally named *P. taxon Forestsoil*, whereas *Phytophthora* sp. 2 represented another novel taxon that has not been identified elsewhere before. A thorough morphological and physiological characterisation as well as a detailed phylogenetic analysis in relation to other *Phytophthora* spp. will be presented.



***PHYTOPHTHORA POLONICA* SP. NOVO ISOLATED FROM POLISH
ALDER STANDS IN DECLINE**

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In a survey of *Phytophthora* associated with alder decline in Poland, several isolates of a homothallic *Phytophthora* sp., which could not be assigned to other taxa including *P. alni* subspecies, were consistently recovered from rhizosphere soil samples. Their morphology and pathogenicity, as well as sequence data for three nuclear regions (ITS rDNA, EF-1 α and β -tub) and a coding mitochondrial DNA region (nadh1), were examined. The new *Phytophthora* species is characterized by moderate to slow growth on carrot agar at 20 °C, high optimal (ca. 30 °C) and maximum (ca. 38 °C) growth temperatures. It forms catenulate, often lateral, hyphal swellings, large chlamydospores in agar media and in soil extract, persistent. Sporangia are ovoid to ellipsoid and non-papillate. Large oogonia with paragynous and sometimes amphigynous antheridia were observed. From pathogenicity tests on alder twigs and on a few Iberian trees, *P. polonica* seems to be a poor inner bark colonizer. Despite being pathogenic to fruits in wound inoculations, no plant diseases attributable to *P. polonica* have appeared in Poland. In a phylogenetic analysis using either Bayesian inference or Maximum Likelihood methods *P. polonica* falls within clade 10 “sensu Cooke et al. (2000)”, together with *P. insolita* and in clade 8 “sensu Kroon et al. (2004)” of the *Phytophthora* genus. This new species was named *Phytophthora polonica* Belbahri L, Moralejo E & Lefort F. sp. nov.

Cooke DEL, Drenth A, Duncan JM, Wagels G & Brasier CM, (2000) A molecular phylogeny of *Phytophthora* and related oomycetes. Fungal Genet Biol 30: 17–32.

Kroon LPNM, Bakker FT, van den Bosch GBM, Bonants PJM & Flier WG (2004) Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. Fungal Genet Biol 41: 766–782.



PRODUCTION OF VIABLE OOSPORES BY *PHYTOPHTHORA RAMORUM*

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Phytophthora ramorum (Werres, De Cock, Man in't Veld) is a heterothallic species. Initial pairing studies revealed that all European isolates were of A1 type while all American isolates were of A2 type. In 2003, a Belgian isolate was identified as a putative European A2 while some A1 isolates were reported in American nurseries, therefore suggesting some possible crossing between both mating types. However, attempts to produce oospores *in vitro* with classical methods were difficult compared to other heterothallic species, therefore suggesting a weak functionality of the sexual system in this *Phytophthora* species.

In order to increase the amount of oospores produced *in vitro*, the quality of the gelling agent and the genotype of the strain were evaluated in pairings between European mating partners. By comparing different agar sources on a carrot based medium, a delay or a failure in the production of oospores was observed in pairings carried out on media supplemented with technical agar. In contrast, oospores were produced on other agar types, the production on media supplemented with agarose being slightly higher. These differences in oospores production were not observed with other heterothallic *Phytophthora* species used as reference material. Besides the gelling agent, the formation of gametangia was also influenced by the genotype of the strains involved in the pairing. A European A1 strain producing very few chlamydospores was found to be a better mating partner than other A1 strains.

In order to characterise the oospores resulting from intraspecific pairings between European strains, a technique was developed to separate oospores from mycelium, chlamydospores and sporangia. The viability of the isolated oospores was evaluated with a coloration method using tetrazolium bromide (MTT). The distribution of oospores of *P. ramorum* in the different classes of coloration (purple, blue, black and colorless) was similar to that found in other *Phytophthora* species, *i.e.* a majority of oospores in a dormancy state (purple coloration). However, interpretations were sometimes questionable, therefore suggesting that this coloration method should be used with caution, and preferably in combination with other parameters to evaluate the viability of oospores. Germinated oospores were observed in several cases. Some of them died after several days. The survival progenies were characterised in terms of mating type, pathogenicity on *Rhododendron* and morphological features.

A macroscopic experiment aiming at determining the origin of the oospores was carried out and suggested that sexual spores are the result of selfing rather than hybridization between both mating partners. Although hybridization represents the main risk to generate new genotypes with changes in pathogenic fitness, selfed oospores could also lead to progenies with new phenotypical characters. Moreover, they are probably more resistant than chlamydospores. They could therefore constitute a source of inoculum which might be more difficult to eradicate.

POPULATION STRUCTURE OF *PHYTOPHTHORA RAMORUM* IN OREGON FORESTS

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Since the discovery of *Phytophthora ramorum* in the forests of southern Oregon in 2001 and despite quarantine and eradication efforts the pathogen continues to spread. Newly infected sites within and the quarantine zone are discovered every year and in 2006 two new quarantine areas were added. Our study aims to track the spread of *P. ramorum* in Oregon forests through population structure using microsatellite markers. We collected samples from infected areas from a variety of hosts, streams and soil from 2001 through 2006. In 2006 we also intensively sampled a number of trees by collecting tissue samples from multiple cankers on one tree. To date, 40 different genotypes from Oregon forests are defined. Our results indicate there is one dominant genotype present in about 90% of the samples in all six years. Another four genotypes occur relatively infrequently each year and the 35 remaining genotypes occur rarely and in only a few years. Within tree results reveal infection from more than one individual in some cases. Our current efforts are aimed at examining the fitness of each of the most common genotypes in an effort to parse apart whether the dominance of one genotype is based on a founder event or the advanced fitness of that particular genotype.



***PHYTOPHTHORA CINNAMOMI* POPULATIONS ON *QUERCUS* FORESTS FROM SPAIN AND PORTUGAL**

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Molecular characterization of *P. cinnamomi* isolates obtained from soil or root samples from declining holm and cork oak trees from different locations of the southwest region of the Iberian Peninsula (Spain and Portugal), the geographical area more severely affected by Mediterranean oak decline, was performed by AFLP (amplified fragment length polymorphism).

