Fifth Sudden Oak Death Science Symposium

MEETING ABSTRACTS

June 19-22, 2012, Petaluma, California, USA

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Fifth Sudden Oak Death Science Symposium
Sheraton Sonoma County - Petaluma
June 19-22, 2012

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Welcome

Welcome to the Fifth Sudden Oak Death Science Symposium. It has been almost 10 years since the first Sudden Oak Death Science Symposium was held in Monterey, CA. At that gathering, no one would have dreamed that *Phytophthora ramorum* would cause an epidemic on Japanese larch (*Larix kaempferi*) in the UK, or even that *P. ramorum* would be detected on *Camellia* and shipped inadvertently to nurseries in the Eastern US. Over the past decade, an estimated $50 million has been invested in research to combat *P. ramorum*, with remarkable progress made in monitoring, diagnosis, genetic fingerprinting, and other areas. Yet, we continue to be surprised, humbled, and challenged by this sightless, legless microbe. What will the next decade bring? Thank you for your help with this fight. Your valuable contributions are helping to protect nature and assist impacted industries, communities, and individuals.

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Conference Sponsors

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California Oak Mortality Task Force
California Department of Forestry and Fire Protection
Sacramento, CA

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Emergency Response, Response and Recovery, Plant Pathogens and Weeds
Center for Plant Health Science and Technology
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University of California, Center for Forestry
Berkeley, California

University of California Cooperative Extension, Marin County
Novato, California

Washington State University, Department of Plant Pathology
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Program of Events Overview

MONDAY, JUNE 18, 2012

7:00 - 8:00 pm Registration and Casual Dessert Reception

TUESDAY, JUNE 19, 2012

8:30 - 9:30 am Registration
9:45 - 9:55 Check In – Field Trip: Sudden Oak Death: Biosecurity Concerns and Forest Restoration
10:00 - 5:00 Field Trip (Lunch Provided)

WEDNESDAY, JUNE 20, 2012

7:00 - 8:00 am Registration
8:00 - 8:10 Welcome
8:10 - 9:30 North American Disease Status
9:30 - 10:00 Break
10:00 - 12:00 International Updates
12:00 - 1:00 Lunch (Provided)
1:00 - 2:15 Review Papers
2:15 - 2:30 Break
2:30 - 3:15 Waterways and Monitoring
3:15 - 3:45 Break
3:45 - 5:15 Concurrent Session: Nurseries
3:45 - 5:00 Concurrent Session: Fire Ecology

5:30 - 7:00 pm CA Oak Mortality Task Force Nursery Committee Meeting (open to all)
7:00 - 9:00 pm Poster Session (nacho bar and no host bar)

THURSDAY, JUNE 21, 2012

8:00 - 8:30 am Registration
8:30 - 10:00 Biology
10:00 - 10:30 Break
10:30 - 12:00 Biology, cont.
12:00 - 1:00 Lunch (Provided)
1:00 - 2:15 Diagnostics and Biology
2:15 - 2:45 Break
2:45 - 4:45 Wildland Management
4:45 - 5:15 Meeting Summary and Future Needs
5:15 Adjourn

5:30 - 7:00 pm Ask the Expert, Evening Session (for the general public)

FRIDAY, JUNE 22, 2012

8:30 - 9:00 am Sign in for Participants only attending Tanoak Session
9:00 - 10:30 What are we Trying to Save? Tanoak History, Values, and Ecology
10:30 - 11:00 Break
11:00 - 12:30 What are we Trying to Save? Tanoak History, Values, and Ecology, cont.
12:30 Adjourn

Oral Presentation Abstracts

(In agenda order)

Field Trip. Special Session.
Sudden Oak Death: Biosecurity Concerns and Forest Restoration

Specialist Plant Imports and Forest Biosecurity

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Numerous 'exotic' tree pathogens are arriving in Europe, North America and elsewhere due to flaws in current international plant health (SPS) protocols. These include lack of protection against the many organisms unknown to science and an emphasis on promoting trade rather than environmental biosecurity; increasing globalisation of the trade in rooted plants; and the failure of regulatory authorities to take meaningful and effective action. The present situation in the UK, for example, is effectively a full blown, though largely un-trumpeted, forest biosecurity emergency. Phytophthoras are particularly well suited to spread on imported plant material and a significant number of current disease outbreaks are caused by introduction of Phytophthoras. It has been estimated that there may be 100-500 unknown *Phytophthora* species in underexplored ecosystems – the "invasives in waiting".

A lot of blame for the biosecurity crisis has been put on high volume plant imports, but, specialist plant collecting nurseries, amateur and professional plant collectors and one-off person to person transfers of imported plants can also be part of the problem. It is now well documented that Chestnut blight was introduced into Britain in 2011 on highly specialised imports of *Castanea sativa*. Small, highly specialised plant imports are suspected to have been involved in the recent introduction of *Phytophthora tropicalis, P. kernoviae, P. niederhauseri* and the new EU2 lineage of *P. ramorum* into the UK. However conclusive evidence is often difficult to obtain. Sometimes this is because affected plant material or relevant documentation has been destroyed. It can also be due to a requirement for official confidentiality about the relevant parcels of imports and the infested propagation sites. The latter practice is questionable and is probably one of the major blocks to achieving adequate plant biosecurity; the need for better education of nurserymen, horticultural journalists and the wider public is another. In reality specialist plant collecting and the standard nursery trade should probably not be viewed separately, but as parts of a wider network. It should also be emphasized that most plant collecting professionals, such as those attached to botanic gardens, usually operate to the highest standards.

Examples of the risk of importing unknown Phytophthoras from underexplored forest ecosystems have come from recent surveys in Asia, in particular in Nepal and Taiwan. Similar studies indicate that Nepal is now inadvertently introducing exotic Phytophthoras into its forest areas. A recent soil survey of a visually healthy forest area in a remote area of western Nepal found two probably endemic forest Phytophthoras. This is in contrast to a survey of a degraded forest area near Kathmandu and close to a nursery specialising in introduced *Castanea* spp. which revealed nine *Phytophthora* species, many of them probably introduced. Although such studies are critical to enhancing our knowledge base in the fight against importing pathogens, up to now they have been fragmentary and carried out largely by committed volunteers. It is time for regulators, funding agencies and plant collectors to get involved.

North American Disease Status Updates

Current Status of Sudden Oak Death in California Forests

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It has been exactly 12 years since the causal agent of sudden oak death (SOD) in California was first isolated. Since June 2000, we have learned a tremendous amount about the biology, epidemiology, ecology, and management of *Phytophthora ramorum* in California forests. At the time of the last SOD symposium in 2009, California was in the midst of a 3- year drought. This drought had important influences on the epidemiology and impact of *P. ramorum* during 2007-2009. In addition to the drought, a large wildfire in 2008 burned in Big Sur, one of the most SOD-impacted areas in the state. These environmental influences on SOD were discussed in a number of talks at the last symposium.

Since 2009, California has had two very wet springs (2010, 2011). Dramatic increases in *P. ramorum* symptoms were noted in a number of studies. Monitoring efforts (e.g., stream baiting, SOD Blitzes, aerial surveys) have detected an expanded geographic range of *P. ramorum* in California forests. New detections in Humboldt and Mendocino counties have confirmed the northward expansion of the pathogen in both counties. While *P. ramorum* has long been established in the central California coast, the pathogen has had a very patchy distribution across the landscape. Recent surveys suggest continued increases in pathogen populations at local scales and movement into previously uninfested areas. There have also been a number of recent efforts in forest disease management in California since 2009. This includes the first large scale 'eradication' attempt. This management effort is currently taking place in northern Humboldt County.

Managing Sudden Oak Death in Oregon Forests, 2001-2011

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Sudden Oak Death, caused by *Phytophthora ramorum*, is lethal to tanoak (*Notholithocarpus densiflorus*) and threatens this species throughout its range in Oregon. The disease was first discovered in coastal southwest Oregon forests in July 2001. An interagency team attempted to eradicate the pathogen through a program of early detection surveys followed by destruction of infected and nearby host plants. Eradication treatments eliminated disease from many infested sites but the disease continued to spread slowly in a predominantly northward direction. During the 10-year period, the disease spread from the initial infestations southward 1.2 miles, and northward and eastward 17.3 and 4.7 miles, respectively. The area under quarantine has expanded five times: from 9 mi^2 in 2001 to 202 mi^2 in 2012. Continued spread of sudden oak death is attributed to the slow development of symptoms in infected trees which hinders early detection, and to delays in completing eradication treatments which allow disease spread from known infestations.

From 2002 to 2006 we discovered an average of 25 new infested sites per year. From 2007 to 2009 the number of new infested sites appeared to stabilize at approximately 60 per year. At this level of disease we were barely able to keep up with eradication treatments. In 2010 the number of new infested sites increased to 83, with many of these in areas where treatment delays had occurred in prior years. In 2011 we detected 172 new sites, nearly triple the three-year average, and one of these sites was 6.5 miles north of the quarantine boundary and 12 miles from the nearest known infested site.

The marked increase in disease in 2010 and 2011 clearly indicated that eradication treatment costs on private lands would exceed available or expected funds. In early 2012 the Oregon State quarantine regulations were revised to reflect the financial reality of managing sudden oak death. The initial goal of complete eradication in Curry County forests is now considered unachievable. Our goal now is to slow further disease spread by: 1) early detection and rapid eradication of new infestations that are epidemiologically important; 2) reducing inoculum levels wherever practical through cost-share projects and best management practices, and; 3) improved education and outreach to prevent spread by humans.

An Overview of *Phytophthora ramorum* in Washington State

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Phytophthora ramorum, the exotic water mold that causes sudden oak death and Ramorum shoot blight, was first detected in Washington state on ornamental nursery stock in 2003. Since then, all three lineages (NA1, NA2, and EU1) have been detected in a total of 48 nurseries in western Washington. The number of positive nurseries has decreased since a high of 25 in 2004 to 3 in 2011. In 2011, two of the positive nurseries had been positive in previous years. The swimming zoospores of this pathogen are commonly spread via water. In 2006, stream baiting revealed that *P. ramorum* had spread from a nursery in Pierce County into a nearby stream. Subsequent yearly stream baiting has resulted in the detection of *P. ramorum* in a total of 11 drainage ditches and/or streams in five western Washington counties. Genotype analysis indicates that all three lineages of this pathogen have spread into waterways and that contamination of waterways has typically resulted from spread of inoculum from nearby positive nurseries. Stream baiting has also shown that once a waterway becomes infested, it remains infested even after successful mitigation steps have eliminated the pathogen from infested nurseries.

In the spring of 2009, infested ditch water resulted in the infection of salal (*Gaultheria shallon*) plants along the perimeter of another nursery in Pierce County. This represents the first time the NA2 lineage has been detected on plants outside of a nursery. In 2010, additional plants were positive on the nursery, and ditch water continued to be positive along the perimeter of the nursery. Composite soil samples collected from along the ditch were also positive in 2010; making this the first location in Washington with evidence that inoculum has spread from a nursery in water resulting in the contamination of soil and infection of natural vegetation. In addition, positive soil has also been detected at 3 trace forward sites where infected plants from a nursery in Thurston County had been planted in the landscape.

The Washington State Department of Agriculture is continuing to monitor nurseries for *P. ramorum* as required by the Confirmed Nursery Protocol, but as of 2012 will no longer monitor waterways and streams outside of nurseries. Stream baiting efforts are still be conducted by the Washington Department of Natural Resources (DNR); with initial 2012 sampling being done on 10 watercourses in 5 counties. In addition to leaf baiting, DNR will be working with the USDA Forest Service on “Bottle of Bait” protocols to assay each of the streams for *P. ramorum*.

