

**2008 APS CENTENNIAL MEETING
MINNEAPOLIS CONVENTION CENTER, MINNEAPOLIS, MN
JULY 26-30, 2008**

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Overviews

Hansen, E. 2008. *Phytophthora* – A day late and a dollar short. *Phytopathology* 98:S187.

Our knowledge of *Phytophthora*, and our tools to manage it have advanced dramatically in the last 100 years. Today we have a much better understanding of the phylogeny, ecology, and pathogenesis of *Phytophthora* than we did then. Today we have selective media and selective fungicides; we have an elaborate regulatory apparatus. But if today's knowledge had been available 100 years ago would it have been used to slow the spread of *P. cinnamomi* in the SE U.S.? If a time machine had allowed Australians in 1950 to see the damage *P. cinnamomi* threatens to native forests and the incredibly diverse heathlands of Western Australia, would they have acted more quickly or with more resolve? How would today's tools have been deployed in 1920 when *Phytophthora* root rot began to kill ornamental Port Orford cedars on the west coast of the United States? Would it have been a different story in 1950 when the pathogen reached the native forests in SW Oregon? The record of the last decade certainly doesn't support any claim that our modern knowledge has changed our behavior significantly when it comes to slowing, let alone preventing, invasive *Phytophthoras*. The problem today is not so much lack of knowledge, but failure of will in a changing world. The pressures of population and globalization, the power of money, and accelerating environmental change keep us off balance and behind the curve.

Hansen, E. 2008. A historical review of *Phytophthora* diseases. *Phytopathology* 98:S196.

In many ways *Phytophthora* has defined the science, the practice, and the promise of plant pathology, from the potato famine to the new world of pathological genomics. *Phytophthora infestans* spawned plant pathology and thrust it onto the world stage, and in 2007, more articles in the APS journals *Phytopathology* and *Plant Disease* addressed *Phytophthora* than any other genus of plant pathogens. Our understanding of pathogenesis has been enhanced by work with *Phytophthora* phytoalexins and elicitors, and now with complete genome sequences for 4+ species available, *Phytophthora* will continue to lead the way in genomics research in plant pathology. *Phytophthora* provided early and dramatic examples of the importance of nomenclature, taxonomy, and today phylogenetics to plant pathologists. *Phytophthora* first demonstrated the dangers of invasive pathogens through the globalization of agriculture, and continues to force the issue as newly recognized exotic *Phytophthora* species threaten wild as well as agricultural ecosystems. *Phytophthora* species have been instrumental for epidemiological research and the development of disease forecasting models. It's not all about potatoes and peppers, either. *Phytophthora ramorum*, described only 7 years ago,

now leads the annual *Phytophthora* citation index. The history of *Phytophthora* and the diseases it causes is the history of plant pathology, and today's research on *Phytophthora* gives a preview of the directions plant pathology will take in the future.

Rizzo, D.M. 2008. *Phytophthora ramorum*: A recent discovery with a large impact. *Phytopathology* 98:S197.

Phytophthora ramorum first came to attention of the plant pathology community as the causal agent of sudden oak death (SOD). Since 1994, potentially millions of tanoak (*Lithocarpus densiflorus*) and oak (*Quercus* spp.) have been killed by this pathogen in coastal forest of California and Oregon. *P. ramorum* is a generalist and has a host range of well over 100 species ranging from ferns to conifers to herbaceous plants and shrubs. On these hosts, *P. ramorum* causes a variety of foliar and branch symptoms. Since its association with SOD in 2000, much research has been conducted on the biology, genetics (including the sequencing of its genome), epidemiology, host-pathogen interactions and ecological impacts of *P. ramorum*. This talk will put *P. ramorum* research and management into historical context through examination of past research on other forest *Phytophthoras* and subsequent impacts of *P. ramorum* research on other *Phytophthoras*. Research on *P. cinnamomi*, *P. lateralis* and European Oak decline set the stage for early research on *P. ramorum* and set the stage for many discoveries concerning SOD. The potential importance of the nursery trade for long distance movement of *P. ramorum* has led to the implementation of national and international regulations and quarantines. This in turn resulted in numerous surveys and monitoring programs for the presence of *P. ramorum*. In the course of these surveys, many new *Phytophthoras* have been discovered in natural ecosystems and nursery settings.

***P. ramorum* - Diagnostics**

Mavrodieva, V.A.; Negi, S.; Picton, D.; Levy, L.; Tooley, P.; Shishkoff, N.; and Luster, D. 2008. Development and validation of a tissue based panel for the *P. ramorum* proficiency testing program. *Phytopathology* 98:S100.

Proficiency testing (PT) is a key element of laboratory accreditation program. A tissue-based PT panel for *P. ramorum*, used by the National Plant Protection Laboratory Accreditation Program (NPPLAP), was developed and validated in 2008 to assess proficiency of diagnosticians at critical stages of the diagnostic for *P. ramorum*. Healthy and *P. ramorum* infected *Rhododendron* 'Cunningham's White' leaves were used to prepare PT samples by lyophilization. Detached rhododendron leaves were dip-inoculated with 5000 sporangia per ml of *P. ramorum* and incubated in plastic moist chambers for 4–7 days at 20°C in darkness. Each batch of lyophilized tissue was characterized by DNA extraction and real-time PCR (TaqMan) analysis of 5–10% of the PT panel samples. Mean (average) Ct values and standard deviation coefficients (STDV) were estimated for the *P. ramorum* (FAM) and plant DNA (Texas Red) markers. To create PT tissue samples at varying concentrations, *P. ramorum*-infected tissue was diluted with healthy tissue at different ratios. The batch of 1:9 infected/healthy tissue ratio had a low STDV and produced a mean FAM Ct approximately 3.3 cycles higher

then the undiluted *P. ramorum* infected batch. At greater ratios (1:99 and 1:999), STDVs were 3.42 and 3.96 respectively. These samples were not used for the panel. Alternatively, healthy plant tissue was spiked with *P. ramorum* culture DNA to produce low-level infection samples. Using this method we obtained a batch with a high mean FAM Ct and satisfactory STDV. Selected PT sample batches were then validated by three analysts to determine PT panel performance. PT panel stability was monitored monthly.