Phytophthora cinnamomi isolates from *Quercus* spp. structured in six populations according to their geographical origin, were separated into two groups (populations) based upon the AFLP genotypic profiles, showing only 20% of similarity between them. The dendrogram calculated from Nei's unbiased genetic distances among populations showed two distinct clusters. Cluster 1 comprises the populations coming from Portugal and southwestern Spain from both *Q. rotundifolia* and *Q. suber*. Cluster 2 comprises populations from the eastern Spanish part of our area of study and also comprises isolates coming from *Q. rotundifolia* and *Q. suber*.

Both populations of *P. cinnamomi* evidenced from the genetic profiles were in good agreement with previous results based upon morphology and temperature-growth relationships of the isolates.

These results suggest that there are two different populations of *P. cinnamomi* causing root rot on *Quercus* species in southern Iberia, one coming from Portugal and now also colonizing the southwestern part of Spain, and a second Spanish population of *P. cinnamomi* infecting oak forests in the eastern part of the geographical area affected by oak decline. Nevertheless, differences on pathological ability between both populations seem to be inexistent.



GENETIC TRANSFORMATION OF *PHYTOPHTHORA RAMORUM* WITH THE JELLY FISH GFP GENE

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The important quarantine organism *Phytophthora ramorum* has been dramatically increasing its host range in the past years and most of the studies concerning *P. ramorum* focus on these issues. Very little is known about the latency period. For sampling and analyzing potentially infected plant material, detailed information on the latency of the disease is of high importance. In order to provide such a tool for the infection process follow-up, we established a reliable method for the stable genetic transformation of *P. ramorum* isolates. The transferred genes were the marker gene nptII for resistance to genitacin and the target gene GFP. The first and most important step in this protocol was to develop a stable system to produce protoplasts from *P. ramorum* tissue. The next step was the transformation of protoplasts with plasmids containing marker gene and target gene, following an improved polyethylene glycol (PEG)-mediated protocol of protoplasts transformation. After transformation, protoplasts were cultivated on a selective medium and allowed to regenerate mycelium. The selected transformants were checked for integration and expression of transferred genes by PCR amplification and the use of anti-GFP antibodies. About forty-three different transformed isolates were produced and then tested for GFP fluorescence, GFP expression and GFP gene integration. They were further tested for their infection potential on Rhododendron plants.



***PHYTOPHTHORA PSEUDOSYRINGAE* FOUND ON EUROPEAN BEECH AND HORNBEAM TREES IN THE UK**

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There is major concern about the spread and impact of *Phytophthora ramorum* and *P. kernoviae* on trees and plants in the UK. Annual national surveys monitoring their presence on *Rhododendron ponticum* are carried out but intensive spot surveys are also done on sites of particular concern. At two such sites in Wales further inspections identified a few trees with bleeding cankers potentially caused by *P. ramorum*. Three very mature trees (>150 yr-old) were affected: two European beech (*Fagus sylvatica*) and a hornbeam (*Carpinus betulus*), all were in close proximity to rhododendrons infected with *P. ramorum*.

Two bark panels were excised from each tree and isolations made from the dead live junctions of necrotic inner bark tissue onto a *Phytophthora* selective medium (SMA). Tissue from the hornbeam was also washed several times before *Phytophthora* could be isolated. Colonies did not show typical morphological characteristics of *P. ramorum*, so they were sub-cultured on fresh CA and sexual structures were examined after 10 d. Plugs taken from the margins of actively growing colonies were also placed in pond water and incubated at 20°C on the laboratory bench to stimulate sporangial formation.

Detailed examination of the cultures and sporangia revealed a homothallic *Phytophthora* species with semi-papillate, caducous sporangia and catenulate hyphal swellings formed in liquid culture. The morphological features correspond to those described for *P. pseudosyringae*. Sequences of the ITS1 and ITS2 rDNA matched those of *P. pseudosyringae* in GenBank (>99%).

Pathogenicity tests conducted on logs 1.2m long, ca. 40cm diam. of beech, included a reference isolate of *P. pseudosyringae* obtained from infected oak trees in Bavaria. *P. pseudosyringae* caused lesions on the beech logs, but isolates originally obtained from beech caused significantly larger lesions (15–20 cm²) than isolates from either hornbeam or oak (2 cm²). All isolates used in inoculations were re-isolated.

This is the first time that *P. pseudosyringae* has been reported from the UK and also the first record of this pathogen on hornbeam. The infected trees at both sites were very close to pathways running through the woodlands with a source of water close by. In Italy, this pathogen has also been recorded from mature beech with bleeding cankers, situated close to pathways. This study raises questions about host specificity and aggressiveness of isolates of *P. pseudosyringae* and the possibility of anthropogenic introduction to the UK through recreational activities.



ROOT ASSOCIATIONS OF *PHYTOPHTHORA RAMORUM* AND *PHYTOPHTHORA KERNOVIAE* IN UK WOODLANDS

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Phytophthora kernoviae and *Phytophthora ramorum*, two pathogens recently introduced to the UK, incite foliar lesions, shoot necrosis, and death of *Rhododendron ponticum*, an invasive weed pervading UK woodlands. In infested woodlands, *R. ponticum* serves as an epidemiologically important host, supporting sporulation of both pathogens. Bleeding cankers on trunks of European beech (*Fagus sylvatica*) caused by either *P. ramorum* or *P. kernoviae* are often associated with neighboring infected *R. ponticum*.

Rhododendron ponticum has been removed from several woodlands as an inoculum management strategy, but the long-term efficacy of plant removal is unknown, in part due to lack of knowledge of pathogen persistence in roots and in emerging seedlings.

The potential for *P. ramorum* and *P. kernoviae* to infect roots of *R. ponticum* in UK woodlands is unknown. To assess pathogen association with rhododendron roots, roots initiated from natural layering were excavated from two sites infested with *P. kernoviae* and one site infested with *P. ramorum*. At each site, four sets of layered roots were sampled, in addition to the associated leaf litter, rhizosphere soil, and symptomatic leaves. In the laboratory, soil and leaf litter were individually baited using leaf disks of *Rhododendron catawbiense* 'Cunninghams White.' Tissue from symptomatic leaves was embedded in SMA agar for isolation of *Phytophthora* spp. Neither pathogen was baited from rhizosphere soil, but both were routinely recovered from leaf litter. *Phytophthora ramorum* was baited from one set of layered roots; *P. kernoviae* was baited from three sets of roots at one site and from two sets at another site.