Phytophthora ramorum Regulatory Program: Present, Past, and Future Direction

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Since the publication of the *P. ramorum* interim rule in 2007, APHIS-PPQ has explored several avenues to obtain consensus on the objectives of the regulatory program and meet its goals. The principal objective of the program is to protect native biodiversity and wild land environment from Sudden Oak Death (SOD) disease. For the past several years, input from stakeholders, better scientific knowledge, and novel detection methods have helped APHIS, in collaboration with their partners, to develop and implement several scientifically-based protocols and remediation strategies that have reduced the risk of the pathogen being moved through shipments of infested nursery stock. Despite the progress made, *P. ramorum* continues to be moved around and has subsequently established itself in a number of retail and wholesale nurseries in several non-regulated states and through these nurseries into streams and waterways. Based on 10 years (2001-2010) of regulatory data, enhanced understanding of the science, and current realities, the general consensus is that a more targeted and focused regulatory framework is needed to reduce the potential for pathogen movement in nursery stock in order to protect valuable forest resources and the nursery industry. In addition to survey and detection within nurseries, it is imperative that Best Management Practices (BMPs) be implemented to avoid the introduction and/or reoccurrence of *P. ramorum* within the nursery production system. APHIS is currently investing in several pilot programs to test and improve implementation of BMPs in nurseries. The paper describes several aspects of a revised regulatory framework being currently discussed and how it can be related to the implementation of Best Management Practices (BMP) and Critical Control Point (CCP) assessment for regulated nurseries.

International Updates

The New *Phytophthora ramorum* Dynamic in Europe: Spread to Larch

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Phytophthora ramorum has been reported from most EU Member States, mainly affecting ornamental plants in nurseries. The most epidemiologically important hosts are those that support abundant sporulation and until recently in Europe this applied primarily to rhododendron and *Vaccinium*. However, following the first findings of *P. ramorum* on Japanese larch (*Larix kaempferi*) in south west Britain in 2009, it soon became clear that infected foliage of Japanese larch could produce abundant numbers of sporangia, as demonstrated in the laboratory [1] and on naturally infected needles, leading to bark infections on larch and other nearby susceptible tree species.

To compare the spore producing potential of larch foliage with other known sporulating hosts (eg *Umbellularia califonica* and *Rhododendron ponticum*), laboratory tests were carried out using shoots of Japanese larch, hybrid larch (*L.* x *eurolepis*) and European larch (*L. decidua*) challenged with zoospores suspensions of *P. ramorum* (EU1 lineage). These tests were carried out at different times of year and have shown that sporulation potential varies with larch species, pathogen genotype and also with the age of the foliage. Japanese larch generally supports the highest levels of sporulation, even exceeding that on *U. californica.* Sporulation on larch needles can also occur in the absence of any symptoms particularly early in the season and in the field, symptoms on infected needles only become visible towards the end of the season just before they are shed. Field performance of the difference larch species also suggests that European larch is more resistant to stem infections caused by *P. ramorum* as resinous bark lesions are only seen occasionally on this tree species. However, somewhat surprisingly laboratory tests indicated that bark of European larch is much more susceptible than Japanese larch bark, suggesting that European larch may escape infection in the field because its needles usually sustain lower levels of sporulation.

[1] Webber JF, Mullett M and Brasier CM (2010). Dieback and mortality of plantation Japanese larch (*Larix kaempferi*) associated by infection by *Phytophthora ramorum*. *New Disease Reports* 22, 19.

Phytophthora ramorum: Operational Experience from the UK

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The summer 2009 findings of *Phytophthora ramorum* on larch in South West England and the subsequent and on-going evolution of the situation across the UK prompted a rapid operational response from the Forestry Commission. This presentation outlines the nature of the woodland, its ownership and management and the unprecedented forest health challenge encountered.

From the initial findings on 6 sites in Somerset, Devon and Cornwall, the incidence of the disease at the end of March 2012 comprised 314 sites, covering 3,434 Ha across the western fringes of UK (England, Wales, Scotland, Northern Ireland), the Isle of Man and the Republic of Ireland. The estimated standing volume of larch contained in these sites is 678,000 m3. This distribution reflects both the favorable climatic conditions for *P. ramorum* and the distribution of larch in UK forests.

The early operational imperative of the Forestry Commission and its partners was to confirm the incidence and extent of the disease. This involved the adoption of aerial surveillance techniques new to the plant health in the UK. The information gathered through this surveillance provided the starting point for ground investigations of individual sites. This approach, combined with scientific investigations carried out by Forest Research, has helped to build an understanding of infection on the site and determine the nature of action required.

Management of the disease is covered by UK & EU plant health legislation and in a forest setting requires the killing (usually felling) of all symptomatic host material up to a minimum of 100m from individual findings and in many cases more. In smaller woodlands this has generally meant all larch being removed. After guidance from Forest Research it was determined that the timber from infected sites could be used by the processing industry under license which sets out conditions for movement and disposal or treatment of residues. Sale of timber has helped to recoup some of the losses of the owners as there is very limited financial assistance available to owners within the current wider plant health policy framework. In general, private woodland owners have been cooperative and supportive even though the costs have mainly been at their personal (at times considerable) expense.

As the situation progressed through 2010 and on into 2011, surveillance coverage increased and its frequency intensified. Refinements to process and protocol were made and more general staff resource co-opted in the parts of the UK (SW England and S Wales) where the situation started to “normalize” and disease management experience was gained. Changes in the disease dynamics also became apparent. During 2010 we were very obviously dealing with a cumulative picture of historic infection. Sites were observed that presented large scale death and dieback (80-90% in some cases).

During 2011 some sites with large scale infection were observed but in general symptoms were more subtle (sometimes down to individual trees) and on a smaller scale demonstrating earlier detection. However, significantly for the UK context new findings were made in the NW of England, and the SW and West Coast of Scotland.

The occurrence of *Phytophthora ramorum* in larch is significant but must be viewed in the wider context of UK larch inventory. Current knowledge of infection equates to a very small percentage of all larch in the UK which means that protection may be afforded to the majority as well as to other sensitive habitats in the wider environment.

This disease has demonstrated the very real consequences of the increasing risks posed by plant pests and pathogens and the need to see them as an integral part of sustainable forest management practice.

EU2, A Fourth Evolutionary Lineage of *Phytophthora ramorum*

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Studies in North America and Europe in the past decade have demonstrated the occurrence of three lineages of *Phytophthora ramorum* informally designated the NA1, NA2 and EU1 lineages. Each lineage appears to represent a reproductively isolated population, but whether they have come from different geographic regions is unknown. Only the EU1 lineage had been found in Europe until recently. EU1 is believed to have been introduced into Europe around 1990. Since then it has spread widely and rapidly across the continent, including the UK and Ireland, via the plant trade.

In 2011 *P. ramorum* isolates from Northern Ireland and a closely adjacent area of western Scotland, mostly from *Larix* but also from *Quercus, Rhododendron and Vaccinium*, were found to have SSR (microsatellite) and *Cox 1* profiles not matching those of any known lineage. Following a phylogenetic analysis with eleven loci they were assigned to a new lineage, informally designated EU2. This analysis indicates the EU2 lineage may be ancestral to the other lineages. No SSR-based intra-EU2 lineage genotypic diversity was detected. All EU2 isolates examined to date have all been of A1 mating type. As this is the same mating type as that of EU1 in Europe, sexual recombination with EU1 lineage genotypes already resident in the UK is unlikely. The earliest isolation dates to 2007. Present evidence points to a recent introduction of EU2 in the context of ongoing phytosanitary emergency measures.

The arrival of EU2 highlights an urgent need to identify the geographic origins of *P. ramorum* in order to understand the organism's natural ecology, the processes that have produced the lineages, and whether further lineages exist. Presently, studying the organism in the context of introduction and invasion, we may only be looking at half the picture.

The Epidemiology of *Phytophthora ramorum* and *Phytophthora kernoviae* at Two Historic Gardens in Scotland

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In Scotland, *Phytophthora ramorum* was first discovered in 2002 and *P. kernoviae* in 2007. There have now been 41 *P. ramorum* outbreaks and 19 *P. kernoviae* outbreaks, with garden outbreaks being largely confined to the west of Scotland where conditions appear to be particularly advantageous to the establishment and spread of these pathogens.

This study has looked at the conditions that facilitate the spread within two historic gardens in the west of Scotland. Garden 1 in Arran has an extensive *P. kernoviae* infection and Garden 2 in Argyll & Bute has a small number of both *P. kernoviae* and *P. ramorum* infections. The objective has been to determine the current situation and assess the relative risks from these pathogens through (i) identifying sources and pathways/mechanisms of potential pathogen spread and quantifying the impact of infection and spread, (ii) quantifying the effects of host, environment, season and climate on life stages present, and (iii) investigating the current measures required for successful eradication and containment of *P. ramorum* and *P. kernoviae*.

The methods utilised for establishing inoculum levels around the outbreak sites include river baiting, spore traps, bait plants and soil sampling. These methods mirror those used in other parts of the UK in order to assess specific risks to Scottish gardens and landscapes.

At the extensive infection at garden 1 *P. kernoviae* inoculum was detected in soil at several points around the garden despite the removal of the host; these inoculum levels remained constant throughout the study period with very little depletion over time. No inoculum was found using the river baiting in this garden however inoculum was detected in the spore traps at one site under a host which is supposed to be infected but remains asymptomatic.

The river baiting was far more successful at detecting *P. ramorum* at garden 2. The small streams were found to contain inoculum on a number of occasions through the study, particularly after heavy prolonged rain. After the removal of host plants, *P. ramorum* in the soil at garden 2 was also found not to deplete over the 2 year study period. The spore traps at this garden proved to be more successful at detecting *P. ramorum* although no seasonal pattern has yet been found. The *Rhododendron* bait plants have been successful at detecting both pathogens at some areas around each garden although they appear to become more readily infected by *P. ramorum*.

These data will be refined and used by the Scottish Government in order to inform decisions on future infection management in Scotland.

Genetic Diversity of *Phytophthora ramorum* in Nursery Trade and Semi-Natural Environment in Scotland

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Phytophthora ramorum was detected in Scotland for the first time in 2002 in official plant health surveys of ornamental nursery stock. From 2007 it was also found in samples from the semi-natural environment such as historic gardens, parks and country estates.

From 2002 until 2011, between 1 and 22 *P. ramorum* isolates were collected from each outbreak site depending on the severity and duration of the outbreak. Altogether, 228 isolates were genotyped from almost every outbreak site in Scotland.

The genetic diversity amongst these isolates was studied using seven microsatellite markers as described by Vercauteren et al., 2010. Thirty multilocus genotypes were identified within the Scottish population, 51 % of the isolates belonging to the main European genotype EU1MG1. 13 of the detected genotypes were unique. Ten of those genotypes were site specific, often represented by single isolates. Three *P. ramorum* isolates, all from the same location, belonged to a new European lineage.

The number of genotypes found in the semi-natural environment was higher than the number found in the horticultural trade (25 vs. 11), probably due to the fact that outbreaks in nurseries are usually detected earlier and are quicker eradicated. Generally, the genetic diversity has increased over the past 10 years in Scotland.

References

Vercauteren, A.; de Dobbelaerre, I.; Grünwald, N.J.; Bonants, P.; van Bockstaele, E.; Maes, M.; Heungens, K. 2010. Clonal expansion of the Belgian *Phytophthora ramorum* populations based on new microsatellite markers. Molecular Ecology 19: 92-107.