Schoedel, B. and Avila, F.J. 2008. Specific immunodetection of *Phytophthora ramorum* and *P. kernoviae*. *Phytopathology* 98:S141.

Phytophthora ramorum is an important pathogen in the USA and Europe; and *P. kernoviae* is causing problems in ornamental plants and trees in Europe. The objective of this project was to develop serological tests for these two pathogens. Mycelium suspensions of *P. ramorum* from Europe and USA isolates; and *P. kernoviae* from Europe were used as antigens to produce monoclonal and polyclonal antibodies. Antibodies were selected for specificity to *P. ramorum* and *P. kernoviae*. The selected monoclonal antibodies do not differentiate between *P. ramorum* and *P. kernoviae*, or among *P. ramorum* isolates from Europe or USA in ELISA format. The polyclonal antibodies recognized antigens of *Phytophthora* and *Pythium* species. Other species of the genus *Phytophthora* and *Pythium* have been evaluated to confirm the specificity of the monoclonal antibodies. Preliminary results have indicated that monoclonal antibodies can be used in ELISA or lateral flow formats.

Sudarshana, P.; Shukla, R.; Abad, G.; Olson, B.R.; and Palm, M. 2008. A summary of diagnostics conducted by the USDA-APHIS-PPQ Molecular Diagnostic Laboratory. *Phytopathology* 98:S152.

The new APHIS-PPQ Molecular Diagnostic Laboratory (MDL) was established to conduct operational diagnostics and confirmatory testing for PPQ program pests including *Phytophthora ramorum* (sudden oak death and ramorum blight), *Liberibacter asiaticus* (citrus greening), *Xanthomonas axonopodis* pv. *citri* (Asian citrus canker) and *Globodera pallida* (potato cyst nematode). Since June 2006 more than 500 *P. ramorum* samples from 21 states were received for confirmatory testing. Nearly 70 samples were determined to be positive for *P. ramorum* using morphology for cultures and a combination of conventional and real-time PCR methods for DNA extracted from plant tissue and water or soil bait samples. *P. ramorum* was detected for the first time on *Magnolia virginiana* which is not currently on the SOD host list. In addition to the program pests, MDL provided molecular diagnostic support for a regional domestic survey for exotic pea leaf minor. The MDL also identified and characterized a number of exotic pathogens from port interceptions, postentry quarantine material and other samples in support of resolving international trade issues. Some examples include first report of *Botryosphaeria lutea* association with maple canker; confirmatory tests for laurel wilt (Red bay wilt) pathogen *Raffaelea* sp., first report of sweet orange scab disease caused by *Elsinoe australis* in Uruguay; bacterial strain differentiation in pineapple heart rot disease caused by *Erwinia chrysanthemi*.

Zeller, K.A.; DeVries, R.M.; and Levy, L. 2008. Head-to-head comparisons of sensitivity and specificity among 5 real-time PCR assays diagnostic for *Phytophthora ramorum*. Phytopathology 98:S179.

In response to Stakeholder requests, we adapted and validated five alternative Real-time PCR diagnostic assays for *Phytophthora ramorum* developed by other laboratories that target DNA loci in the ribosomal repeats, mitochondrial DNA, and in individual single-copy genetic loci for use in the Cepheid Smartcycler™. We have compared relative sensitivity and specificity of the methods by testing on a common set of >130 previously diagnosed environmental samples. Three of the 5 tested diagnostic methods failed to cross-react with DNAs from closely related *Phytophthora foliorum*, *P. hibernalis* or *P. lateralis*. However, each of these three methods also displayed lower sensitivity for target DNA of *P. ramorum* than do the validated conventional nested PCR assay and ITS-targeting Real-time PCR assay. Two of the three methods (mitochondrial *Cox* locus, and genomic Elicitin locus) are able to reliably detect *P. ramorum* DNAs to >50fg of target DNA. When combined with the currently used ITS-targeting Real-time PCR assay, either of these two Real-time PCRs should reduce the frequency of false positive results on initial tests of environmental samples for *P. ramorum*. The current ITS and Elicitin Real-time assays are multiplexed with internal control amplicons for mitochondrial and genomic DNAs, respectively, that replicate the internal controls in the current diagnostic system that make this combination desirable.

***P. ramorum* - Genetics**

Goss, E.M. and Grunwald, N.J. 2008. Ancient isolation and independent evolution of the three clonal lineages of the sudden oak death pathogen *Phytophthora ramorum*. Phytopathology 98:S61.

Sudden oak death, an emerging disease caused by the exotic pathogen *P. ramorum*, is responsible for extensive mortality of oaks and tanoaks in Northern California as well as economic losses to U.S. and European nurseries due to its infection of common ornamental plants. In its introduced range, *P. ramorum* occurs as three distinct clonal lineages. The two common lineages are opposite mating types, but oospores are not readily produced in culture and they have not been observed in the field. We inferred the evolutionary history of *P. ramorum* from DNA sequence variation at five nuclear loci using coalescent-based approaches. We found that the lineages have been diverged for at least 11% of their history, an evolutionarily significant amount of time roughly estimated to be on the order of 165,000 to 500,000 years. There was also strong evidence for historical recombination between the lineages, indicating that the ancestors of the *P. ramorum* lineages were members of a sexually reproducing population. Due to this recombination, the ages of the lineages varied within and between loci, but analyses suggested that the European lineage may be older than the North American lineages. The divergence of the three clonal lineages of *P. ramorum* supports a scenario in which the three lineages originated from different geographic locations that were sufficiently isolated from each other to allow independent evolution prior to introduction to North

America and Europe. It is thus likely that the emergence of *P. ramorum* in North America and Europe was the result of three independent migration events.

P. ramorum - Nurseries

Grunwald, N.J.; Larsen, M.; and Goss, E.M. 2008. Genotypic diversity of *Phytophthora ramorum* in U.S. nurseries. *Phytopathology* 98:S63.