A second objective focused on investigating the potential for infection of *R. ponticum* seedlings in a woodland cleared of *R. ponticum* in 2005 for management of *P. kernoviae*. Nineteen seedlings were excavated from the woodland and all foliar lesions were sampled for pathogen isolation. Rhizosphere soil and roots were independently baited with rhododendron leaf disks. *Phytophthora kernoviae* was recovered from foliar tissue on 2 seedlings, from roots of 5 seedlings, and from two samples of rhizosphere soil.

The results suggest that both *P. ramorum* and *P. kernoviae* are associated with *R. ponticum* roots in infested UK woodlands. Furthermore, the presence of inoculum in litter but rarely in soil suggests that the pathogens may infect the roots, rather than simply persist on the rhizoplane. Further research is needed to assess the frequency of root associations and to histologically visualize root infections of these two pathogens. These preliminary data suggest that the potential persistence of these pathogens in roots and litter should be considered when managing the diseases in infested woodlands.



“MAL DEL CIPRÉS” DISEASE: ANALYSIS OF THE ASSOCIATION BETWEEN AERIAL SYMPTOMS AND VITALITY OF TREES

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Austrocedrus chilensis is an indigenous Cupressaceae of the Patagonian Andes forests in southern Argentina and Chile that suffers a disease called “mal del ciprés”. *Phytophthora austrocedrae*, a new *Phytophthora* species, was recently shown to be the main cause of the disease. The progressive withering and subsequent defoliation of the tree, which finally dies, has been traditionally considered the main symptom. Other symptoms are basal resinous exudates and red-brown necrotic lesions in the inner bark extending up the bole from killed roots. Brown cubic rots in roots and sapwood are many times associated with dead or dying trees.

Although the symptomatology of the disease has been described as a slow process of defoliation that culminates with the death of the tree, dying and recently dead trees with abundant foliage are frequently observed. This shows that sudden death with little or no previous defoliation also happens. On the other hand, trees with crowns looking almost healthy but with many big lesions at the root collar, and defoliated trees with few or no lesions at the root collar have been observed several times.

Traditionally, it was thought that crown transparency is a suitable measure of the vitality of a tree. Nevertheless, the contradictory observations previously mentioned make such a supposition doubtful and make it difficult to assess the extent of disease in a tree or a stand. Since an accurate method for evaluating the vitality of affected trees is essential for establishing management and conservation strategies in affected forests, an evaluation of symptomatology and its association with tree vitality was done. The aims of this work were to analyze the association between aerial symptoms and vitality and to adjust the method used for determining vitality of trees affected by “mal del ciprés”.

Sixty trees, in three different vitality classes, of a stand affected by “mal del ciprés” were evaluated. Percentage of defoliation was estimated visually, with an accuracy of 5%, using two different methods. The pattern of defoliation, the presence of yellow or red leaves in the crown, cankers and resinous exudates in the stem, and other features that could be related to vitality were also recorded. Bark was removed to expose inner bark and the vitality at the collar root was determined as the percentage of the perimeter with dead tissues. Then the root system was excavated to expose roots in a perimeter of 1m from the base of the tree. Bark of main roots was removed and vitality of each root was evaluated as percentage of tissues dead/affected. Vitality of root system was estimated as the total percentage of affected tissues of main roots. The association between aerial symptoms and tissue vitality was evaluated through a correlation analysis between each of the three variables (defoliation, vitality at root collar and vitality of roots). The association between root collar and root vitality and the other recorded symptoms was also evaluated through a correlation analysis. This work presents the results of the study and a discussion on the reliability of aerial symptoms for estimating vitality of trees affected by the “mal del ciprés”.



THE *PHYTOPHTHORA* SPECIES KNOWN AS “PG CHLAMYDO”

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Phytophthora taxon Pg chlamydo is perhaps the second most abundant *Phytophthora* species in the world, after *P. gonapodyides*, although it is commonly misidentified. Pg chlamydo is frequently encountered in streams and rivers in western North America, Argentina, China, and Europe. It has occasionally been recovered from forest soil and was once isolated from a bole canker on a tanoak tree; it was pathogenic to tanoak in artificial inoculation.

Pg chlamydo resembles *P. gonapodyides* in culture, and is related to that species. It is apparently sterile, not itself forming sexual structures even when paired with tester isolates of known mating type. Sporangia are non-descript nonpapillate, similar to other species in the *P. megasperma/P. gonapodyides* ITS clade. It is distinguished from *P. gonapodyides* by the formation of chlamydospores in culture. It has also been misidentified as *P. lateralis*, *P. drechsleri* or *P. cryptogea*, although the latter species are heterothallic, and readily distinguished with a mating test.

ITS-DNA sequences of isolates from Oregon, California, Argentina, and France were identical, but at least three mitochondrial genotypes were distinguished.



**VIRULENCE BEHAVIOUR AND GENE EXPRESSION PATTERNS OF A
PHYTOPHTHORA CINNAMOMI-CINNAMOMIN SILENCED TRANSFORMANT**

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Phytophthora cinnamomi is an oomycete parasite of *Quercus suber* roots. This pathogen secretes abundantly cinnamomins, proteins from the elicitin family. It was demonstrated that these proteins interact with several lipidic molecules, namely sterols but their biological role remains unknown.

The silencing of the gene coding for β -cinnamomin was induced by genetic transformation of protoplasts by chemical methods (liposomes and CaCl₂/PEG) with an antisense sequence of the β -*cin* gene (our recent work). Selection of transformants was achieved through co-transformation with a gene conferring resistance to hygromycin B. The presence of the transgenes was certified by PCR. The absence of the protein in the culture media was confirmed through *western blotting* using monoclonal antibodies anti- β -cinnamomin, and the absence of the coding mRNA was proven by means of real time RT-PCR quantification. The genetic expression of the genes coding to the other elicitins was shown to decrease as well.

Greenhouse pathogenicity tests were now carried out in parallel in *Q. suber* plants with the only stable cotransformant obtained (antibiotic resistant and with the β -*cin* gene silenced), with a simple transformant (antibiotic resistant) and with the wild type isolates. The results revealed that the β -*cin* silenced isolate has a decreased virulence; and that this impaired virulence could not be caused solely by the presence of the resistance gene.