Genotypic Diversity of European *Phytophthora ramorum* Isolates Based on SSR Analysis

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Genotyping of NA1 isolates of *P. ramorum* has provided valuable information regarding the introduction, evolution and the pathways of spread of this pathogen in North America. So far, genotyping of European isolates of *P. ramorum* has only been reported for the Belgian and Spanish populations. Until the epidemic of *P. ramorum* in larch, the European *P. ramorum* population was mostly confined to nurseries. This population may have different rates of evolution and selection and different pathways of spread than the NA1 population, which is mostly derived from the natural environment. The objective of this study was to genotype and analyze a wider European collection of *P. ramorum*, which was in part made possible via the collection of DNA samples as part of the EU COST action FP0801.

In total, over 1300 samples from 17 European countries were analyzed using seven EU1-polymorphic microsatellite loci. The majority of the samples were collected after 2001 from *Rhododendron* in nurseries. At least 66 EU1 genotypes were identified. Approximately 64% of the isolates belonged to multilocus genotype EU1MG1, which was present in all countries. Isolates with single repeat shifts in the most variable markers were the second-most abundant and widespread, but at frequencies of less than 6% and in a maximum of 11 countries. As in the NA1 population, the structure of the genotype network is indicative of a clonal expanding population that accumulated microsatellite mutations after a single introduction of the EU1MG1 genotype. The population structure and genetic diversity was similar in most of the countries represented by a sizeable number of samples. The population structure of the UK isolates is unique in that it is characterized by a large subpopulation with a unique mutation in one of the markers, a mutation that presumably arose relatively soon after the introduction of the pathogen in the UK. Based on the finding of unique genotypes in time and space, local evolution followed by national or international spread was also observed for other countries. In general, however, the level of diversity was too small and the amount of (inter)national exchange was too extensive to draw detailed conclusions on the primary origin of specific isolates. There were no clear indications of sexual recombination within the EU1 lineage. Three isolates with highly deviating marker profiles were identified. Further analysis of these isolates revealed that they belong to a new (EU2) lineage, as detailed in a separate report.

Review Papers

Beyond *Phytophthora ramorum* - The Other Phytophthoras in Western Forests

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Little is known about indigenous *Phytophthora* species in natural ecosystems although increasing evidence suggests that a diverse, trophically complex *Phytophthora* community is important in many forests. In Oregon, 36+ *Phytophthora* species have been identified from forests. Globally, the number of described species has steadily increased, with a dramatic spike in recent years as new species have been split from old and new species have been discovered through exploration of new forest habitats triggered by the *P. ramorum* epidemics. In Oregon, almost 2/3 of the species were recently described. Forest soil, streams, and the upper canopies of trees are now being explored for *Phytophthora* diversity and a new appreciation for the ecological amplitude of the genus is emerging. Around the world ten to fifteen species are regularly identified in surveys of temperate forest soils. Taxa in ITS clade 6 are especially numerous in forest streams and may be saprophytic in this habitat. In the upper canopies of trees in western tanoak forests three or four *Phytophthora* species (including *P. ramorum*) with similar pathogenic behavior are encountered. Species from all three habitats, soil, streams, and upper canopies, occasionally cause lethal bole cankers on trees. A very few become invasive under circumstances we can't yet predict.

Preventing the next invasion by a *Phytophthora* species is as much a political and economic challenge as a biological one. We have learned much through the *ramorum* experience about the importance (danger) of the nursery trade as pathway for invasion, and in the United States the nursery industry has responded to reduce the threat. Recent emphasis on Best Management Practices and Oregon's "Grower Assisted Inspection Program" assure that nursery stock in interstate commerce is healthier, and less likely to harbor pathogens. Success with the larger horticultural nurseries shifts the focus, however, to gaps in the protective education programs. Smaller native plant nurseries supplying stock for wildland rehabilitation and the agency botanists that buy from them would both benefit from focused plant health training.

Landscape Epidemiology of Emerging Infectious Diseases in Natural and Human-Altered Ecosystems

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A central challenge to studying emerging infectious diseases (EIDs) is a landscape dilemma: our best empirical understanding of disease dynamics occurs at local scales while pathogen invasions and management occur over broad spatial extents. The burgeoning field of landscape epidemiology integrates concepts and approaches from disease ecology with the macro-scale lens of landscape ecology, enabling examination of disease across spatio-temporal scales in complex environmental settings. We review the state of the field and describe analytical frontiers that show promise for advancement, focusing on natural and human-altered ecosystems. Concepts fundamental to practicing landscape epidemiology are discussed, including spatial scale, static versus dynamic modeling, spatially implicit versus explicit approaches, selecting ecologically meaningful variables, and inference versus prediction. We highlight studies that have advanced the field by incorporating multi-scale analyses, landscape connectivity and dynamic modeling. Future research directions include understanding disease as a component of interacting ecological disturbances, scaling up the ecological impacts of disease, and examining disease dynamics as a coupled human-natural system.

Process and Pattern in the Emergence of *Phytophthora ramorum*

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The invasive sudden oak death pathogen *Phytophthora ramorum* emerged repeatedly since its first detection in the 1990s in the U.S.A. and Europe. This paper will explore recent research by several groups documenting the patterns observed and mechanisms inferred to explain these patterns. Briefly, three distinct clonal lineages are recognized named NA1, NA2, and EU1 named consecutively after the continent of origin on which they where first found, namely North America (NA) and Europe (EU). While all three clonal lineages are found in Canada and the U.S.A., Europe to date only has the EU1 clonal lineage. Detailed phylogeographic analysis has documented that the introduction of the NA1, NA2, and EU1 clonal lineages originated from separate populations. The NA1 lineage was introduced into California from an unknown source population, while the NA2 and EU1 lineages were introduced into the Pacific Northwest (either British Columbia or Washington). While the source population for the NA2 introduction remains to be established, coalescent analysis supported introduction of the EU1 lineage into North America from Europe. Further emergence of *P. ramorum* lineages is likely, given the observed repeated emergence of *P. ramorum* over the last two decades and appears to be driven by shipment of infested nursery material.

Waterways and Monitoring

New Insights into the Ecology of *Phytophthora ramorum* in Streams

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Many *Phytophthora* species, including *Phytophthora ramorum*, have been reported from surface waters such as canals, streams, rivers, ponds and reservoirs, frequently in association with infested agricultural or natural landscapes, but often also in the absence of apparent sources of inoculum. The persistence of such plant pathogens in water bodies has significant implications for their spread and management in both agricultural and more natural contexts.

The regularity and abundance with which *Phytophthora* species are recovered from streams and rivers, often in the absence of apparent terrestrial infestations, strongly suggests that these organisms can complete their life cycle in aquatic environments, yet the biological underpinnings of this phenomenon remain effectively unknown. What Phytophthora propagules occur in surface waters? How frequent and widespread are they in such environments? From what substrates do they originate? What is their potential to infect plant material in this environment? To address these questions, we've undertaken experiments focusing on *P. ramorum* in both natural streams and controlled environments.

To determine the potential of leaf litter as a substrate for *P. ramorum* in streams, we exposed freshly picked rhododendron leaves as well as those killed by drying or freezing to natural inoculum in infested streams and additionally to laboratory- produced inoculum in controlled environment experiments. Results indicated that *P. ramorum* may be limited biologically and ecologically from colonizing degraded leaf litter in aquatic environments. To evaluate the potential of aquatic and riparian plants as a source of *P. ramorum* inoculum in aquatic environments, surveys for cryptic infections will be conducted this season.

To determine if *P. ramorum* propagules can arrive and adhere passively to substrates in streams, we incubated glass microscope slides in two infested streams. Colonies of *P. ramorum* and *P. gonapodyides*-like spp. were recovered from glass slides exposed in streams, indicating that passive adherence of propagules to substrates in streams occurs.

Though occasional direct observation of zoospore cysts from stream water filtrate and recovery of *Phytophthora* spp. by baiting from water samples in collection bottles indicate the occurrence of zoospores in stream water, it is not clear if these propagules are free swimming at the time of collection, or if other propagules also occur. To better characterize the nature of Phytophthora propagules in flowing stream water, we will attempt selective isolation of propagule types based on differences in size of Phytophthora spores and lack of a cell wall in zoospores.

Eliminating *Phytophthora* Species from Stream Water Throughout the Year with Algaecides

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In previous research, we demonstrated that algaecides, particularly those with copper-based active ingredients, are very effective at eliminating *Phytophthora* spp. from artificially- and naturally-infested water samples. In January 2010, we initiated a year-long study to investigate efficacy of four commercial algaecides with different active ingredients at eliminating propagules of *Phytophthora* spp. naturally occurring in two streams in western South Carolina at monthly intervals. The four algaecides were applied at the highest recommended label rate: K-Tea (1 ppm of copper hydroxide), Captain (0.8 ppm of copper carbonate), Algimycin (1 ppm of copper citrate), and GreenClean Liquid (25 ppm of hydrogen dioxide). Each algaecide is registered for use in various water systems—including irrigation ponds, natural waterways, and potable water. Each month at each stream, 10 L of stream water was placed in each of fifteen 20-L buckets; three buckets were treated with each of the four algaecides, and three buckets were not treated (controls). All buckets remained in the stream to maintain ambient temperature of the stream water. Before the water was treated (0 h) and at 2 and 4 h after treatment, three aliquots of 200 ml of water from each bucket were passed through membrane filters and membranes were inverted onto PARPH-V8 selective medium to detect propagules of *Phytophthora* spp. Mean numbers of colony-forming units (cfu) in 200-ml aliquots (i.e., propagule densities) were determined.

Propagules of *Phytophthora* spp. were detected in both streams in all months and in all non-treated water samples. In Stream 1, mean propagule densities in non-treated water at 0 h ranged from 1.1 to 30 cfu (mean 18.6 cfu); 30 cfu was the maximum number that could be counted with accuracy, so actual densities may have been greater. In Stream 2, mean propagule densities ranged from 1.2 to 24.8 cfu (mean 8.3 cfu). The three copper-based algaecides completely eliminated propagules of *Phytophthora* spp. from water at 2 h after treatment, so no propagules were detected at either 2 or 4 h. GreenClean Liquid was not as consistent or as effective. In Stream 1, propagules were detected in five monthly samples at 2 h (0.8-2.6 cfu) or 4 h (0.1-0.6 cfu) after treatment. In Stream 2, propagules were detected in Mar (0.7 cfu) and May (2.6 cfu) at 2 h after treatment and in May (1.6 cfu) and Jun (0.1 cfu) at 4 h after treatment. Propagules were not detected after treatment with GreenClean Liquid in all other samples. Algaecides continue to show promise for eliminating propagules of *Phytophthora* spp. from naturally-infested water.

In a supplemental study, we compared the efficacy of each algaecide at two reduced rates (based on the range of rates listed on labels) to the maximum label rate. Reduced rates were 0.4× and 0.04× for GreenClean Liquid and 0.5× and 0.25× for the copper products. Each reduced rate was tested twice during the year—once in each stream. Reduced rates of the copper-based algaecides were as effective as the maximum rate at eliminating propagules of *Phytophthora* spp. from naturally-infested stream water; no propagules were detected after treatment at any rate. However, GreenClean Liquid was not as effective; propagules were not completely eliminated by reduced rates, and propagule density was reduced significantly only by the 0.4× rate. Therefore, lower rates of the copper-based algaecides appear to be effective at eliminating propagules of *Phytophthora* spp. from water, but GreenClean Liquid was not effective when applied at the reduced rates used in this study.