Phytophthora ramorum is the causal agent of sudden oak death, responsible for the rapid decline of tanoak and coast live oak in California coastal forests. It also causes Ramorum blight in many common ornamentals, including *Rhododendron*, *Viburnum*, *Pieris*, *Syringa* and *Camellia*. Genetic variation in *P. ramorum* is structured into three clonal lineages, designated EU1, NA1, and NA2. EU1 is generally mating type A1 while all tested NA1 and NA2 isolates have been mating type A2. All three clonal lineages have been isolated from U.S. nurseries. We have been routinely genotyping *P. ramorum* isolates found in U.S. nurseries using microsatellite loci that exhibit variation within and between lineages. The clonal lineage of each genotyped isolate is posted to a public website along with additional information about the isolate, such as the host species and its county and state of origin (<http://oregonstate.edu/~grunwaln/index.htm>). We have found that NA1 continues to be the most common lineage isolated from infected nursery stock in the U.S. Our genotyping revealed the first incidence of EU1 in California in 2006, where it appeared in the same nursery as NA1. In 2007 we found the two lineages on different leaves of the same plant in an Oregon nursery. EU1 was found in all three Pacific coast states in 2007, whereas the NA2 lineage was limited to Washington State. Continued genotyping of nursery isolates will be critical for monitoring migration of the clonal lineages and the emergence of any new or recombinant lineages.

Parke, J.L.; Grunwald, N.; Lewis, C.; and Fieland, V. 2008. A systems approach for managing *Phytophthora* diseases in production nurseries. *Phytopathology* 98:S121.

Nursery plants are susceptible to several diseases caused by *Phytophthora* species. Nursery plants are also important long-distance vectors of non-indigenous pathogens such as *P. ramorum*. Pre-shipment inspections have not been adequate to ensure that shipped plants are free from *Phytophthora*, nor has this method informed growers about sources of contamination in their nurseries. We applied a new approach based on Hazard Analysis of Critical Control Points (HACCP) for systematically detecting sources of *Phytophthora* contamination in four Oregon nurseries. We identified critical control points (CCPs) in commercial production systems and sampled bimonthly over a 15-month period. Plants, potting media, containers, irrigation water, and can yard substrates were sampled at all stages of production. Putative *Phytophthora* isolates were tested with genus-specific PCR and identified to species by direct sequencing of the internal transcribed spacer (ITS) rDNA. The most frequently encountered species were *P. cinnamomi*, *P. syringae*, *P. citricola*, *P. cryptogea*, *P. gonopodyides* and *P. citrophthora*. Results showed that healthy container plants often became contaminated when set out on contaminated can yard substrates. Used containers were sources of contamination at all four nurseries, as was water from irrigation ponds at two nurseries. After identifying

CCPs where contamination occurred, we worked with nursery managers to develop best management practices (BMPs) specific for each nursery. Sampling will continue after BMPs are implemented to determine if this approach is successful in reducing *Phytophthora* contamination.

Roubtsova, T.V. and Bostock R.M. 2008. Impact of episodic root stress on the susceptibility of *Rhododendron* sp. and *Viburnum tinus* to *Phytophthora ramorum*. *Phytopathology* 98:S136.

Phytophthora ramorum attacks members of the Fagaceae, causing foliar blight and dieback on many forest and nursery species. To examine root infection by *P. ramorum* and the potential role of mild abiotic stress in disease predisposition, experimental systems were established with *Rhododendron* sp. and *Viburnum tinus*. Experiments were conducted in two formats: modified hydroponic culture and standard potting soil. Roots of plants were exposed to NaCl stress prior to inoculation under four treatment regimes: 1) salt-stressed, non-inoculated; 2) non-stressed, non-inoculated; 3) salt-stressed, inoculated; and 4) salt-stressed, non-inoculated. Plants in hydroponic culture were exposed to 0.2 M NaCl/0.02 M CaCl₂/0.5x Hoaglands for 12 hours and then returned to 0.5x Hoaglands. Potted plants were treated with a soil drench of 0.2 M NaCl/0.02 M CaCl₂ for 12 hours, and then flushed with water to remove the salt. Roots were then inoculated with zoospores of *P. ramorum*. In hydroponic plant cultures, the two *P. ramorum* isolates tested were similar in pathogenicity on *Rhododendron* and *Viburnum* plants, with root and stem lesions developing within one week after inoculation (10⁴) zoospores/ml) in salt-stressed roots. Non-stressed, inoculated plants became symptomatic after two weeks. Microscopic examination of roots from both species revealed that their tips were covered with sporangia of *P. ramorum*. On potted *Rhododendron* plants, disease developed in salt-stressed roots, with death of the plant occurring within four weeks after inoculation. Non-stressed plants survived for 6–8 weeks following inoculation. The implications of episodic stress in root infection by *P. ramorum* and disease development in nursery ornamentals will be presented.

Shishkoff, N. 2008. Sporulation on plant roots by *Phytophthora ramorum*. *Phytopathology* 98:S145.

Phytophthora ramorum has been shown to infect the roots of many of its foliar hosts. Methods of detecting inoculum in runoff and of quantifying root colonization were tested using *Viburnum tinus*, *Camellia oleifera*, *Quercus prinus*, *Umbellularia californica*, and *Epilobium ciliatum*. Plants grown from seed or cutting in Turface monmorillonite clay granules were inoculated with a sporangial suspension (15 mL per pot at 500 sporangia/mL) and after 24 hours, uprooted, washed, and transplanted to fresh Turface (100 mL volume). Runoff was collected periodically and aliquots plated on selective media to quantify inoculum of *P. ramorum*; at the end of the assay, roots were plated on selective media to determine colonization. In some trials, plant roots were examined at the end of the experiment, and in *Viburnum*, it was easy to see sporulation on root tips. Dissection of *Viburnum* roots revealed embedded chlamydospores. Other host roots, could be heavily pigmented or extremely fine, and signs of the pathogen were not often

seen, even in heavily infected material. *P. ramorum* was commonly detected from runoff of all tested plants. In 32 *Viburnum* trials over the course of a year, an average of 41 propagules per pot (4 propagules per mL of runoff) were recovered from runoff from plants seven days after inoculation, with a high of 358 propagules/pot (24 propagules/mL runoff). The significance of such sporulation in the epidemiology of the pathogen needs further study.