Genetic expression profiles obtained by cDNA-AFLP disclosed marked differences between the cotransformant and the wild isolate. Sequencing of some fragments differentially expressed showed that these are potentially associated with the expression of the β -*cin* gene; among them, a gene associated with the fatty acids metabolism is particularly important.



**THE SPATIAL AND TEMPORAL DYNAMICS OF *PHYTOPHTHORA LATERALIS*
RELATED MORTALITY ON *CHAMAECYPARIS LAWSONIANA* AFTER
10+ YEARS ON TWO FIELD SITES**

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Chamaecyparis lawsoniana (Port-Orford cedar, POC) is distributed throughout southwest Oregon and Northern California, located predominantly along river drainages. A non-native root pathogen, *Phytophthora lateralis*, was first detected in a Seattle Nursery in 1923 and is responsible for extensive mortality across the range of POC. The spatial structure of infection was examined in the Page Mountain area (Siskiyou Mountains, Oregon/California, USA) using dendrochronology methods (Jules et al, 2002) and the Smith River National Recreation Area (California, USA) using GIS methods (Ritts, 2003). There is no published data on the effects of site and micro-site variation on the patterns of *P. lateralis* infection and its relative virulence using a uniform population background. Two replicated field trials (Quosatana and Flannigan sites) were established in 1993 in the Siskiyou National Forest planted with seedlings from 28 open-pollinated families from a subset of 200 phenotypic resistant selections chosen in the late 80s. A single seedling from 28 open-pollinated families was planted in each plot. The trees were planted in a circular plot consisting of an outer ring and an inner ring centered around a dead POC to provide a consistent and uniform inoculum hazard. A group of 4-8 plots were planted within 15ft. of a neighboring plot and groups were separated by 100 to 200ft. The trees were monitored for mortality (*P. lateralis* and other factors) over 10 + years and *P. lateralis* isolation/confirmation was attempted in the first couple years after planting establishment and again 10 years later. There are significant differences among families in the level of mortality over 10 years, with family 510015 showing greater survivorship than the most susceptible families. However the survivorship functions are not different among families because survival curves have a similar shape but plateau at different mortality levels. Site by family interactions and site effects are not significant but the group (clusters of plots) effects are significant in the ANOVA. Survivorship analysis found significant difference across sites, groups, and plots. The Quosatana site has higher mortality (88%) than Flannigan site (74%) after 10 years and the survivorship functions are different. The absolute level of mortality is different between groups and plots with plot mortality ranging between zero and 100%. The data suggests that the density *P. lateralis* in the early years of the planting was not uniform within or between sites reflected by the differences in mortality. There are some weak associations of group and plot mortality with the surrounding vegetation composition. *P. lateralis* was isolated from most plots in the first couple years after planting and 10 years later the pathogen is detectable in less the half of the plots indicating the density of *P. lateralis* spores are significantly reduced or now absent in some areas of the plantings.

BIOCONTROL OF *Phytophthora cactorum* IN *Aralia elata* Seem

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Aralia elata Seem have been used fresh shoots in spring as edible vegetable due to their special flavor and nutrition for health in Korea. Recently, the roots of the species were infected and damaged seriously by *Phytophthora cactorum* as the pathogen of soil born disease. In order to study the potential of biocontrol using antifungal bacteria, strains showing antifungal activity against *P. cactorum* were collected from soil of Chinju and Gyeongju area, Korea. Out of three hundred, six strains showed antifungal activity *in vitro* against *P. cactorum*. For identification of the strains, morphological characteristic and 16S rDNA sequences were determined. This work is almost first attempt for biological control of *P. cactorum* in *A. elata* Seem to reduce rejection of consumers.



**ADAPTABILITY AND SUSCEPTIBILITY OF OAKS TO
PHYTOPHTHORA QUERCINA UNDER DROUGHT AND NITROGEN STRESS**

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The effects of global climate change are nowadays a frequently discussed problem. Among them especially temperature increase and drought stress during the growing season can predispose plants to diseases. Therefore a project of the Deutsche Bundesstiftung Umwelt (DBU) was started in 2005 to investigate the influence of changing environmental factors to autochthonic tree species as well as their adapted and potential pathogens.

The purpose of the present study is to examine the effects of controlled stress factors, particularly drought and nitrogen stress, on the susceptibility and adaptability of two to three years old *Quercus petraea* plants to infection by *Phytophthora quercina*. The host-pathogen interactions of two different German proveniences of *Q. petraea* under controlled greenhouse conditions have been studying since 2005. First results about the differences in the response of the species to drought and nitrogen stress are compared and discussed.



**PREVALENCE AND DEVELOPMENT OF DISEASE ON COAST REDWOOD
SEEDLINGS CAUSED BY *PHYTOPHTHORA RAMORUM***

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Coast redwood (*Sequoia sempervirens*) is a host for *Phytophthora ramorum* but it is unclear if the pathogen represents a significant disease risk to this tree species. In an on-going field experiment, we are examining the prevalence of infection and the development of symptoms on coast redwood seedlings in naturally infested sites in southern Humboldt Co., California.

In November 2006, healthy redwood seedlings were placed amid tanoak and bay trees naturally infected with *P. ramorum*. Disease incidence and symptom development are being observed monthly, and every two months, a subset of redwood seedlings is destructively sampled to investigate the location and extent of tissue colonization by *P. ramorum*. In order to correlate inoculum levels across field sites with disease development, rainwater is being collected and baited with rhododendron leaves to determine presence and frequency of positive *P. ramorum* rain traps.

Results of this study will help evaluate the risk of *P. ramorum* to coast redwood seedlings and inform land managers of the potential for reforestation of infested sites with this species.



EPIDEMIOLOGY OF THE ALDER *PHYTOPHTHORA*

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Since the beginning of the nineties, *Phytophthora alni* a new hybrid species, induces major declines along European watercourses. To evaluate the long-term impact of the disease on the alder population, we set up in 2002 a permanent survey area along the Sarre river (Moselle). All alder over 1.3m high were censused and mapped along about 4 km of river and their health status and growth was annually determined. Each year, new tree reaching 1.3m high was recruited in the sample.

In this study, we used those data to document the speed of disease evolution on the trees, based on survival analysis. Additionally, we proposed an estimation of local tree infection probability based on kernel estimators. The disease is characterised by presence of trunk cankers and decline of the crown. The trunk cankers might better reflect the present infection activity while the crown decline might more reflect past infection, being slower to appear. We thus based local tree infection probability on the likelihood of new canker cases.