Comparison of In Situ and In Vitro Baiting Assays for *Phytophthora ramorum* Survey of Waterways in the Southeastern USA

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In situ baiting with whole, intact leaves of *Rhododendron* spp. has been employed since 2006 by the National *Phytophthora ramorum* Early Detection Survey of Forests (National Survey). Using this method, *P. ramorum* was detected for the first time in National Survey waterways draining 12 infested ornamental crop nurseries in AL, FL, GA, MS, NC, and WA as well as many forest areas in CA and OR. In situ baiting periods lasting 1 to 3 weeks allow sampling large volumes of water over time but also can result in loss of bait leaves from storm surges and vandalism. In situ baiting also requires two site visits for a single bait set (once to deploy and once to retrieve) and sustained stream flow over the entire baiting period. An in vitro assay without these limitations was evaluated in experimental applications, and it has been effective at recovering *P. ramorum*. Therefore, we used both the in situ and in vitro baiting assays simultaneously for the 2011 National Survey for 12 *P. ramorum*-infested waterways in five states in the southeastern USA to compare relative performance under field conditions.

In situ baiting was conducted according to the established National Survey protocol with three baiting periods during each of the spring and autumn seasons (six in all). Eight water samples were collected for the in vitro assay—at the same times as leaves were deployed or retrieved for in situ baiting. For the in vitro assay, two 800-ml water samples were collected in 100-ml aliquots, and each sample was placed in a 1-liter Nalgene screw-top bottle. Each sample was baited immediately with 20 leaf pieces and one whole, non-wounded leaf of *Rhododendron maximum*. Bottles were capped, placed on their sides, and held for 3 days at 18-22°C in the dark. Baits then were removed, rinsed in distilled water, and blotted dry. Leaf pieces were processed immediately for detection while whole leaves were placed in moist chambers for up to 14 days to allow lesion development. Two detection methods were used for both assays—isolation on selective PARPH-V8 medium and nested or real-time PCR. Relative assay performance was determined by comparing detection results for sample sets collected at the same time.

P. ramorum was recovered by one or both assays at least once during the year in 11 of 12 waterways surveyed with substantially more detections occurring during spring (24) than autumn (14). There were 67 cases in which both assays were conducted and relative performance could be compared. *P. ramorum* was recovered by one or both assays in 32 of these cases (48%). Out of the 32 positive cases, the pathogen was recovered by both assays in 14 cases (44%) while each assay alone recovered the pathogen in nine cases (28%). However, pathogen recovery by each baiting assay differed considerably by season. During spring, there were 20 comparable cases in which *P. ramorum* was recovered by one or both assays. In vitro baiting recovered the pathogen in seven cases without corroboration by the in situ assay while in situ baiting recovered the pathogen in two cases without in vitro corroboration. The pathogen was recovered by both assays in 11 additional cases. Relative performance of the two assays was exactly the inverse during autumn: two pathogen recoveries by in vitro baiting only, seven recoveries by in situ baiting only, and three recoveries by both methods (12 in all). More importantly, *P. ramorum* would have escaped detection altogether in five streams had the in vitro assay not been used. Implications of these findings for modifications to the National Survey protocol will be discussed.

Nurseries

Pathways of Spread of *Phytophthora ramorum* in a Simulated Nursery Setting: An Update

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European phytosanitary measures as applied to nurseries require that potential host plants within a radius of 2 m of a *P. ramorum*-infected plant must be destroyed and that remaining host plants within a radius of 10 m cannot be traded until they are inspected and found to be pest-free at further specific inspections. Despite the wide application and acceptance of these distances, they are not based on data regarding the in-field spread of this pathogen. Our previous study reported at the SOD4 meeting demonstrated that direct aerial spread between potted plants in nurseries is rare and limited in distance. As an extension of this reported work, we have performed a study with two objectives: 1) to test the relative importance of direct versus indirect spread of *P. ramorum* in nurseries and 2) to test the movement of *P. ramorum* from symptomatic plants to the root ball of neighboring plants via the water film. Such movement could eventually lead to long-distance spread of the pathogen via the nursery trade in latently infected plants.

Experiments were conducted at a mock nursery plot under specific biosafety conditions. The plot was lined with an impermeable film, a common practice in potted plant production in Europe. *Rhododendron* was chosen as the test plant due to its susceptibility and its prevalence as a nursery host for *P. ramorum* in Europe. Pathogen dispersal was monitored from individual infected potted plants placed in the middle of a circle of healthy detector plants. The rate at which the disease spread onto the detector plants was monitored in replicated experiments. Indirect splash dispersal (via the water film on the plastic ground cover and back to the leaves) as well as direct aboveground plant-to-plant dispersal (via air or via leaf-to-leaf splashing) were investigated by selective physical blocking of such pathways. Indirect dispersal via the drain water film was at least as important as direct dispersal. Contamination of the drain water film was confirmed using leaf baits and direct PCR-mediated detection at significant distances from the source plants. This showed that indirect spread via the water film could take place over larger distances than direct dispersal. Movement of the pathogen from the water film into the root ball of detector plants was demonstrated using leaf baiting of the root balls combined with physical blocking of this pathway.

These data suggest that in nurseries, direct aerial dispersal of *P. ramorum* can be relatively less important than spread via the (drain) water film: the pathogen can spread over several meters when an impermeable surface cover is present. The presence of such a cover could therefore be considered as a factor when quarantine actions are taken. Drain water films can contribute not only to indirect aerial plant-to-plant spread but also to root ball infection. Such infections could add to the long distance spread of the pathogen via latently infected plants.

Detection and Survival of *Phytophthora ramorum* in *Rhododendron* Root Balls and Survival in Rootless Substrates

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Rhododendron is an important host for the spread of *Phytophthora ramorum*. Disease symptoms can develop on the upper plant parts of *Rhododendron* growing in contaminated soil or growing substrate, but root symptoms have never been observed in naturally infected *Rhododendron*. Nevertheless, the pathogen has been isolated from asymptomatic *Rhododendron* roots. Latently-infected *Rhododendron* plants can therefore be an important source of inoculum for the spread of *P. ramorum*.

The detection and survival of *P. ramorum* were studied in three experiments. In experiment 1, discs of infected leaf material were buried below the surface of either 1) various undisturbed soil columns or 2) a growing substrate for *Rhododendron*. The soils and the growing substrate were incubated for 33 months outdoors under quarantine conditions. Recoverability was assessed during several seasons. In experiment 2, artificially inoculated *Rhododendron* root balls were sampled over time using a new baiting technique. Roots were also examined for disease symptoms and for *P. ramorum* infection. In experiment 3, *Rhododendron* plants from a commercial nursery were cultivated for 25 months under quarantine conditions in a greenhouse. Outdoor temperatures were simulated. Symptom development was monitored and samples from the root balls were examined using various baiting tests.

Long-term survival of *P. ramorum* could be demonstrated in all three experiments. The roots did not show any disease symptoms in spite of the infection detected.

Reference

Vercauteren, A.; Riedel, M.; Maes, M.; Werres, S.; Heungens, K. *Submitted.* Survival of *Phytophthora ramorum* in *Rhododendron* root balls and in rootless substrates (submitted to Plant Disease)

Acknowledgments

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Effect of Oomatistatic Compounds and Biological Control Agents on Production of Inoculum and Root Colonization of Plants Infected with *Phytophthora ramorum*

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In this study, *Viburnum* cuttings were treated with oomatistats (Subdue Maxx®, Banol®, and Aliette®) at standard rates for use as soil drenches or with biological control organisms (*Streptomyces lydicus* formulated as Actinovate ® SP and used as a soil drench, and *Trichoderma asperellum* formulated in wheat bran and used as a top dressing) 4 days after roots were infected with *P. ramorum*. The amount of inoculum in runoff samples taken weekly for 5 weeks was studied using a quantitative assay analyzed as a mixed model regression, and the percent colonization of roots at the end of each experiment analyzed by a General Linear Model. Experiments were run three times for each compound or biological control agent, except for *Trichoderma*, which was run twice. Root-infected *Viburnum* cuttings treated with Banol® did not show any reduction in inoculum production compared to nontreated cuttings and there was no significant difference in root colonization at the end of the experiment. Aliette®-treated *Viburnum* cuttings gave off significantly less inoculum than nontreated plants at all sampling dates (days 7, 14, 21, 28 and 35; $p \leq 0.02\text{-}0.0001$), and root colonization was significantly reduced ($p \leq 0.01$). Subdue Maxx significantly reduced inoculum at all sampling dates ($p \leq 0.02\text{-}0.0001$), and reduced root colonization ($p \leq 0.0001$). When Actinovate® SP was applied as a soil drench to root-infected cuttings significantly less inoculum was released than from nontreated ones at all sampling dates ($p \leq 0.002\text{-}0.0001$), and root colonization was reduced ($p \leq 0.05$). When *Trichoderma* in wheat bran was applied as a top-dressing to pots containing root-infected cuttings runoff contained significantly less inoculum than nontreated plants at all sampling dates ($p \leq 0.0001$), and root colonization was reduced ($p \leq 0.0001$). These results suggest that biological control agents are effective as Subdue Maxx® and Aliette® at reducing inoculum production and root colonization in experiments lasting 35 days, and are more effective than Banol.

A Technique for Determining Inoculum Threshold for the Spread of *Phytophthora ramorum* in Irrigation Water

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Phytophthora ramorum was first found in a Washington nursery in 2003 and has since been detected in 48 nurseries in the state. All three genetic lineages of *P. ramorum* have been found in Washington nurseries, and in several streams near infested nurseries. Once infested, streams remain positive for *P. ramorum*, even after mitigation steps have been taken at the nursery and the pathogen can no longer be detected at the nursery site. The movement of the NA2 lineage of *P. ramorum* to salal and soil outside of a nursery via contaminated water in 2009 and 2010 illustrates the importance of this pathway as a means of spreading of *Phytophthora* pathogens from nurseries to plants and soil in the landscape. Nationally, there is concern about the potential risk of spreading *P. ramorum* via the irrigation of plants with infested water. Although research in California and Europe has shown that plants in nurseries can be infected when overhead irrigated with water infested with *P. ramorum*, it is unknown what levels of inoculum are present in streams in Washington and how much is needed for infection of plants or infestation of soil to occur via irrigation. Being able to quantify inoculum levels in waterways and understand the inoculum threshold necessary for infection will assist the nursery industry and regulatory agencies in making decisions about the level of risk in using *P. ramorum* infested water for irrigation in Washington State.

To test whether plants overhead irrigated with contaminated stream water would become infected, a "shower" apparatus was constructed. A bilge pump was attached to a floating platform and pumped water through a sprinkler onto plants. Humidity was maintained by enclosing the plants in the fiberglass "shower stall". A timer was included to allow for irrigation at predetermined intervals, and the apparatus was powered using a car battery.

The "shower" was first tested in the biocontainment unit at WSU-Puyallup. Potted rhododendron seedlings were placed in the chamber and overhead irrigated with a zoospore suspension of *P. ramorum*. After 4 weeks, the plants were sampled for *P. ramorum* by culturing and qPCR. A small amount of *P. ramorum* DNA was present on three samples, one sample was positive for *P. ramorum* in culture, and only one plant had symptoms of *P. ramorum* infection. More than 80% of soil and root baits were positive for *P. ramorum*. In this small study, soils and roots had very high levels of colonization by *P. ramorum* after overhead irrigation with a large amount of inoculum. Plants had very low levels of infection. The apparatus was then deployed at a site where leaf baiting has been positive for *P. ramorum* in 2009 and 2010. Rhododendron plants were placed into the shower and bait bags were deployed upstream from the pump for three, one-week intervals in June 2011. After exposure, plants were taken to WSU-Puyallup and placed in the biocontainment unit for 4-6 weeks. All samples were negative for *P. ramorum* using culturing methods and qPCR. *P. ramorum* was found in the stream earlier in the year by WA DNR, but possibly June was too late for detection. Several other Oomycetes were isolated from leaf baits and identified using DNA sequence analysis. Based on these preliminary results, it appears that inoculum levels of *P. ramorum* were very low or nonexistent in the stream that was sampled, or that it was too late in the season to detect it. Water temperature in the ditch was 15 C at deployment of the shower, which is in the range for *P. ramorum* growth. However, other Oomycete species present may be more competitive than *P. ramorum* under these conditions. Plans are underway to use the "shower" apparatus in addition to baiting, filtration and qPCR methods for quantifying *P. ramorum* inoculum in streams and to determine whether these inoculum levels are sufficient to cause disease on plants.