P. ramorum - Wildlands

Brennan, J.; Cummins, D.; Kearney, S.; Choiseul, J.; Cahalane, G.; and Nolan, S. 2008. Investigating the threat of *Phytophthora ramorum* to Ireland: The current situation. *Phytopathology* 98:S25.

Phytophthora ramorum is a serious pathogen of trees and ornamental plants, causing a disease known as sudden oak death (SOD) in the USA, where it has had major environmental & economic impacts. Plants affected by *P. ramorum* show a range of symptoms such as leaf blight, stem canker, and tip dieback. The pathogen was first detected in the USA in the mid 1990s, Europe 2001 (it was first named in 2001 but the disease was first detected in early 1990s) and Ireland in 2003. *Phytophthora ramorum* is subject to plant health controls in the EU under Commission Decision 2002/757/EC (& amendments). The organism has been isolated from over 30 plant species worldwide including a number which have significant commercial and amenity value in Ireland, particularly *Rhododendron* and *Viburnum* spp. To date *P. ramorum* has not been detected on tree species in Ireland, however there is strong concern however that Irish trees could become infected. Extensive surveys have been carried out by the Department of Agriculture, Fisheries & Food (DAFF) from 2003 to present. Since 2003 over 4000 samples were collected from nurseries, garden centres, public parks and forests around Ireland. *Phytophthora ramorum* was detected in all years [positive samples: 8% (2003), 2% (2004), 19% (2005), 10% (2006) & 16% (2007)]. In 2003, *P. ramorum* was only found on *Rhododendron* and *Viburnum* spp., however by 2007 the presence of *P. ramorum* was confirmed on five plant genera (*Rhododendron*, *Viburnum*, *Camellia*, *Photinia* & *Magnolia*). *Phytophthora ramorum* has also been found on wild *Rhododendron* spp. On going surveys are being carried out by Horticulture and Plant Health Division and the Forest Service of DAFF. Eradication & containment measures are being implemented in accordance with EU legislation.

Dileo, M.V.; Bostock, R.M.; and Rizzo, D.M. 2008. **Ecophysiological factors** mitigating *in planta* survival of *P. ramorum* in California bay laurel. *Phytopathology* 98:S46.

Phytophthora ramorum, the causal agent of sudden oak death, has altered the community structure of coastal California forests by dramatically increasing the mortality rates of keystone species such as tanoak and oaks. In these ecosystems, bay laurel (*Umbellularia californica*) has been found to be the most important reservoir host for this pathogen both by supporting the majority of pathogen sporulation from ubiquitous, non-lethal foliar infections in the winter wet season and also by providing shelter during the dry,

Mediterranean summer. The proportion of symptomatic bay leaves from which *P. ramorum* can be successfully isolated typically decreases during the summer. This putative loss of infection appears to occur to a greater extent within mixed-evergreen than redwood-tanoak forests. A field study was conducted during the summers of 2005, 2006 and 2007 to address these observations and to assess associations between summer survival of *P. ramorum* within bay laurel leaves and environmental, topographic and physiological variables. Isolation success from symptomatic leaves was tracked in 50 trees within 12 sites in the Sonoma and Mayacmas mountain ranges and compared to temperature, vapor pressure deficit, elevation, insolation, canopy exposure, leaf area, leaf water potential, and lesion area data. The resulting model describes environmental and physiological constraints on the summer survival of *P. ramorum* and will assist in the development of sudden oak death risk assessments.

Fichtner, E.J.; Rizzo, D.M.; Kirk, S.; Whybrow, A.; and Webber, J. 2008. Root infections of *Phytophthora ramorum* and *Phytophthora kernoviae* in UK woodlands. *Phytopathology* 98:S53.

Rhododendron ponticum, an invasive weed pervading UK woodlands, supports prolific sporulation of *Phytophthora kernoviae* and *Phytophthora ramorum*. The long-term efficacy of *R. ponticum* removal from woodlands as an inoculum management strategy is unknown, in part due to lack of knowledge of pathogen persistence in roots and emerging seedlings. The potential for both pathogens to infect *R. ponticum* roots was investigated. Adventitious roots from shoot layers and associated leaf litter, as well as rhizosphere soil, and foliage were collected from infested sites in 2007. Soil, leaf litter, foliage, and surface sterilized roots were baited with rhododendron leaf disks for *Phytophthora* spp. The potential for infection of *R. ponticum* seedlings in a woodland cleared of *R. ponticum* in 2005 for management of *P. kernoviae* was similarly investigated. Emergent seedlings were excavated and their surface-sterilized roots and foliage were assessed for infection by *P. kernoviae*. Neither pathogen was routinely baited from rhizosphere soil, but both were frequently recovered from leaf litter. Both pathogens were recovered from adventitious roots, and *P. kernoviae* was recovered from roots of emergent seedlings. *P. kernoviae* was routinely recovered from roots of otherwise asymptomatic seedlings. The results suggest that the persistence of these pathogens in roots and litter should be considered when managing the diseases in infested woodlands.

Jinek, A.; Simard, M.; Brière, S.C.; Watson, A.K.; Tweddell, R.J.; and Rioux, D. 2008. Susceptibility of six eastern Canadian forest species to *Phytophthora ramorum*. *Phytopathology* 98:S75.

Phytophthora ramorum (Pr), a recently described pathogen, causes sudden oak death, ramorum leaf blight and ramorum shoot dieback. The list of ornamental and wild plant species that are naturally infected by Pr exceeds 120 host species. Absent in the wild in eastern North America, there is concern that Pr could be introduced and spread into this area. To help assess this risk, detached leaves/needles of six eastern Canadian forest species were inoculated with Pr and the amount of necrosis and sporulation was evaluated. *Abies balsamea*, *Acer saccharum*, *Betula alleghaniensis* (Ba), *Fraxinus*

americana (Fa), *Larix laricina*, and *Quercus rubra* (Qr) were the species tested whereas *Rhododendron* ‘Nova Zembla’ (Rh) served as positive control. With broad-leaved species (BLS), Ba and Fa were the most susceptible but sporulation was only significant on Qr, which was similar to that on Rh. Compared with the BLS, the amount of necrosis on needles was higher in both conifer species concomitantly with a higher level of sporulation. Real-time PCR results suggested that the amount of Pr DNA was higher in BLS than in conifer tissues. In addition, it clearly appeared that the young leaves of Ba, Fa and Qr were more susceptible than the older leaves.