We then checked that that it is important to take into account the lag between infection and decline of the crown. For that, we estimated the likelihood that a tree with a healthy crown will decline, given trunk dbh and the local likelihood of new canker cases in the previous years. The lag depend on the tree size, the seedling showing very quick crown decline in area with high likelihood of new canker cases while large tree decline more slowly. We used the fitted function to identify trees that appeared healthy, but nevertheless had a high likelihood of being already infected. These trees were compared to healthy trees with little risk of being already infected (according to our model), slightly declining trees, severely declining trees and dead trees. *P. alni* presence in the soil was estimated by baiting followed by PCR at the basis of the studied trees in order to determine which of them are major inoculum producers. The results show that *P. alni* is detected in larger amount at the basis of healthy trees with a high probability of being already infected and moderately declining trees compared to severely declining trees. By contrast, *P. alni* is seldom detected at the basis of healthy trees that we predict to be uninfected and of dead trees. We conclude that it is very important for modelling the disease evolution to take into account asymptotic uninfected trees as they are major inoculum producers.



**MONITORING *PHYTOPHTHORA RAMORUM* DISTRIBUTION IN STREAMS
WITHIN COASTAL CALIFORNIA WATERSHEDS**

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One hundred eighty-seven sites were established in perennial watercourses and sampled for one to four years between 2004 and 2007 to monitor for the presence of *Phytophthora ramorum* (Pr) throughout coastal central and northern California watersheds as well as portions of the Sierra Nevada mountain range. In 2007, 132 sites were monitored, including 65 new sites. The majority of the monitored watersheds have limited or no Pr at this time, but are near the epidemic range of Pr and/or are considered high-risk for invasion by Pr. Three currently infested watersheds in Sonoma, Humboldt, and Monterey Counties were included as a baseline for successful recovery of Pr. *Rhododendron* leaves were placed in mesh bags and secured in watercourses for 1 to 3 week intervals to bait for *Phytophthora* species. Recovered symptomatic leaves were plated on *Phytophthora*-selective medium (PARP-H) and monitored microscopically.

Pr has been detected at 37 total stream monitoring sites, including all sites with *a priori* knowledge of nearby forest infestation. Pr was detected at 23 streams sites without prior knowledge of adjacent forest infestation in Humboldt, Contra Costa, Mendocino, Monterey, and Santa Cruz counties. Forest infestations have thus far been confirmed at only nine of these sites; surveys are underway to identify the source(s) of inoculum for the other sites. Additionally, Pr was recovered as far as 25km downstream from known forest infestations. This year (2007) was an unusually dry year in California which impacted our recovery of Pr from watercourses. Pr was detected in only 15 streams this year; seven of those were new sites for 2007. We recovered no Pr from four streams that were positive for Pr in 2006. At the most heavily infested sites, the frequency and quantity of recovery of Pr was greatly reduced in 2007.

Stream monitoring has extended the southern range of Pr in Monterey County and the northern range in Humboldt County. All sites in the Sierra Nevada remain negative for Pr. With culturing and molecular sequencing we have identified several other *Phytophthora* species within these watersheds; *P. gonapodyides* is the most commonly detected species and was isolated from at least 60 sites. Streams were monitored year-round in 2004 and 2005 and revealed a distinct seasonality associated with Pr recovery. Therefore, in 2006 and 2007 watersheds were monitored monthly, February through June, during the peak seasonal period.



**REAL TIME PCR PROTOCOLS FOR ENVIRONMENTAL MONITORING OF
PHYTOPHTHORA ALNI AND ITS THREE SUBSPECIES**

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Appeared on the UK at the end of the 1980's, *Phytophthora alni* has severely reduced alder populations in Western Europe and has been emerging as a major threat to alder stands in Poland in the past years. For addressing Phytophthora epidemics, which spread in the wild, environmental monitoring must be made easier, quicker and cheaper than classical description or PCR methods. The objective of the work presented here was to provide with a set of real time PCR assays for environmental monitoring of *Phytophthora alni*, fulfilling the desired requirements. We present here the development and evaluation of a set of real-time PCR assays for the LightCycler real time hot air thermocycler (Roche Diagnostics), using primers that target the Sequence Characterised Amplified Regions (SCARs) previously developed for *P. alni*. The real time PCR format allowed to perform a melting curve analysis of the PCR products once amplification was completed and to offer an additional characterization of the targeted SCARs. The availability of rapid and specific assays for the detection and characterization of *P. alni*, such as provided in the present study, should facilitate the detection, control of outbreaks and the occurrence and distribution of the different subspecies of this pathogen in Poland. However care should be taken when conducting environmental studies using these oligos since we show a clear cross contamination with at least *P. gonapodyides* and *P. quercina*, two species frequently encountered in alder stands.



REQUIREMENTS FOR THE AERIAL DISPERSAL OF *PHYTOPHTHORA RAMORUM*

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Phytophthora ramorum is an aerially-disseminated pathogen threatening coastal California and Oregon forests. It produces deciduous sporangia on infected leaves and twigs of foliar hosts, notably bay laurel (*Umbellularia californica*) and tanoak (*Lithocarpus densiflorus*). The pathogen is regularly recovered in rain splash from sporulating canopy infections, an important inoculum source leading to the infection of neighboring vegetation. While this leads to local intensification of the disease, the distances between known disease foci indicate that different dispersal mechanisms are responsible for pathogen spread at larger scales. Movement of infested soil or plant material has largely been attributed as the cause of new, distant infections. The focus of this work, however, is on the potential for dry air, turbulent dispersal of *P. ramorum* sporangia.

Sporangia production and dry air dispersal from aerial parts of plants are unusual traits in this genus, however they are not unprecedented. *P. infestans*, causal agent of potato late blight, and *Peronospora tabacina*, the cause of blue mold of tobacco, are two oomycete models for the dispersal and spore characteristics we are studying in *P. ramorum*. Initial work has demonstrated that Rotorod spore samplers can be used to capture *P. ramorum* sporangia in experimental conditions, and that sporangia trapped on the rods can be quantified by microscopic observation, in culture, and with RT-PCR. Continuing research is exploring the environmental conditions and timing for release of sporangia, the survival of sporangia after exposure to atmospheric conditions, and other requirements for sporangial deposition, germination, and successful infection.