Examining Fungicide Resistance and Pathogenicity Among Clonal Lineages in *Phytophthora ramorum*

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Phytophthora ramorum, the fungus-like pathogen which causes sudden oak death, is a threat to the PNW nursery industry. Because this is a quarantine organism, the destruction of plants and mitigation treatments resulting from a positive *P. ramorum* detection has caused millions of dollars in losses to the commercial nursery industry in California, Oregon and Washington. Some nurseries have gone out of business as a result of *P. ramorum* being found on their property. Mefenoxam (Metalaxyl-M, Subdue MAXX®) is one of the most commonly used chemicals for controlling *Phytophthora* species on ornamentals and other crops. It has been shown to be effective when used preventatively against *P. ramorum* and is inhibitory to both mycelial growth and sporulation. Unfortunately, resistance to this chemical can develop in *Phytophthora* spp.

The objectives of this study were to examine 85 isolates of *P. ramorum* in the WSU culture collection for resistance to mefenoxam and to evaluate the fitness of sensitive and resistant isolates. Lesion size on wounded rhododendron leaves was used to examine relative pathogenicity of *P. ramorum* isolates.

Isolates of *P. ramorum* were collected from 12 nurseries in Washington, one in Oregon, and one Christmas tree farm in California. Isolates from Washington nurseries included those collected from symptomatic plant material, soil and stream baits, and from trace-forward sites where plant material purchased at the nursery was planted into a landscape. Isolates from the NA1, NA2, and EU1 lineages were tested, in addition to four isolates that were a co-mingling of NA2 and EU1. The co-mingled isolates were acquired from several rhododendron plants at an infected nursery and, although they contain *P. ramorum* of both lineages, do not appear to be hybridizing or sexually reproducing.

All isolates of *P. ramorum* were grown on media amended with varying concentrations of mefenoxam. Isolates were considered to be sensitive to the fungicide if there was scant or no growth at 1 ppm a.i. and resistant if there was significant growth at 1 ppm. Only a few of the isolates tested showed resistance to mefenoxam, and these belonged to the EU1 lineage and originated from one nursery and its trace-forwards.

When the relationship between pathogenicity and fungicide sensitivity was examined, three groups were observed: isolates showing some resistance to the fungicide, isolates with low pathogenicity, and the remaining isolates not having characteristics of the other two groups. The fungicide resistant group was composed entirely of isolates from the EU1 lineage and originated from Nursery #41. The isolates of low pathogenicity were mostly of the NA1 lineage, with two from the co-mingled EU1/NA2 samples and one EU1 isolate from nursery #44. Most of the weak NA1 isolates originated from nursery #35. A positive relationship between pathogenicity and fungicide sensitivity was seen when the first two groups were removed from the analysis ($R^2 = 0.3877$), suggesting that there may be some trade-offs between fungicide resistance and pathogenicity. However, the EU1 isolates showing resistance were some of the most aggressive. A strong connection between phenotypic characteristics such as fungicide sensitivity and pathogenicity, and the originating nursery was seen in this study.

How does *Phytophthora ramorum* Infect *Rhododendron* Leaves?

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In Europe *Rhododendron* is a very important host for the spread of *Phytophthora ramorum*. To get better knowledge on the infection of these hosts detached leaves of the Rhododendron cultivar 'Catawbiense Grandiflorum´ (CG) and the *R. insigne* hybrid `Brigitte´ (B) were inoculated with a zoospore suspension of a *P. ramorum* isolate of mating type A1 and A2, respectively. Development of leaf necrosis was calculated after different incubation periods (between 3 hours until several weeks after inoculation) by measuring the size of the necrosis. To study the development of gametangia, leaves of both Rhododendron cultivars were inoculated on the lower leaf surface with drops of zoospore suspensions of both isolates. Different application and incubation variants were tested to induce gametangia production. The incubation period for the gametangia induction was no longer than 12 days. For microscopical observation representative leaf pieces from both Rhododendron cultivars were cut out at each rating. With the lower leaf side up the leaf pieces were placed on slides and stained with Calcofluor White (0.02%) for one minute.

CG developed much bigger leaf necrosis than B. *P. ramorum* invaded the leaf tissue via the stomata by the germ tubes of the zoospores. The trichomes on the B leaves seem to prevent the germ tubes from detecting the stomata. Appressoria-like structures could be observed. On the infected leaves new hyphae grew out of the stomata. Sporangia and chlamydospores developed on the mycelium originated from the applied zoospores as well as on the hyphae growing out of the stomata after infection. They could be observed mainly on the necrotic leaf areas. The observations indicate that *P. ramorum* can start a new life cycle from the inner tissue of infected Rhododendron leaves. Only single oospores developed within an incubation period of 12 days. They could only be observed on leaves inoculated with two single drops of the two *P. ramorum* isolates and after incubation in the dark.

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Fire Ecology

Sudden Oak Death-Caused Changes to Surface Fuel Loading and Potential Fire Behavior in Douglas-fir-Tanoak Forests

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Field observations and anecdotal evidence suggest that sudden oak death (SOD), a disease caused by the pathogen *Phytophthora ramorum*, may alter fuel loading in affected forests. Though it is reasonable to assume that a disease resulting in leaf blight, dead branches, and tree mortality would increase forest fuels, little work has been done to support or quantify this important issue. We compared fuel loading in SOD-infested Douglas-fir-tanoak forests of northwestern California to 1) assess whether the continued presence of this pathogen alters surface fuel loading, 2) model potential fire behavior in affected stands, and 3) evaluate potential impacts on firefighting response in infested areas.

Recognizing that *P. ramorum* has not been present in California long enough for us to fully capture its effect on fuels, we supplemented sampling of pathogen-killed stands with those killed by herbicides. Herbicide treatments included in the study selectively targeted tanoak, one of the most vulnerable hosts of *P. ramorum* and the species of interest in this study; the lethal effects of both herbicide and *P. ramorum* on tanoak rendered the two treatments comparable.

Fuel loadings were greater in diseased than in undiseased stands, yet great variability was observed and differences were not significant. However, fuel loads observed in herbicide-treated stands were significantly greater than in control stands ($P < 0.001$); total weight of downed woody debris (all size classes) approximately doubled with the herbicide treatment ($\bar{x} = 106.3$ Mg ha^{-1}) over the control condition ($\bar{x} = 58.1$ Mg ha^{-1}). The increasing trends in herbicided and diseased plots were similar, suggesting that fuel loading in diseased plots may continue to increase relative to control plots over a longer time horizon than observed. Fuel models based on the observed surface fuel accumulations in herbicide-treated and diseased plots predict that for some early-to-mid-phase (2-8 years) herbicide-treated forests, and for late-phase (8 years plus) diseased forests, rates of spread, flame lengths, and fireline intensities could increase significantly over the baseline, challenging effective firefighter response and requiring alternative approaches to fire suppression. These results, in addition to the relatively high background surface fuels observed in the control stands, highlight the need for fuels treatments and effective disease management strategies in infested stands and as sudden oak death expands throughout a broader region.

Survival of *Phytophthora ramorum* Following Wildfires in the Sudden Oak Death-Impacted Forests of the Big Sur Region

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In June 2008, dry lightning storms across California ignited the first wildfires known to occur in forests infested with *Phytophthora ramorum*. The largest fires were located in the Big Sur region of the central coast (Monterey County), where more than 100,000 ha ultimately burned. Big Sur is one of the most botanically and ecologically diverse areas in California, yet its forests were among the earliest infested by *P. ramorum* and are some of the most devastated by sudden oak death in the state. A large network of long-term forest monitoring plots established in Big Sur to study the feedbacks between *P. ramorum* and the environment provided important pre-fire data, while a post-fire survey on burn severity indicators quantified forest impacts immediately post-fire in a subset of the plot network. The pre- and post-fire data from the Big Sur plot network allowed for the rare opportunity to study the interactions between a destructive, invasive forest pathogen and wildfire.

In this study, we addressed the prediction that the persistence of *P. ramorum* in burned, previously-infested forests would differ across the Big Sur landscape due to local site conditions. We accomplished this objective by asking and testing the following questions: 1) which pre-and post-fire forest characteristics affected *P. ramorum* recovery? 2) Did the frequency of *P. ramorum* isolation differ between burned and unburned plots or among hosts and host tissue types? To accomplish the research objectives of this study, we completed intensive *P. ramorum* surveys in 2009 and 2010 within 63 plots in the Big Sur network -- 45 of which were burned and 18 of which were unburned -- that were known to contain *P. ramorum*-infected trees at the time of plot establishment. Following the determination of *P. ramorum* presence in the plots via standard culturing techniques, we used 25 plot-based variables consisting of burn severity, pre-fire host density, pre-fire disease level, and landscape factor measurements as predictors in the analyses of *P. ramorum* recovery following the wildfires.

The wildfires of 2008 failed to eradicate *P. ramorum* from the Big Sur landscape, even in those areas that burned with great intensity. However, there was a much lower likelihood of recovering *P. ramorum* from burned plots than unburned plots both one and two years following the fires, which suggests that fire did at least reduce the abundance of the pathogen. Recovery of *P. ramorum* in burned plots was positively correlated with the number of California bay laurels (*Umbellularia californica*) expressing symptoms of *P. ramorum*-infection prior to the fires, further highlighting the importance of this sporulating host to the establishment, spread, and persistence of the pathogen. Patchy burn patterns that left green, *P. ramorum*-infected bay laurels amidst the charred landscape may have allowed these trees to serve as inoculum reservoirs that could infest newly sprouting vegetation. Unexpectedly, two other *Phytophthora* spp., *P. pseudosyringae* and *P. nemorosa*, were frequently isolated from new vegetative growth in burned plots that were not known to contain these pathogens prior to the fires.

Collateral Damage: Fire and *Phytophthora ramorum* Interact to Increase Mortality in Coast Redwood

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Understanding how natural and anthropogenic disturbances interact is of growing importance because of the increasingly important role disturbances such as habitat fragmentation, the introduction of exotic species, or global change play in structuring ecological communities. *Phytophthora ramorum*, the causal agent of the emergent disease sudden oak death (SOD), is an invasive pathogen causing widespread tree mortality in coastal forests of California and Oregon. In the absence of the pathogen, species composition in these forests is shaped by a number of biotic and abiotic factors, including the endemic disturbance of wildfire. Large wildfires in California in 2008 provided an opportunity to test the interactions between *P. ramorum* and wildfire and their separate or joint impacts on forest composition. Here we ask whether the interacting effects of *P. ramorum* and wildfire increase mortality for three dominant species in redwood forests that differ greatly in their vulnerability to the pathogen and to the effects of fire.

The 2008 Basin Complex and Chalk fires in Big Sur, CA, burned more than 40% of the 280 forest monitoring plots established throughout the region in 2006-2007. In 2009 we surveyed every stem that had been alive at plot establishment in 2006-07 to determine mortality one year following the fire. In particular, we examined the mortality of tanoak, bay laurel, and coast redwood in 61 redwood forest plots, half of which were infested with *P. ramorum*. Tanoak and bay laurel are both competent sporulating hosts for the pathogen and sensitive to wildfire, but only tanoak suffers lethal bole canker infections. Redwood may become infected with the pathogen, but it is an epidemiologically unimportant host in the spread or impacts of the disease, and is generally resistant to wildfire.