Nagle, A.M.; Garbelotto, M.; and Bonello, P. 2008. Differences in constitutive and induced expression of two phenolic compounds in coast live oaks susceptible and resistant to infection by *Phytophthora ramorum*. Phytopathology 98:S111.

Phytophthora ramorum is the causal agent of sudden oak death (SOD), a devastating disease of tanoaks and oaks in California and Oregon. Apparent resistance to infection by *P. ramorum* in coast live oak (CLO) has been observed in natural populations and in laboratory inoculation trials. No practical controls for this disease are available, therefore characterization of natural resistance is highly desirable. In a preliminary test, we used HPLC analysis to evaluate branch phloem phenolic profiles for CLO’s previously identified as relatively susceptible (S) (N = 4) or resistant (R) (N = 5) to *P. ramorum*. Separate sets of branches from the same trees were wound-inoculated with *P. ramorum*. Two compounds, identified as tyrosol and catechin, were present in constitutively higher amounts in R than in S trees, but due to the low replication the differences were not significant. However, a significant overall negative correlation was found between lesion length and tyrosol concentration ($r = -0.667$, $N = 9$, $P = 0.026$). These preliminary findings may be important in establishment of chemical biomarkers, which has great significance in applications such as screening of oak germplasm for resistance to SOD. Follow-up studies in different seasons and with trees exhibiting prolonged resistance in the field under high disease pressure are planned.

Parke, J.L.; Oguchi, A.; Fichtner, E.J., and Rizzo, D.M. 2008. Viability of *Phytophthora ramorum* after passage through slugs. Phytopathology 98:S121.

Phytophthora ramorum, the causal agent of sudden oak death, produces abundant chlamydospores; however, understanding the role of these potential survival structures in the disease cycle is difficult due to the low and variable rate of chlamydospore germination. Oospore germination of other *Phytophthora* spp. may be stimulated by passage through the alimentary canal of snails, but the effects of chlamydospore ingestion by molluscs are unknown. The viability of *P. ramorum* colonies was investigated after passing them through the alimentary canal of banana slugs (*Ariolimax* spp.) and grey garden slugs (*Derocerus reticulatum*). Slugs that were fed V8 agar cultures of *P. ramorum* produced feces that contained hyphae and chlamydospores. Broth-grown hyphae and chlamydospores were also applied to strawberries and fed to slugs. Slug feces from both sources plated onto *Phytophthora* selective media yielded *P. ramorum* colonies. Microscopic observation showed that many chlamydospores in fecal samples either germinated directly, often with multiple germ tubes, or indirectly to form

sporangiophores. Sporangia production was abundant on fecal surfaces. Tanoak leaves inoculated with feces from culture-fed slugs became infected by *P. ramorum*. These results suggest that a portion of chlamydospores and hyphae that pass through the alimentary canal of slugs remain viable. The potential effect of slugs on chlamydospore germination and the possible role of slugs in disease transmission will be investigated.

P. ramorum - Treatments

Colburn, G.C. and Jeffers, S.N. 2008. Toxicity of commercial algaecides to *Phytophthora ramorum*. Phytopathology 98:S40.

Oomycetes like species of *Phytophthora* are more closely related to brown algae than they are to fungi. Therefore, commercial algaecides (with copper compounds as active ingredients) used to manage algae in natural and commercial waterways might be useful in managing *P. ramorum* in similar settings. Chlamydospores of A1 and A2 isolates of *P. ramorum* were produced on mycelia grown in clarified V8 broth; cultures were sonicated to kill hyphae and free chlamydospores. Sporangia were produced by growing isolates on V8 agar and placing agar plugs in a sterile soil-extract solution, and zoospores were released after a cold temperature shock. Chlamydospores (5×10^3 spores/ml), sporangia (2.5×10^3 sporangia/ml), and zoospores (1×10^5 spores/ml) were exposed to commercial rates of two algaecides (0.8 ppm of copper carbonate and 1.0 ppm of copper-triethanolamine + copper hydroxide) for 0, 0.5, 2, 4, 8, and 24 hr. For each treatment, propagules were washed to remove algaecides and were collected on membrane filters. Filters were inverted on PAR-V8 selective medium, and plates were placed at 20C. For both isolates, zoospores were not viable after 30 min of exposure to either algaecide. Compared to the control, viabilities of chlamydospores and sporangia of both isolates were reduced significantly at 2 and 4 hr of exposure to the algaecides; no chlamydospores or sporangia remained viable at 8 or 24 hr of exposure. Consequently, algaecides have potential to manage *P. ramorum* in natural and commercial waterways

Other *Phytophthoras*

Ahonsi, M.O.; Banko, T.J.; Doane, S.R.; Demuren, A.O.; Copes, W.E.; and Hong, C.X. 2008. *Phytophthora nicotianae* zoospores evade pressure and agitation stress but are completely destroyed by CO(2) injection. Phytopathology 98:S10.

Phytophthora nicotianae is a known pathogen of numerous herbaceous and some woody ornamental plants, and is commonly isolated from recycled irrigation ponds. Zoospores are the most important propagules of *Phytophthora* spp. Using simulated recycled irrigation water we investigated the survival of *P. nicotianae* zoospores as affected by hydrostatic pressure, agitation, and aeration with CO(2) or air. Exposing zoospores to hydrostatic pressure of 840 kPa for 8 min or agitation of mixing intensity $G = 6483$ 1/s for 4 min did not kill any zoospores. However, bubbling CO(2) into zoospore-infested water at 110.4 ml (0.2 g)/min for 5 min consistently killed up to 81% of the zoospores. Further extending CO(2) injection up to 30 min did not increase percent zoospores killed although fewer were killed with a shortened injection time. When we exposed zoospores

to CO₂ pressure of 630 kPa (16.3 g CO₂) or 70 kPa (3.85 g CO₂) for 30 seconds or longer, percent zoospore kill did not differ from one another and did not differ from bubbling CO₂ at 110.4 ml/min for 5 min. In contrast, when the same treatments were done using pressurized air in place of CO₂, all zoospores survived. In further experiments, when we minimized cyst formation during zoospore-infested water preparation by avoiding vigorous shaking, CO₂ injection consistently resulted in over 98% zoospore kill. We concluded that the percent zoospores not killed by CO₂ injection in previous experiments were zoospores that had encysted before exposure to CO₂. Similarly, hydrostatic pressure and agitation treatments induced cyst formation and consequently allowed 100% survival. Results indicate that CO₂ treatment may be a promising alternative technology for disinfecting recycled irrigation water contaminated with *P. nicotianae*.