**PHYSIOLOGICAL CHANGES AND GENE EXPRESSION ON EUROPEAN BEECH
(*FAGUS SYLVATICA*) INFECTED WITH *PHYTOPHTHORA CITRICOLA***

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In the last decade *Phytophthora citricola* Saw. was described as an important and aggressive pathogen on European beech (*Fagus sylvatica* L.). Inoculated beech seedlings, grown under controlled conditions, showed a significant decrease of photosynthesis, transpiration and water potential after three days. Typical wilt symptoms on leaves were observed only after a consistent reduction of these parameters. Sugar contents of roots of infected plants were significantly lower in infected plants as compared to control seedlings. A negative correlation between photosynthesis, transpiration and water potential and the increase of *P. citricola* in roots was demonstrated. In addition synthesis of ethylene of infected plants was increased two days after inoculation compared to controls. Expression of genes of the primary and secondary metabolism, as well as of defense-related genes, is ongoing in order to get more detailed information of the studied host-parasite interaction.



CHARACTERIZING THE COMMUNITY OF *PHYTOPHTHORA* SPECIES IN AN OREGON FOREST STREAM

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Phytophthora species are best known as pathogens of agricultural crops, or invasive pathogens destroying forests. Little is known about indigenous species, especially in wild ecosystems. Previous work showed that *Phytophthora* species are relatively abundant in natural streams in forests, but the species present are poorly characterized, and their ecology is essentially unknown. The aim of this work was to compare methods for the collection, identification, and enumeration of *Phytophthora* spp. in streams. Three methods of isolate collection were compared (leaf baits, pear baits, and filtration), and species identification was carried out using morphological and growth characters, internal transcribed spacer (ITS-DNA) sequencing, sequencing of the mitochondrial COX spacer region, and single strand conformational polymorphism analysis (SSCP). Stream sampling was conducted at five locations in the Oak Creek Drainage near Corvallis Oregon. This approach enabled us to get a broad idea of *Phytophthora* diversity in a natural stream.

Water filtration was the most efficient way to collect *Phytophthora* isolates, since it provided the greatest number of isolates and the greatest species diversity. It also enabled quantification of propagules. Between 15 and 20 *Phytophthora* propagules /liter of stream water were usually detected. Four hundred isolates were collected and characterized. At least 11 different species, five of which could not be named, were characterized. *P. gonapodyides* was most numerous, followed by taxon Pgchlamydo, and an unidentified taxon.



***PHYTOPHTHORA RAMORUM* TISSUE COLONIZATION STUDIED WITH
FLUORESCENCE MICROCOPY**

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The proceeding worldwide spread and the expanding host spectrum of *P. ramorum* has become a serious threat to natural plant communities. To encounter this threat detailed knowledge about infection pathways and tissue colonization is essential. To analyze these issues, histological studies of infected tissue with epifluorescence microscopy have been started. For first infection tests *Rhododendron* has been taken as a model host. Root infection of potted *Rhododendron* cuttings with the *P. ramorum* isolate BBA 9/95 (ex-type strain) were started. Inoculation of the non injured plants was done by application of a zoospore suspension onto the surface of the pot soil. The plants were then incubated with 16 hour light at 20°C in a quarantine chamber. Samples of healthy looking plants and plants with typical symptoms were taken and fixed. Unstained hand-cuttings were analyzed with fluorescence microcopy. The development of zoosporangia on the leafsurface was analyzed using the vital stain FUN[®]1 (Molecular Probes). Natural autofluorescence of *P. ramorum* and plant tissue is enhanced using the fixing fluid FAA (formaline-aceto-alcohol). Epifluorescence images showing *P. ramorum* structures in different tissues and in different stages of disease development are presented. An overview of the development of *P. ramorum* in *Rhododendron* root, twig and leaf tissue is offered for discussion.



PHYTOPHTHORA SPECIES IN FINLAND

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Potato late-blight epidemics caused by *Phytophthora infestans* have been reported in Finnish newspapers since 1847. The pathogen has caused considerable losses in potato fields in southern Finland. Chemical control against this pathogen started in the 1970's. A new, more aggressive population containing both mating types was introduced to Finland in the late 1980's. The sexual reproduction of the pathogen has also produced some strains resistant to fungicides.

Phytophthora cactorum was first time isolated in Finland in the early 1990's from strawberries suffering from leather rot. Since then the pathogen has caused losses in strawberry production as an agent of crown rot as well as causing stem lesions on silver birch in forest nurseries. Fosetyl-Al is used to control the diseases on both host plants.

We have also baited *P. cactorum* from a natural pond from which a forest nursery is taking its water for irrigation. The *P. cactorum* strains in the pond were tested by Random Amplified Microsatellite (RAMS) analysis to be genetically similar to those causing symptoms on birch in the nursery.

Phytophthora ramorum is a regulated pathogen in Finland. It was isolated in the spring in 2004 from imported plants of *Rhododendron* spp. and afterwards also from a domestic ornamental plant nursery. It has been found every year since then on plant material transported to Finland from other EU countries. In surveys for *P. ramorum* on Finnish *Rhododendron* cultivars, also another *Phytophthora* sp. was isolated in 2004 and 2005. It was confirmed as *Phytophthora inflata* by morphology and sequence analyses. In pathogenicity tests, both *P. inflata* and *P. ramorum* caused also necrotic lesions on *Alnus glutinosa*, *A. incana* and *Betula pendula*, but *P. ramorum* was less pathogenic than *P. inflata*. *P. inflata* was also able to infect *Picea abies*, *Vaccinium myrtillus*, *V. uliginosum*, *V. vitis-idaea*, *V. angustifolium* and *Fragaria x ananassa*. *Pinus sylvestris* was shown to be resistant to both *Phytophthora* species.