In the absence of fire, all three species had very little mortality between 2006-07 and 2009, except for tanoak stems in pathogen-infested plots. All three species had increased mortality in burned plots, but mortality in tanoak and bay laurel was much higher than in redwood. Redwood mortality varied greatly by tree diameter: for stems >60 cm DBH there was negligible mortality, and, below 10 cm DBH, there was > 80% mortality, regardless of pathogen presence. Surprisingly, mortality risk increased dramatically for redwood stems 10-60 cm DBH in infested, burned plots relative to uninfested plots. For example, mortality of 25 cm DBH stems more than doubled, for stems approximately 20-40 cm DBH in the presence of the pathogen. Standard predictive models of tree mortality following fire that use only tree size and bark thickness were not sufficient to predict redwood mortality in infested areas. Instead, it was necessary to include a characterization of the SOD impacts to the forest to successfully predict redwood mortality.

Elevated fire-related redwood mortality in the presence of *P. ramorum* is not due to any direct effect of the pathogen on redwood, but rather the ways in which SOD influences fire severity through impacts on forest structure and fuel availability. This study demonstrates the important and surprising impact two interacting disturbances can have on a species that is otherwise resistant to the impacts of either disturbance alone.

Dynamics of Dead Wood Following Emergence of Sudden Oak Death

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Sudden oak death continues to impact forests notable for high fire risk and contiguous host communities such as the Big Sur and northern coastal California regions. As the disease emerges in stands and landscapes with large amounts of tanoak biomass, woody debris will also increase. This increase in fuels and the possibility of increased C release may impact wildfire and regional C management goals. We documented rapid reductions of tanoak biomass and accumulation of course woody debris (dead wood >10 cm diameter) in a set of plots dominated by redwood[1]. We measured log and snag (standing dead trees) mass, snag-fall rates, and tanoak wood decomposition rates and combined these data with detailed measurements of pathogen prevalence and mortality rates. In pathogen invaded stands, snag mass was 22.4 Mg ha-1 while mean log mass was 11.5 Mg ha-1. In comparison, masses in an uninvaded stand were 0.27 and 1.16 Mg ha-1 of snags and logs respectively. Woody debris mass and accumulation rates are principally driven by the amount of pre-disease tanoak biomass and the prevalence of infection in tanoak and California bay laurel. Previous work showed that tanoak infection and mortality rates are also largely driven by tanoak size distribution and the local prevalence of infection in sporulation supporting hosts[2]. As these are the same drivers of woody debris levels, we adapt epidemiological models to investigate the temporal patterns and duration of elevated woody debris in a geographically broad and diverse host landscape. Overall, sudden oak death generates lower maximum amounts of woody debris and these materials accumulate at slower rates compared to better studied disturbances such as harvesting and wildfire. We identify two patterns critical to management of disease impacted forests. First, the duration and maximum amounts of woody debris can be predicted with relatively simple stand level measurements of tanoak densities and tree size along with density of bay laurel. Second, sudden oak death is likely to result in a long period of increased woody debris levels when compared to other disturbances such as wildfire.

1. Cobb, R.C., Chan, M.N, Meentemeyer, R.K., Rizzo, D.M. 2012. Common Factors Drive Disease and Coarse Woody Debris Dynamics in Forests Impacted by Sudden Oak Death. Ecosystems. Volume 15. 2: 242-255.

2. Cobb, R.C., Filipe, J.A.N., Meentemeyer, R.K., Gilligan, C.A., and Rizzo, D.M. 2012. Ecosystem transformation by emerging infectious disease: loss of large tan oak from California forests. Journal of Ecology. DOI: 10.1111/j.1365-2745.2012.01960.x.

Biology

Combining Field Epidemiological Information and Genetic Data to Comprehensively Reconstruct the Invasion Genetics of the Sudden Oak Death Agent *Phytophthora ramorum* (Stramenopila: Oomycetes) in California

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Previous attempts at reconstructing the entire history of the Sudden Oak Death (SOD) epidemic in California have been limited by: 1) incomplete sampling; 2) the inability to include singleton samples; and, 3) over-collapsing of non-spatially contiguous yet genetically similar samples into large meta-samples that confounded coalescent analyses.

Here we employ a complete sampling coverage of 832 isolates of *Phytopthora ramorum* (the causative agent of SOD) from 60 Californian forests, genotyped at nine microsatellite loci. We have expanded upon and refined previously established analytical strategies. These strategies included iterative collapsing of genetically indistinguishable populations based upon minimum Φ_{ST} into highly-resolved sets of spatially contiguous populations; the construction of a robust framework of genetic relationships among major populations; and, the use of age of infestation as a constraint on coalescent analyses. We produce a convincing picture of the entire history of the epidemic that differs from the one presented in previous works and that allows the positioning of small but important infestations represented by single genotypes within the resulting robust genetic framework.

Results indicate that in most counties multiple introductions of *P. ramorum* have occurred, and that the most significant sources of further infestations can be identified as being either very large wild outbreaks or small outbreaks in densely populated areas. The study also identified minor introductions, some of them relatively recent, that may be linked to infected ornamental plants. Finally, using archival isolates collected soon after the discovery of the pathogen in California, we corroborate that the epidemic likely resulted form 3-4 core founder genotypes that rapidly evolved from a *single* genotype.

Host Induced Phenotypic Diversification in *Phytophthora ramorum*

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Forestry, agriculture, and native ecosystems face ever-increasing threats by invasive plant pathogens. Due to population genetic bottlenecks, invasive microbe populations often have limited genetic variation and propagate clonally. Despite the limited opportunities for sexual reproduction, microbial populations may still display diverse phenotypic variation. This attribute may be responsible for the tenacity and adaptability of introduced pathogens in non-native environments. The invasive oomycete pathogen, *Phytophthora ramorum* is the causal agent of sudden oak death. The primary lineage of *P. ramorum* in California forests is known to display a wide range of virulence and cultural morphology. We found large variation in molecular profiles in pure cultures of the pathogen. Surprisingly, gene expression patterns were associated with originating host species, but not with DNA fingerprints. In comparison with *P. ramorum* isolates originating from foliage of California bay laurel, those from stems of oak trees tended to show elevated transcription of transposable elements (TEs) and reduced transcription of genes belonging to the crinkler effector family. We suspected that the oak host environment was responsible for phenotypic diversification. In order to establish causality, we examined phenotypes of re-isolates, which were recovered from trunks of mature canyon live oaks. These oaks had been inoculated with isolates derived from bay laurel six to nine months prior to re-isolation. Approximately 30% of re-isolates showed the non-wild type (nwt) colony morphology, which has been shown to be associated with isolates derived from oaks. On the other hand, the original isolates used for inoculum consistently showed wild type (wt) colony morphology. Global mRNA profiling confirmed that some of the re-isolates growing on petri plates showed expression patterns typical to those isolated form oak trunks. Because oak is a non-transmissive dead-end host for *P. ramorum*, our observations are congruent with an epi-transposon hypothesis; i.e., physiological stress is triggered on *P. ramorum* while colonizing oak stems and disrupts epigenetic silencing of TEs. This then results in TE reactivation and possibly genome diversification without significant epidemiological consequences. We propose the *P. ramorum*-oak host system in California forests as an ad hoc model for epi-transposon mediated diversification.

Susceptibility of Larch, Hemlock, and Sitka Spruce to *Phytophthora ramorum*

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The recent determination that *P. ramorum* is causing bleeding stem cankers on Japanese larch (*Larix kaempferi syn L. leptolepis*) in the UK and that inoculum from this host appears to have resulted in disease and canker development on other conifers, including western hemlock (*Tsuga heterophylla*), Douglas-fir (*Pseudotsuga menziesii*), grand fir (*Abies grandis*), and Sitka spruce (*Picea sitchensis*), potentially has profound implications for the timber industry and forests in the U.S. Pacific Northwest (PNW). Douglas-fir and hemlock are the most important timber species in the PNW, and represented 73% and 9%, respectively of the 2003 Oregon timber harvest. Larch is planted on a much smaller scale in both states. Most of the larch in both states occurs east of the Cascade mountains, but there is a small amount of larch grown in the western portions of Oregon and Washington. Given that these areas are considered to be at high-risk for the spread of *P. ramorum* because of the presence of *P. ramorum* in nurseries and suitable climatic conditions, a clearer understanding of the susceptibility of the conifers found to be infected in the UK is necessary to assess the risk that *P. ramorum* may develop in PNW forests.

An experiment was conducted to examine the susceptibility of new growth on European (*L. decidua*), Japanese, eastern (*L. laricina*) and western larch (*L. occidentalis*), western and eastern hemlock (*T. canadensis*), Sitka spruce and a coastal seed source of Douglas-fir to isolates of the three known lineages (NA1, NA2, and EU1) of *P. ramorum*. Due to the discovery in the UK that *P. ramorum*-infected Japanese larch needles can sporulate heavily in the late summer and early fall, we also conducted an experiment to determine the susceptibility of late-season European, Japanese, eastern and western larch and Douglas-fir foliage to infection by this pathogen.

The same sources of container-grown seedlings or saplings were used in both experiments. Five trees or branches of each species were inoculated with each isolate of *P. ramorum* by spraying the foliage with a suspension of zoospores (10^5spores/ml). Seedlings or branches that were sprayed with water alone served as the control. After 5 to 6 days in a moist chamber within a biocontainment unit, symptoms were monitored on the plants which were maintained at 19-20C with 24 hr light.

In the spring inoculation trial, the percentage of inoculated seedlings with symptoms ranged from 20 to 100%. Symptoms ranged from infection of individual 2011 needles or needle bundles, shoot dieback that extended into the previous year's stems in the form of lesions, to mortality. All of the conifer species exhibited some level of symptom development, but symptom severity and recovery of *P. ramorum* was most common on the four larch species. Although all of the larches were susceptible, the western larch had the highest level of mortality and percentage of isolation positives for all the genotypes. Of the isolates used, the NA2 isolate caused symptoms on all of the conifers, while symptoms developed on 75% and 50% of the conifers inoculated with the EU1 and NA1 isolates, respectively.

There were minimal symptoms on the late-season inoculated trees and they were the same as those on the controls (e.g., a few gray needles, yellow/brown needle tips, a faint lighter-colored needle banding, single yellow needles, etc). Isolations from symptomatic needles 8 weeks after inoculation resulted in only a single positive recovery of *P. ramorum* from the proximal end and center of a single, partially gray needle from Japanese larch inoculated with the NA2 isolate.

Variation in Susceptibility to *Phytophthora ramorum* Infection in a Range-Wide Collection of Pacific Madrone Seedlings

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Pacific madrone (*Arbutus menziesii*) is an important evergreen hardwood tree species in Pacific Northwest forests that provides food and habitat for wildlife as well as erosion control in disturbed areas. It is also a host for the invasive pathogen *Phytophthora ramorum*. Pacific madrone is a wide-ranging species, covering a large latitudinal gradient from California to British Columbia. Very little is known about the genetics of this species, such as its variation in resistance to pathogens and adaptation potential in the face of changing climate.

Seedlings of Pacific madrone grown from seed collected from the range of the species were inoculated with *Phytophthora ramorum* to examine susceptibility to this pathogen within the species. Seedlings grown from 81 seed sources from 36 sites within 7 ecoregions in the range of Pacific madrone were used. Zoospore inoculum was prepared from one isolate from each of the three clonal lineages of *P. ramorum* and sprayed on six-month-old seedlings in plug trays. Seedlings were incubated under optimum conditions for infection, and disease incidence and severity was rated after 8 days.