Hwang, J.; Oak, S.W.; and Jeffers, S.N. 2008. Variation in population density and diversity of *Phytophthora* species in streams within a forest watershed. *Phytopathology* 98:S70.

Water samples from a portion of the Davidson River watershed in western North Carolina were collected and assayed to help determine the number of sample sites needed to effectively survey watersheds for *Phytophthora* species. The sampled watershed covers 32.6 km² and consists of nine sub-watersheds, each drained by an individual stream that runs into the Davidson River. Seven streams, each in a separate sub-watershed, and the drainage point at the lower end of the Davidson River were sampled twice, in Sep and Oct 2007. Samples (1 liter) from all streams were collected within a 30-min period to minimize variation that may be associated with time of day. Nine 100-ml aliquots were filtered from each sample and filters were inverted onto PARPH-V8 selective medium; colonies of *Phytophthora* spp. were counted after 3 days at 20C, and numbers of colony-forming units (cfu) were calculated. Densities of *Phytophthora* spp. were lower in the streams draining the upper sub-watersheds. In Oct, the mean density of *Phytophthora* spp. from the three upper streams was 5 cfu/liter while a mean density of 52 cfu/liter occurred in three lower streams. Six known species--*P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. gonapodyides*, *P. heveae*, and *P. pseudosyringae*--and three previously unidentified species were recovered from streams in the watershed. The diversity of species varied among streams and was greater in Oct than in Sep. In Sep, five species were detected within the watershed and three of these were recovered at the lower drainage point. In Oct, nine species were detected in the watershed and five of these were recovered at the drainage point

Ivors, K.L. and Greene, M.D. 2008. Identifying *Phytophthora* species isolated from stream baits in North Carolina. *Phytopathology* 98:S72.

Ten locations throughout North Carolina were initially established as part of the National *Phytophthora ramorum* Early Detection Survey in Forests. Non-wounded *Rhododendron* leaves were placed in mesh bags as baits and secured in perennial watercourses for seven to fourteen day intervals. Recovered symptomatic leaves collected from May through November 2007 were plated on selective media. Putative *Phytophthora* isolates were

provided to our lab from the North Carolina Department of Agriculture and identified using ITS sequencing. Isolation of *Phytophthora* species varied by location and time of sampling. This collection of over 100 isolates included multiple representatives of the four described species *P. gonadopodyides*, *P. citricola*, *P. citrophthora* and *P. cryptogea*, and eight clades of previously undescribed *Phytophthora* species. Future work will include additional stream monitoring and ITS identification of isolates, as well as addressing the survival, spread and significance of these undescribed species in North Carolina watercourses and natural ecosystems.

Kim, S.; Nikolaeva, E.V.; Park, S.; and Kang, S. 2008. First report of *Phytophthora hedraïandra* in Pennsylvania. *Phytopathology* 98:S82.

Phytophthora hedraïandra has been recently identified for the first time in PA. The pathogen was isolated from *Rhododendron catawbiense* showing twig blight symptoms collected from three different PA counties from June 15 to July 22, 2004 as part of the PPQ national *P. ramorum* survey. The pathogen was isolated from 12 samples comprised of 'Roseum Pink,' 'Roseum Elegans,' and unknown cultivars that originated from Virginia, Oregon, and unknown sources. The cultures grown on PARP at 20°C were tentatively identified as *P. cactorum* by morphology, and stored in hemp-seed water (one culture plug from V8-200/two seeds/10 ml sterile distilled water). Lately, the pathogen was identified as *P. hedraïandra* based on its sequences of the ITS region. Koch's postulate was satisfied by inoculating *R. catawbiense* 'Roseum Pink' using the 12 isolates of *P. hedraïandra*. A detached leaf on moistened paper towel in a plastic bag was inoculated with a 5-mm disc of the inoculum on V8 200 agar and then incubated at 27°C for 7 days, 12 h light cycle (Each isolate replicated 4x). The lesion size averaged 1,215 mm² (SD ± 606 mm²). No symptoms were observed on control leaves that were inoculated with V8 200 agar plugs. *P. hedraïandra* was reisolated from the lesions. This first detection of *P. hedraïandra* in PA is from limited areas and further study is needed for comprehensive risk assessment.

Kong, P. and Hong, C. 2008. Quorum sensing operates in *Phytophthora nicotianae*. *Phytopathology* 98:S85.

The term quorum sensing was introduced to describe the control of gene expression in bacteria species in response to cell density. Bacteria produce, detect and respond to hormone-like signal molecules called autoinducers to coordinate communal behaviors. Most autoinducers (e.g. acyl-homoserine lactones, AHL) promote intraspecies communication, but autoinducer 2 (AI-2) allows interspecies communication and regulates gene expression of many important behaviors including virulence. Apart from bacteria, no organism has been shown to have a quorum-sensing system involving AI-2. Here we show operation of quorum sensing involving AI-2 in *Phytophthora nicotianae*. Using two autoinducer reporters, we demonstrated that zoospores produce an AI-2-like signal but not AHL. We also demonstrate chemical communication among zoospores prior to or during plant infection by *P. nicotianae*. Autoaggregation and plant infections that usually require a high concentration of zoospores occurred at a low concentration or single spore level when provided with zoospore free fluid (ZFF) from a highly

concentrated suspension. Moreover, zoospores at low concentration did not move toward to plant tissue unless supplied with ZFF. These results indicated *Phytophthora* species may share a similar quorum sensing mechanism with bacteria although their autoinducers may be produced through different pathways. This mechanism may allow *Phytophthora* species to maximize infection potential by use of widespread bacterial autoinducer (AI-2) in nature. It may thus be possible to develop novel methods to control *Phytophthora* diseases through interfering with the pathogen's communication systems.