SCREENING WILD CHERRY (*PRUNUS AVIUM*) MICROPROPAGATED CLONES FOR RESISTANCE TO FOUR *PHYTOPHTHORA* SPECIES

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Prunus avium L., wild cherry, is a valuable component of diversity in mixed coniferous forests and temperate broadleaf forest ecosystems in Europe. Wild cherry is also one of the most prized species for wood production, and as a result it has been increasingly planted both in artificial stands and in natural forests. A number of *Phytophthora* species have been associated with root rot of wild and cultivated cultivars of cherry trees under different environmental conditions. *In vitro Phytophthora* pathogenicity assays are commonly used in order to avoid soil contamination and to speed up the selection procedure to obtain resistant plants. For these assays the cited Authors used callus tissue cultures, however, the symptom assessment could lead to questionable results far from the natural host-pathogen interactions. Thus, the purpose of this paper was to determine *in vitro* the pathogenicity of four *Phytophthora* isolates of several species (*P. alni* ssp. *uniformis*, *P. megasperma* var. *megasperma*, *P. citrophthora*, and *P. cinnamomi*) on micropropagated wild cherry genotypes, previously selected for having superior phenotypic characters and adapted to different Italian environments, by using plantlets 10 cm high cultivated in sterile environment. Host susceptibility was evaluated in accordance with a disease score scale, taking into account the percent of yellowing/wilting. All the wild cherry clones resulted highly susceptible to *P. citrophthora*. The *P. cinnamomi* virulence varied according to the challenged clone, while *P. alni* ssp. *uniformis* and *P. megasperma* var. *megasperma* were able to cause only modest symptoms. Three of the clones showed resistance to the last three pathogens. The results are consistent with what reported in literature. The method is functional and quick, moreover any contamination risk of the environment by the pathogens spores is avoided. For these reasons it could be considered for early resistance screening tests. On the other hand, the need of a specific protocol for regeneration and multiplication of each clone acted as a limiting factor in terms of number of challenged clones and replicates of each clone.



INVOLVEMENT OF *PHYTOPHTHORA* SPECIES IN THE DECLINE OF BEECH (*FAGUS SYLVATICA*) IN THE SOUTHERN PART OF BELGIUM

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During the last decade, typical symptoms of *Phytophthora* diseases were observed in beech stands of several European countries. The main symptoms were the presence of bleeding cankers on the stem, a low crown density as well as the yellowing of foliage and the small size of leaves. Several species of *Phytophthora*, such as *Phytophthora citricola*, *P. cambivora* and *P. cactorum*, were reported as the causal agents.

In order to evaluate the implication of the different *Phytophthora* species in beech decline in the southern part of Belgium, a monitoring was undertaken with the help of managers of public and private forests. *Phytophthora* strains isolated from beech of different stands as well as from soil were characterized through morphological and molecular analyses (PCR-RFLP of ITS). All the isolated strains were identified as *P. cambivora* which is considered as one of the most aggressive *Phytophthora* species involved in beech decline in Europe.

Molecular analysis was also directly applied to necrosed tissues of bleeding beeches and enabled the detection of additional cases. All positive cases exhibited a profile characteristic of the *P. cambivora* species, except for one of the sampled trees showing a different RFLP profile. Identification of the involved species is ongoing.

Regarding *P. cambivora*, both mating types (A1 and A2) were identified, sometimes in the same sampling site. Ornamented oogonia characteristic of the *P. cambivora* species were produced by pairing A1 and A2 strains isolated from the same site. Oospores from intraspecific pairing were characterized in terms of viability and germinability.



PHOSPHITE APPLICATION AS AN EXPLORATIVE TOOL FOR UNDERSTANDING AND CONTROLLING *EUCALYPTUS GOMPHOCEPHALA* (TUART) DECLINE IN SOUTHWEST WESTERN AUSTRALIA

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Eucalyptus gomphocephala is a Mediterranean forest canopy species endemic to a narrow (5-10 km wide) coastal strip approximately 300 km in length in south-west Western Australia. *E. gomphocephala* is undergoing a significant decline that was first identified as a spot decline in 1994 and now occurs throughout large sections of its remnant distribution within Yalgorup National Park, in some areas resulting in 100% mortality. The reduction of this keystone species represents a significant modification to the associated ecosystem. Modifications to hydrology, fire regimes, entomological pressures, and fungal and Pythiaceus soil pathogens have been identified as possibly contributing to the decline syndrome. The potential of phosphite (phosphonate), nutrient and insecticide treatments to reverse the decline in tree health was assessed as (a) a method for controlling the decline and (b) a method for diagnosing possible causal agents. Phosphite has been successfully used to control *Phytophthora* and Pythiaceus soil pathogens by inducing a host defense response within the plant. Stem injection of declining *Eucalyptus gomphocephala* in the present study has resulted in improved canopy health and vigor, indicating that *Phytophthora* and/or other Pythiaceus microorganisms may be playing a role in the decline. The impact of phosphite application on nutrient uptake and fine feeder root concentration was also assessed.



AN EVALUATION OF STREAM MONITORING TECHNIQUES FOR SURVEYS FOR *PHYTOPHTHORA* SPECIES IN VICTORIA, AUSTRALIA

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In Australia, surveys for *Phytophthora* species generally rely on either soil or plant testing to confirm their presence in an area. Stream monitoring has become an important part of early detection surveillance systems for *Phytophthora ramorum* in the USA. In the present study, water monitoring methods used for the detection of *Phytophthora* species in streams in the USA were evaluated in surveys in June 2006 and March 2007 at 8 sites and 4 locations to the east of Melbourne, Australia.

At each site, five leaves from four different bait types were floated in mesh bags in the streams for periods varying from one to three weeks depending on the bait type (total 160 baits). In the first survey in June, bait types included Rhododendron leaves (undamaged and cut in a herringbone pattern), mature *Eucalyptus regnans* leaves and *E. sieberi* cotyledons, a bait used often for isolation of *Phytophthora cinnamomi* from soil in Victoria. In the March survey, *Pittosporum undulatum* leaves were added as baits and only undamaged Rhododendron leaves used, along with the other eucalypt baits. One litre of water was collected from each site at the end of each baiting period and filtered in the laboratory using 0.2µm Metrical membrane filters. Lesions that developed on the bait samples, and the complete filters, were plated directly onto P₅ARP(H) agar. DNA sequencing of the rDNA ITS region was carried out on purified cultures of *Phytophthora* species isolated from the plates.

Phytophthora species were isolated from all sites, bait types and filters used in the study. Isolated species included *Phytophthora cinnamomi*, *P. citricola*, *P. gonapodyides*, undescribed *Phytophthora* species from clades 6 and 7, and an unknown, potentially new clade. Species isolated varied with season, bait and filter used. This highlights the need to use a variety of parameters for stream monitoring when targeting a wide range of *Phytophthora* species.