There were significant differences in both incidence and severity of foliar infection caused by each of the three isolates of *P. ramorum*. The NA2 isolate was the most aggressive, EU1 intermediate, and NA1 least aggressive. Cluster analysis of incidence and severity ratings for seed sources of Pacific madrone formed three groups: resistant (24%), intermediate (40%), and susceptible (36%). There was no interaction between *P. ramorum* isolates and susceptibility of madrone seedlings: if plants from a given seed source were resistant to one isolate of *P. ramorum*, they were resistant to the others. There was no relationship between susceptibility and the ecoregions where seed was collected, but there were differences in disease incidence and severity on seedlings among individual sites.

Five replicated field trials that include all of the seed sources used in this trial have been installed in CA, OR and WA. One of these sites is located near Ben Lomond, CA, which is an area where *P. ramorum* is established in the landscape. Monitoring of this plot for *P. ramorum* infection will provide an opportunity to verify the results of the laboratory susceptibility studies under field conditions.

Diagnosis and Management of *Phytophthora ramorum* Canker in Canyon Live Oak, an Atypical Bole Canker Host

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Diagnosis of SOD in tanoak (*Notholithocarpus densiflorus*) and several oak species (*Quercus agrifolia, Q. parvula* var, *shrevii, Q. kelloggii*) is facilitated by the fact that infected trees commonly develop obvious bleeding bark cankers. In contrast, canyon live oak, *Q. chrysolepis,* exhibits a different pattern of symptom expression, which has made it difficult to identify and document *P. ramorum* bole cankers in this species.

In 2002, *P. ramorum* was shown to cause dieback in small (<2 cm diameter) branches of *Q. chrysolepis*, but bole cankers on mature *Q. chrysolepis* were not observed. In 2006, we suspected that SOD was the cause of mortality in a cluster of *Q. chrysolepis* on Bolinas Ridge in Marin County that were amid *P. ramorum*-infected *Umbellularia californica*. In 2008, mortality and large trunk cankers were seen in large mature *Q. chrysolepis* in the Los Trancos Open Space preserve in Santa Clara County. Symptomatic *Q. chrysolepis* had bole cankers with *Annuluhypoxylon thouarsianum* sporulation and extensive flat-headed borers and ambrosia colonization. Several rounds of sampling from affected *Q. chrysolepis* failed to yield *P. ramorum,* which was present on foliage of the locally abundant *U. californica*. After ruling out other possible causes of tree decline, we surveyed the affected stand and found a high association between the presence of symptoms in *Q. chrysolepis* and proximity of *U. californica.* Positive PCR results for *P. ramorum* were obtained from cankers on two symptomatic trees after further sampling, but no positive cultures were obtained.

We subsequently tested pathogenicity of *P. ramorum* on detached large-diameter logs of *Q. chrysolepis* under lab conditions using established protocols. Large dark brown cankers developed in the phloem of inoculated canyon live oak logs within 2 weeks after inoculation and *P. ramorum* was readily reisolated from canker margins. This provided the first clear indication about the internal appearance of early stage *P. ramorum* cankers on this species. However, the detached log assay does not indicate what types of external symptoms would be seen on infected trees in the field. In August 2010, we initiated a field test by inoculating 18 *Q. chrysolepis* (25 cm average DBH) and two *Q. parvula* var, *shrevii* (to serve as positive controls) at a site in San Mateo County. Each tree was inoculated with two different local *P. ramorum* isolates and a control (sterile agar only) inoculation. Symptoms were evaluated periodically over an 18 month period, and subsets of trees were sampled 5 and 9 months after inoculation. Cankers of varying sizes developed at all *P. ramorum* inoculation points on *Q. chrysolepis* that have been sampled. *Phytophthora ramorum* was reisolated from even small cankers nine months after inoculation. Only 9 of 36 (25%) *P. ramorum* inoculation points developed any bleeding, and the duration of active bleeding was short. One tree, which developed a large canker within four months, had significant amounts of bleeding. On remaining trees, any bleeding was limited to a few miniscule spots visible only on close inspection.

With the information gleaned from inoculation studies, we have successfully isolated *P. ramorum* from several naturally-infected *Q. chrysolepis,* but isolation efficiency has remained very low. We were able to identify these recent cankers by the small amounts of bleeding present. However, our results suggest that many infected trees do not bleed and cankers in these cryptically-infected trees cannot be seen until they are colonized by secondary organisms. This has complicated ongoing management studies to prevent *P. ramorum* infection in *Q. chrysolepis*. Our results raise the possibility that *P. ramorum* bole cankers in species that develop little or no bleeding may remain unrecognized.

Roads Are Not Significant Pathways for SOD Spread in Oregon

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Control measures for forest Phytophthoras often focus on reducing the spread of infested soils, including closing roads and washing vehicles. Similar measures are suggested for *P. ramorum*, despite its evident aerial dispersal: stay out of areas of wet soils, and clean clothing and equipment when entering or leaving infested areas. It remains unclear, however, if these measures have limited the spread of *P. ramorum* in Oregon and California.

We used two approaches to study the risk of spread of *P. ramorum* along roads in the Oregon quarantine area. First was a spatial analysis of infected sites relative to the road network. Second, was a ground survey of roads in the infested area. For the spatial study we used GPS coordinates for all *P. ramorum* positive trees identified between 2001 and 2010 within the North Fork Chetco River study area. Clustered positive trees were reduced to a single site coordinate defined as the centroid of all trees located within 60 m of one another. GIS road layers were obtained from the POC-GIS regional distribution maps. Distance from each centroid to the nearest road was calculated with a spatial join relating points (site) to line features (roads).

To test our null hypothesis that SOD sites were no closer to roads than expected by chance, random points were generated separately within 1-km wide regions spaced horizontally throughout the study area; the proportion of points created was identical to the proportion of SOD sites found in each region. The distance of each random point to the nearest road was calculated with a spatial join as with the true dataset. Statistical likelihood of observing the true median distance under randomness was computed with a restricted randomization test comparing the observed median distance of SOD sites to roads to 10,000 reiterations of the random dataset.

Road segments were surveyed during the rainy season in 2011 and again in 2012 in a series of transects, on foot. The road segments were in heavily trafficked areas passing through concentrations of SOD sites. Water was collected from mud puddles on the roads and baited for *P. ramorum*. Symptomatic roadside vegetation was also collected and tested for *P. ramorum* infection.

The spatial analysis showed no association between roads and SOD sites. The median distance from SOD centroids to roads was not significantly different from the median distance expected under randomness. In the first year of road sampling, *P. ramorum* was not recovered from road puddles or susceptible plants growing within splash distance from roads except when they were growing immediately beneath an infected over story tree. Analysis of second year data is continuing.

Results to date indicate that roads are not important dispersal pathways for *P. ramorum* in Oregon. This is probably testament to the effectiveness of the sanitation protocols incorporated in the SOD eradication program, as well as evidence of the epidemiological limitations that the harsh road environment forces on *P. ramorum* survival and sporulation.

The Effect of *Phytophthora ramorum* on the Physiology and Xylem Function of Young Tanoak Trees

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Tanoak *(Notholithocarpus densiflorus)* is highly susceptible to *P. ramorum*. Symptoms include stem cankers, shoot dieback, and foliar blight. The mechanism by which *P. ramorum* kills the trees is not known, however. In this study we aimed to determine what physiological factors contribute to tanoak mortality when trees are infected with *P. ramorum* and to investigate the relationship between elicitin secretion and disease symptoms. In growth chamber experiments, we investigated how photosynthesis, stomatal conductance, water usage, stem specific hydraulic conductivity, and starch partitioning were affected following inoculation of young tanoak trees with *P. ramorum* isolates that differed in elicitin secretion.

In experiments with two-year-old tanoak saplings, stems of 60 trees were wound-inoculated with one of three treatments including a high elicitin expressing *P. ramorum* isolate (PR-07-058[NA2]), a low elicitin-expressing *P. ramorum* isolate (4353[NA1]), or a noninoculated wounded control. Physiological parameters including photosynthesis and water usage were measured weekly for 5 weeks. Sets of trees from each treatment were sampled destructively twice monthly to measure the conductive properties of stem xylem tissue and to examine for the presence of tyloses. There was a large difference between the high and low elicitin treatments in stem hydraulic conductivity as early as week two of the experiment. Significant treatment differences were also observed in tree mortality. Trees inoculated with the high elicitin expressing isolate died sooner and at a higher rate than trees inoculated with the low elicitin expressing isolate. Photosynthesis and stomatal conductance began to decline three weeks after inoculation. This experiment was potentially compromised by differences in growth rate of the two isolates, so the experiment was repeated with high- and low-expressing NA2 isolates with similar growth rates.

Tanoak seedlings (2- or 3-months-old) were inoculated with one of three treatments: PR-05-002 (high elicitin-producing isolate), PR-05-166 (low elicitin-producing isolate), or a sterile V8 agar plug. Photosynthesis, stomatal conductance, water usage and stem specific hydraulic conductivity were measured twice weekly for two weeks, when inoculated trees began to die. Net photosynthetic rate and stomatal conductance were significantly reduced in both sets of inoculated trees as compared to the wounded control trees by day 12 after inoculation. Stem specific hydraulic conductivity was significantly reduced as early as 7 days after inoculation. There were few significant differences between the low-elicitin expressing and the high-elicitin expressing treatments.

The rapid decline in stem hydraulic conductivity in *P. ramorum*-inoculated trees, with a concomitant reduction in net photosynthetic rate and stomatal conductance, is consistent with the hypothesis that *P. ramorum* interferes with stem water transport. No clear role for elicitin secretion in pathogenesis was observed.

Screening Gulf Coast Forest Species for Susceptibility to *Phytophthora ramorum*

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Phytophthora ramorum, the causal agent of sudden oak death in California oak woodlands, poses a threat to woody plants in the rest of the U.S., including the Gulf Coast area, which is regarded as a high threat location. Several plant species native to the Gulf Coast forest were tested for reaction to *P. ramorum*, including yaupon (*Ilex vomitoria*), spice bush (*Lindera benzoin*), Southern magnolia (*Magnolia grandiflora*), sweetbay magnolia (*Magnolia virginiana*), black willow (*Salix nigra*), baldcypress (*Taxodium distichum*), Virginia creeper (*Parthenocissus quinquefolia*) from two sources (Louisiana and Maryland), and Eastern baccharis (*Baccharis halmifolia*). This study was conducted at the USDA- ARS Biosafety containment greenhouse facility at Ft. Detrick, Maryland. Foliage of four plants for each species tested was inoculated with a suspension of 50,000 zoospores per ml until run-off. Inoculated plants were placed in a dew chamber at 20 ºC for 5 days. After incubation period, leaf lesion areas were assessed for necrosis. The average percentage of leaf area necrosis was 5.0, 0.2, 8.6, 1.5, 1.1, 0.2, 32.1, 4.9, and 27.9 for inoculated baldcypress, black willow, sweetbay magnolia, Virginia creeper (Louisiana genotype), Virginia creeper (Maryland genotype), Eastern baccharis, Southern magnolia, spicebush, and yaupon, respectively, while 4.2, 0.3, 0.3, 3.1, 1.1, 0.4, 0.6, 1.2, and 0.1% for non-inoculated control plants, respectively. Comparison of inoculated with non-inoculated plants showed significant differences ($P \leq 0.05$) for yaupon ($P=0.0008$), Southern magnolia ($P=0.001$), and sweetbay magnolia ($P=0.0009$).