McLaughlin, I.M.; Jeffers, S.N.; and Waldrop, T.A. 2008. Long-term effects of fuel reduction treatments on the incidence of *Phytophthora* spp. in soil of a hardwood forest in the southern Appalachian Mountains. *Phytopathology* 98:S102.

The accumulation of fuels is one of the main contributors to forest fires. The National Fire and Fire Surrogate study was initiated in 2000 to investigate the effects of fuel reduction treatments on a variety of ecosystem parameters in forests across the United States. We have been studying the effects of these treatments on the incidence of *Phytophthora* spp. in forest soils of the southern Appalachian Mountains. The study site was located in Polk Co. in western North Carolina and was composed primarily of hardwood and southern pine trees. Four fuel reduction treatments were applied to plots in each of three replicate blocks: prescribed burning, mechanical fuel reduction, fuel reduction followed by burning, and a non-treated control. Ten sub-plots (20 m × 50 m) were established in each treatment plot of each block. Representative, composite soil samples were systematically collected from sub-plots before treatments were applied in 2002, after one application of all treatments in 2004, and after a second application of prescribed burning in 2007. A baiting bioassay, using camellia leaf pieces and hemlock needles as baits, was used to assay soil sub-samples for *Phytophthora* spp. Only two species of *Phytophthora* were recovered throughout this investigation; *P. cinnamomi* was recovered from 33% of sub-plots in 2002, 31% of sub-plots in 2004, and 57% of sub-plots in 2007 while *P. heveae* was recovered from less than 1% of the sub-plots at each sample period. Fuel reduction treatments did not significantly affect the incidence of *Phytophthora* spp. in sub-plots over the 5-yr duration of this project. However, incidence in all sub-plots combined was greater in 2007 than in either 2002 or 2004. Overall, fuel reduction treatments did not have any immediate or long-term effects on the incidence of *Phytophthora* spp. in forest soil during this study.

Olson, H.A. and Benson, M. 2008. Characterization of *Phytophthora* in North Carolina greenhouse ornamentals. *Phytopathology* 98:S116.

Root rot, crown rot, and foliar blight, caused by species of *Phytophthora*, are common diseases on ornamental crops and are ongoing problems in greenhouse production. Greenhouse facilities in North Carolina were surveyed to expand the data from a 2001–2002 survey. Symptomatic plants were collected, and direct isolation from plant material was conducted on a selective medium. To date, *Phytophthora* isolates have been identified as *P. cryptogea*, *P. drechsleri*, *P. nicotianae*, and *P. tropicalis* using morphology and sequences of the ITS region. Incubation at 35°C has been used to distinguish *P. cryptogea* (no growth) and *P. drechsleri* (growth). Utility of this assay was

evaluated with collected isolates. Results were inconclusive. Isolates of *P. cryptogea* did not grow; however, not all *P. drechsleri* isolates grew at 35°C. Mefenoxam sensitivity was tested using growth on mefenoxam-amended medium. Isolates of *P. cryptogea* and *P. tropicalis* were sensitive; whereas, *P. drechsleri* isolates were insensitive. Mefenoxam sensitivity of *P. nicotianae* ranged from sensitive to insensitive and depended on the geographic origin of the isolate. The *P. cryptogea* isolates from the 2001–2002 survey were characterized further by ITS and *cox* sequencing. Inconsistencies between the ITS RFLP, ITS sequence, and *cox* sequence identifications are being explored. In-depth characterization of the species present will further our understanding of the impact of *Phytophthora* on the floriculture industry in North Carolina.

Santamaria, L. and Mmbaga, M.T. 2008. A survey for Phytophthora diseases in mid-Tennessee nurseries: Identification and characterization. Phytopathology 98:S140.

Phytophthora diseases impact trees and shrubs in nursery production and landscape settings, but identification of species in mid-Tennessee nurseries have not been done. A survey of Tennessee nurseries was started in 2006 and results from eight nurseries sampled will be discussed. Samples of plant tissues from symptomatic plants, rhizosphere soil and water from irrigation ponds or creeks were evaluated for Phytophthora. Direct isolation of Phytophthora from plant tissues and baiting system for Phytophthora from soil and irrigation water were used in this survey. A total of 660 samples were processed using Phytophthora semi selective media (PARPH). The pathogens isolated were characterized morphologically and using DNA analysis following standard PCR protocols with universal primers ITS1/ITS4. Results of the first 100 samples DNA sequence analysis (Davis Sequencing, Davis, CA) showed that *Phytophthora* spp. was the major organisms in 37% of the samples. Other fungi isolated included *Pythium* 13%, Uncultured endophytes 9%, *Pestalotiopsis* spp. 6%, *Alternaria* 6%, *Absidia/Heterobasidion* 6%, *Fusarium* spp. 6%, *Phoma* spp. 4%, and 12% miscellaneous genera including *Botryosphaeria*, *Ampelomyces*, *Glomerella*, *Giberella*, *Paraconiothyrium* spp. and *Verticillium*. Most of the *Phytophthora* species were isolated from soil and water (36%), and only 1% was from plant tissue. 28% of *Phytophthora* spp. are unclassified *Phytophthora* according to GenBank information. Several *Phytophthora* species were often found in one nursery. Example: PC nursery had 4 species, *P. cinnamomi* and *P. cryptogea* associated with Juniper, *P. nicotianae* (Cotton Easter), and unclassified *Phytophthora* spp. from their irrigation water. Another nursery had *Phytophthora* spp., and *P. cryptogea/megasperma*. Species of *Phytophthora* in the irrigation water were different from those isolated from the soil or plant tissue. More intensive sampling is needed to determine the association of pathogens in irrigation water with disease incidence in the irrigated fields. Species in irrigation water have the potential to infect susceptible hosts during irrigation. Some of the other fungi isolated from plant tissue, soil and/or water are known pathogens. Their pathogenicity and role in disease complexes will be evaluated.