Stream baiting has potential as a valuable method to determine the presence of a range of *Phytophthora* species in catchments across Australia. It also provides a valuable tool for early detection of *Phytophthora* species, and needs to be included in Australia's network of quarantine surveillance activities. The method may also be potentially applicable to the monitoring of stream and dam water for use in irrigation and fire fighting activities, and of water runoff from gravel pits and nurseries so as to identify the presence of the pathogens, and reduce their spread across Australia.



MOLECULAR TESTING UNCOVERS NEW *PHYTOPHTHORA* TAXA FROM NATURAL ECOSYSTEMS IN WESTERN AUSTRALIA

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Verification of mapping of the extent of *Phytophthora* dieback disease, based on shadowless colour aerial photography, involves the routine testing of soil and root samples collected from beneath dying, *Phytophthora*-sensitive native plant “indicator species” for the presence of the pathogen. In addition to *P. cinnamomi*, other isolates have been recovered on selective agar following the baiting of soil, or the direct plating of plant tissue, during these operations. These have been identified, using morphological characters, as *P. citricola*, *P. megasperma*, *P. cryptogea*, *P. drechsleri*, *P. nicotianae* and *P. boehmeriae*.

The recent advent and availability of DNA sequencing techniques for the identification of *Phytophthora* species has enabled the testing of new isolates that were difficult to identify from their morphology, as well as a range of historical isolates dating back to the 1980s from the Department of Environment and Conservation culture collection.

DNA was extracted from pure cultures grown on cornmeal agar and the Internal Transcribed Spacer (ITS) regions of the rRNA were amplified using primers ITS6 and ITS4. BLASTn searches of sequence data were conducted in GenBank to determine the most closely related *Phytophthora* spp. Sequences were then aligned and parsimony and distance analyses conducted in PAUP. Based on phylogenetic analysis, seven potentially new and undescribed taxa of *Phytophthora* can be distinguished. Several of these are morphologically indistinguishable from known species (eg *P. citricola*, *P. megasperma*, *P. cryptogea*). In some cases the new taxa are indeed most closely related to the known species (eg *P. citricola*), but in others their DNA sequences show that they are not closely related to the morphologically similar species (eg *P. megasperma*).

Phytophthora inundata, described in Europe in 2003, has been identified based on phylogenetic analysis from several locations in the south-west where it has been associated with dying native plants. Some of these isolates have been stored since the 1980s. One of the new species, with morphology similar to *P. citricola*, but most closely related phylogenetically to *P. bisheria* and *P. multivesiculata*, has been isolated from dying 1- to 2-year-old jarrah (*Eucalyptus marginata*) seedlings in rehabilitated open-cut bauxite mine pits.

Further work is planned to describe the new taxa and their relationships, and to test their pathogenicity, so that an estimate of the level of threat they pose to native vegetation can be made.



LONG-TERM STORAGE OF *PHYTOPHTHORA* CULTURES IN WATER

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Long-term storage of cultures of *Phytophthora* species is a challenge for any lab managing a working collection of isolates. Storage in liquid nitrogen is generally considered to be optimal for archival storage, and successful recovery of most species is regularly achieved after many years. Nitrogen storage has its drawbacks, however, especially for a working collection. It requires species specific freezing conditions, and thawing must be done carefully. Equipment is bulky, and regular addition of liquid nitrogen is necessary. Storage in vials in water at room temperature is an efficient, effective alternative for many collections. Isolates are grown on agar, and plugs are removed from the colony margin and placed in sterile water in 1.5 ml plastic tubes, with or without pieces of sterilized hemp seed. Tubes are kept in coded racks at room temperature, in the dark. Agar plugs can be removed one at a time as needed and plated on selective agar to resume growth. In a recent test, we had nearly 100% recovery of several *Phytophthora* species, including *P. ramorum*, after 7 years in water storage.



OCCURRENCE AND IMPACT OF *PHYTOPHTHORA* SPECIES IN FOREST TREES IN HUNGARY

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Occurrence of *Phytophthora* species and their phytopathological role have been investigated in forest stands in Hungary since 1999. Decline symptoms, specific stem lesions and unspecific top drying signs were surveyed in forest stands of different tree species.

The isolation of *Phytophthora* was carried out from soil samples taken from around the diseased trees by baiting with *Prunus laurocerasus* leaves on selective medium PARPNH. The isolates were identified by morphological and molecular way. The morphological characters were observed in cultures growing on carrot agar medium. The formation of sporangia was induced by flooding the cultures with soil extract. The molecular identification was performed by sequencing the ITS regions of rDNA and comparing the sequences with the known *Phytophthora* sequences accessible in GenBank database. The pathogenicity of the isolates was tested by wound inoculation in the stem of seedlings and by root infections.

Phytophthora species were found in *Alnus glutinosa* with bleeding stem lesions and crown drying symptoms, in *Juglans nigra*, *Quercus petraea* and *Q. cerris* with crown drying symptoms. The morphological and molecular identification resulted in 8 *Phytophthora* species in *Alnus* (*P. alni*, *P. citricola*, *P. gonapodyides*, *P. inundata*, *P. megasperma*, *P. sp.1*, *P. sp. 2.*, *P. sp. 3.*), 4 in *Juglans* (*P. cactorum*, *P. citricola*, *P. hedraiandra*, *P. sp.1*) and 2 in *Quercus* (*P. citricola*, *P. gonapodyides*). The artificial inoculations caused well-delimited bark necrosis in the stem of the seedlings, the largest by *P. alni* in alder and *P. citricola* in black walnut, but generally not exceeding 3-4 cm length. Root infections resulted in lesion and reduction of the fine roots, most pronounced by *P. citricola* in black walnut. The impact of *Phytophthora* species on the healthy condition of the forest trees proved to be most important in planted *Alnus glutinosa* and *Juglans nigra* stands situated in wet sites and flood areas respectively. A community of *Phytophthora* species occurs in the rhizosphaera of these trees causing root and collar rot and bleeding stem lesions in alder and fine root reduction manifesting by crown drying in black walnut. The appreciation of the role of *Phytophthoras* in oak decline needs further research because of their fewer occurrences in the oak drying cases. The identified *Phytophthora* species, except of *P. cactorum*, were recorded first time in Hungary during this research work.

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