Wang, S.; Lyles, L.; Garneni, S.; Carlos, W.J.; and McKie, P. 2008. Phytophthora species associated with silver maple bleeding canker in northern Nevada. Phytopathology 98:S166.

A bleeding canker disease, symptomatically similar to sudden oak death, was first noticed in 1999 on silver maple trees (*Acer saccharinum*) in northern Nevada. The disease has caused decline or loss of both young and mature trees. In an effort to identify the cause, pieces of fresh phloem and xylem tissue were collected from the margin of lesions underneath the canker area, and then placed on pimarinic-ampicillin-rifampicin-PCNB agar to isolate *Phytophthora* species. Six isolates (SM1-SM6) obtained from different locations represent two groups morphologically. Five of them (SM2-SM6) have identical morphology similar to *P. cactorum*, and one (SM-1) differs from other isolates. To confirm their identities, regions of rDNA including partial 18S ribosomal RNA gene, ITS1, 5.8S ribosomal RNA gene, ITS2 and 28S ribosomal RNA gene were amplified from selected isolates, subcloned into pGEM®-T vector, and then sequenced using T7 promoter and SP6 upstream primers. DNA sequences of *P. cactorum*-like isolates generated a minimum of first 97 hits of deposited *P. cactorum* sequences and had 99% nucleotide identities by a BLAST search of the GenBank database. DNA sequence of SM-1 matches mostly the sequences of 7 isolates of an undescribed *Phytophthora* species followed by 74 hits of deposited *P. citricola* sequences. Thus, the identities of these isolates were confirmed as *P. cactorum* and an undescribed *P. citricola*-related species. Both species are believed to be the primary cause of silver maple bleeding canker in northern Nevada.

Weiland, J.E.; Nelson, A.H.; and Hudler, G.W. 2008. Aggressiveness of *Phytophthora cactorum* and *Phytophthora citricola* isolates on European beech and lilac. *Phytopathology* 98:S168.

Inoculation experiments were conducted to compare the aggressiveness of *Phytophthora cactorum* and *P. citricola* isolates on European beech and lilac seedlings grown in a greenhouse. The isolates were obtained from bleeding cankers on European beech from 5 cities (Albany, Ithaca, Oyster Bay, Plainview, and Rochester) in New York. Isolates of *P. citricola* were subdivided into 2 clades (*P. citricola* 1 and 2) based on distinct differences within selected DNA sequences. Stems, roots, and leaf disks of both hosts were inoculated with 3 single-spore isolates of *P. cactorum*, 4 of *P. citricola* 1, and 3 of *P. citricola* 2. Stems were inoculated with colonized agar plugs, roots via infested soil at 3 inoculum levels, and leaf disks with a zoospore suspension. Disease incidence was independent of isolate in all inoculated stems and leaf disks (100%), but was dependent on isolate in the soil infestation assay (0–100%) for both hosts. Severity (canker length, rate of mortality, and affected leaf disk area) was dependent on isolate regardless of inoculation site (stem, root, or leaf, respectively) or host, with *P. cactorum* isolates usually causing less necrosis than either clade of *P. citricola*. However, the range of disease severity caused by isolates of *P. citricola* 1 was similar to that of *P. citricola* 2. Lilac was less severely affected by inoculation than beech, regardless of isolate. No effect of inoculum level on root infection was observed.

Widmer, T.L. 2008. Comparing New Zealand and United Kingdom isolates of *Phytophthora kernoviae*. *Phytopathology* 98:S171.

Phytophthora kernoviae was discovered in the United Kingdom in 2003 and identified as a new species in 2005. Recent DNA sequence studies identified two unknown *Phytophthora* isolates collected in the 1950s and 2002 in New Zealand as *P. kernoviae*. The purpose of this study was to compare two isolates originating from New Zealand (PK-1 and PK-2) and two isolates originating from Cornwall, UK (PK-3 and PK-4). Mycelial growth on agar plates was similar for all isolates at 5, 10, 15, 20, 25, and 30°C. Sporangial production was about five to 10 times higher in liquid cultures for PK-3 and PK-4 than PK-1 and PK-2. However, isolate PK-3 could not be induced to release zoospores. *P. kernoviae* is homothallic and easily produces oospores in culture. Oospore production was similar for PK-1, PK-2 and PK-4, which were about three times higher than for PK-3. Inoculation of Rhododendron leaf disks with sporangial or oospore suspensions showed little difference in necrosis among the isolates, except for PK-3, which showed very little necrosis. Whole plant inoculations of *Magnolia stellata* and *Rhododendron* “Cunningham’s White” showed higher necrosis when inoculated with sporangia of PK-1 and PK-2. These results show differences between the New Zealand and UK isolates of *P. kernoviae*. Future tests should include at least one isolate from each geographic location.

Miscellaneous

Levesque, C.A.; de Cock, A.W.A.M.; Robideau, G.; Desaulniers, N.; and Bala, K. 2008. The Oomycota. *Phytopathology* 98:S184.

Oomycetes are no longer part of the Eumycota, or true fungi. Although oomycetes are different from true fungi in many ways, the two groups still have many common ecological features. Molecular taxonomy and phylogenies have confirmed for the most part the traditional classification of oomycetes. The two main orders of Saprolegniales and Peronosporales are still well separated by phylogenies. Most important plant pathogen genera such as *Pythium* and *Phytophthora* are still monophyletic and their species morphological taxonomy is generally supported by molecular analyses. There are a few exceptions though. There are some species that are being split (e.g. *Py. irregulare*), genera that are within a genus clade (e.g. *Pythiogeton*), and clades that might require a new genus status (e.g. *Pythium vexans* clade). Most phylogenetic studies of oomycetes have been done with the ribosomal DNA cistron and mitochondrial cytochrome oxidase genes but multigene phylogenies were performed in *Phytophthora*. These were made possible by the large amount of genome sequence information available for different species of this genus. The genome of *Pythium ultimum* was recently sequenced, opening new possibilities of multigene studies in Peronosporales. There are also efforts to sequence the genome of *Saprolegnia parasitica* which would greatly facilitate broader phylogenetic studies in oomycetes.