Phytophthora ramorum in Coast Live Oaks: Search for Resistance and Mechanisms

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Despite the presence of *Phytophthora ramorum* in northern and central California forests since at least 1994, asymptomatic coast live oaks (*Quercus agrifolia*) still remain in heavily infected stands. Coast live oak infection and mortality rates of 5% y^{-1} and 3% y^{-1}, respectively, observed in long term monitoring plots in Marin County, give no indication that individual trees appear to evade infection. In some study plots, more than half the mature coast live oaks have died since 2000.

Inoculation studies consistently find continuous canker length distributions, varying in size from negligible to girdling. Separate inoculation studies of coast live oaks in Marin County, and of Shreve oaks (*Q. parvula* var. *Shrevei*) in Santa Cruz County found similar continuous canker size variation. Larger cankers are more likely to be attacked by ambrosia and bark beetles, as are larger diameter infected trees. Beetle attacks reduce survival considerably and may overcome any resistance in an infected tree, suggesting that trees with smaller cankers are not only expressing effective defenses against the pathogen, but may minimize or avoid the deleterious effects of beetle attacks. The continuous size distribution throughout populations is consistent with quantitative, multi-gene, and potentially durable resistance to the pathogen.

In an effort to establish a quantitative basis for estimating resistance to the pathogen, we developed a logistic regression model to predict the survival probability of coast live oaks as a function of canker length. The model predicts that trees in the Marin County inoculation study with canker lengths <21 cm, measured 9 months after inoculation, have >80% probability of survival after 7 years.

The Marin and Santa Cruz County studies were conducted in areas where *P. ramorum* had been established and were therefore 'prescreened' for susceptibility. To minimize the possibility that the true response to inoculations would be affected by prior exposure, we initiated a study in a naïve forest. In 2010 we selected 600 mature coast live oaks in two stands in Briones Regional Park, Contra Costa County, sampled the phloem for chemical analysis, and inoculated 150 with *P. ramorum*. The relationship between phloem chemistry, canker length, and survival will be investigated. The remaining trees will be monitored for their response to natural infection in future years to determine if phloem chemistry can be a reliable predictor of survival.

Metabolite Profiling to Predict Resistance to *Phytophthora ramorum* in Natural Populations of Coast Live Oak

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Sudden oak death, caused by the invasive alien oomycete pathogen *Phytophthora ramorum,* continues to shape the dynamics of coastal populations of oak and tanoak in California and tanoak in southwestern Oregon. Over the last decade, high mortality rates have been reported in natural populations of coast live oak (CLO; *Quercus agrifolia*) in California, raising concerns for the integrity of important coastal ecosystems. However, it is now recognized that in spite of high infection and mortality rates, asymtomatic CLO have persisted.

Though the mechanisms of persistence are not known, putative phenolic biomarkers of oak resistance to the pathogen have been identified and include tyrosol hexoside pentoside, ellagic acid, and total phenolics. To test the accuracy of these biomarkers in predicting resistance within natural populations of CLO, we quantified biomarkers in 600 mature trees from 2 naïve stands in Briones Regional Park, East Bay Regional Park District, Contra Costa Co., California. 150 of those trees were then inoculated with *P. ramorum* to assess their resistance, by measuring resulting cankers approximately ten months after inoculation and quantifying biomarkers. We applied a predictive model to estimate survival based on canker lengths. This model predicted that 14% of inoculated trees have an 80% probability or greater of being resistant; however, only 48% of those trees contained one or more biomarkers at or above resistant threshold levels. Current efforts are focused on identifying novel biomarkers, which may better predict resistance in naïve populations of CLO. Additionally, to account for phenology as a potential source of variation in CLO phenolics, we measured biomarkers every season from December 2010 to November 2011 in 14 randomly selected, control trees. We found no significant difference between seasons in the phenolic biomarkers tyrosol hexoside pentoside and ellagic acid.

Mitigation of disease, via exploitation of host resistance, is one of the most effective and economically feasible management strategies for keystone tree species in forested ecosystems. The ability to identify resistant trees in natural populations of CLO can thus be incorporated into disease management plans with efforts aimed at preserving resistant trees which could otherwise be lost to urban encroachment, fire, or other destructive disturbances.

Diagnostics and Biology

Comparative Mitochondrial Genomics and the Development of a Genus and Species Specific Diagnostic TaqMan Assay for *Phytophthora*

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With the recent history of the impact of exotic *Phytophthora* species on forest ecosystems and nursery plant production the ability to accurately and rapidly identify this pathogen is essential for making regulatory and management decisions. The ideal molecular diagnostic marker system for this purpose would be sensitive, highly accurate, have both species specific as well as genus specific capabilities and provide a systematic approach for design of species-specific markers. In addition to these detection capabilities it would be important that the assay be highly reproducible between labs and not be dependent on narrow amplification parameters for accuracy. In an effort to design such a diagnostic assay a comparative genomics approach was undertaken to identify gene order differences that could be used for developing a highly specific and sensitive assay that has both genus and species specific detection capabilities.

The mitochondrial genome was selected as the target region for this study due to its comparatively higher copy number (thereby enhancing the sensitivity) and the level of interspecific variation among closely related species that had been observed in prior studies. The mitochondrial genomes of 19 *Phytophthora* spp. and 15 *Pythium* spp. were sequenced and gene order compared with other mitochondrial genomes of Oomycetes and plants deposited in GenBank. Three loci were identified where a unique gene order was conserved in *Phytophthora* and these regions were sequenced for a wide range of species to evaluate their utility for marker design. Based on the level of interspecific variation observed in the spacer region separating the two genes and a highly conserved region in one gene that allowed for design of a genus specific TaqMan probe, one locus was chosen for further development. A multiplexed TaqMan assay has been developed that includes 1) a plant target as a positive control to demonstrate the extracted DNA is amplifiable and 2) a genus specific amplicon that has annealing sites for both a genus and species specific TaqMan probe. Genus specific amplification has been confirmed for all *Phytophthora* species tested (106+) with the exception of *P. alticola, P. bisheria* and *P. frigida*. Tests with a wide range of *Pythium* and plant species has confirmed the specificity of *Phytophthora* specific amplification. TaqMan probes have been validated for 14 species (including *P. ramorum, P. kernoviae*, and *P. alni*) and 5 additional are under evaluation (including *P. europaea, P. pinifolia*, and *P. quercina*). The locus has been sequenced for approximately 700 isolates representing over 106 species and *in silico* analysis suggests it may be possible to develop species specific TaqMan probes for 75+ species. Techniques for sequencing the genus specific amplicon from environmental samples have been developed and a BLAST database set up to facilitate species identification. A second locus with a smaller amplicon size was also examined and while it did not exhibit the same level of interspecific sequence variation in the spacer region, this locus was amplified in all *Phytophthora* spp. Validation of the marker systems with environmental samples is currently in progress.

Testing the Importance of Understory *Phytophthora ramorum* Infection as a Means of Primary Disease Establishment in Oregon Forests

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Phytophthora ramorum infested soils have been suggested to be a source primary inoculum in natural ecosystems. Implicit in this pathway is the need for infection of understory vegetation as a means for pathogen establishment, from which *P. ramorum* may spread onto adjacent hosts in rain splash. While useful in detecting sudden oak death (SOD) associated tree mortality, the aerial survey methods currently utilized by the Oregon eradication program are not reliable for detection of understory infection. *P. ramorum* can be recovered from soils at sites treated as part of the SOD eradication program (Goheen et al. 2008), as well as from streams within infested watersheds during all seasons of the year (Sutton et al. 2009). It is unknown, however, to what extent these inoculum sources are responsible for establishing new disease foci, posing a risk for the continued spread of *P. ramorum* in Oregon.

We took two approaches to assess if soil- or stream-borne inoculum is contributing to the establishment of *P. ramorum* infection in understory vegetation: 1) survey of infested streams to discern the extent of stream-side infection, and 2) a spatial analysis to assess if understory infection occurs independently of overstory mortality. For both studies, we postulated that the presence or absence of disease gradients in the understory may indicate if infection arose from understory inoculum sources, or from symptomatic, overstory tanoaks presumed to have canopy infection. Transects were established adjacent to streams known to harbor inoculum, or around symptomatic overstory tanoaks. Along each transect the presence of major foliar hosts was noted; symptomatic foliage was gathered and plated in selective media to discern the presence or absence of *P. ramorum* or other *Phytophthora* spp. with increasing distance away from inoculum sources.

Despite the abundance of understory hosts and other *Phytophthora* spp., *P. ramorum* was not recovered along streams bearing inoculum, except when associated with overstory mortality. A strong disease gradient was detected around SOD positive overstory tanoaks, indicating spatial dependence upon overstory sources. Our results have implications for the dispersal epidemiology and containment of *P. ramorum* in natural ecosystems.

Determining Landscape-Scale Changes in Forest Structure and Possible Management Responses to *Phytophthora ramorum* in the Mt. Tamalpais Watershed, Marin County, CA

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The Marin Municipal Water District's (MMWD) 18,500 acre Mt. Tamalpais Watershed in Marin County, CA has the dubious distinction of being one of the earliest and most extensively *Phytophthora ramorum* impacted zones in California. Rapid die-offs of tanoaks (*Notholithocarpus densiflorus* var. *densiflorus*) were first documented in 1995.With funding support from the US Forest Service, MMWD initiated an assessment of landscape-scale changes in forest structure and understory floristics relative to *P. ramorum* spread in the Mt Tamalpais Watershed. This assessment looked at changes in the extent and severity of diseased stands over a 5- year period as well as changes in understory vegetation. There specific questions were addressed to inform the development of a response strategy: (1) What Sudden Oak Death (SOD)-related changes have already occurred? (2)What future SOD related impacts are likely or where is SOD likely to spread? (3) What is the status of natural regeneration in SOD-impacted stands? An additional benefit from this project was revision the of SOD-impacted portions of the 2004 vegetation map for the Mt. Tamalpais Watershed to more accurately reflect stand conditions in 2009.

Analysis of true color aerial imagery of the watershed indicates the spatial extent and severity of SOD-related tree mortality expanded between 2004 and 2009 from 8750 acres to 10,700 acres. This represents 83% of all habitat on the watershed with a principal component of tan oaks, coast live oak, black oak (*Q. kelloggii*), canyon live oak (*Q. chrysolepis*), or Shreve's oak (*Q. parvula* var. *shrevei*). The largest unimpacted stand in 2009 was 150 acres in size. In both 2004 and 2009, canopy mortality was most pronounced in tanoak-dominant assemblages in the western Bolinas Ridge portion of the watershed. Much of the expansion between 2004 and 2009 is due to a spatial increase in coast live oak mortality in the southern and eastern portions of the watershed. Type conversions from one recognized vegetation association to another were detected for 2075 acres of habitat where tanoak ceased to be a primary component.

Ground sampling identified changes in understory shrub and herbaceous cover relative to disease progression. Shrubs overall showed a 38% increase in stands with lessening disease severity; were nearly unchanged areas where disease severity remained stable; and increased 21% in areas where disease severity increased. Evergreen huckleberry *(Vaccinium ovatum)* increased 19% where the SOD severity changed, but decreased 10% in moderate-severely infected plots where the disease level remained unchanged. Weedy grasses increased by 143% regardless of SOD severity, while native grasses also increased 131%. Recruitment of replacement species was not observed.

Quantification and mapping of both canopy and understory changes relative to SOD infection on the watershed are informing MMWD staff in the assessment of response options. With little un-impacted susceptible habitat remaining, containment or inoculation options are unlikely to be meaningful. Initiation of active revegetation remains premature as continued tree failure hinder access and likely survival of plantings.

Long-Term Monitoring of Disease Progression and the Population Genetics of *Phytophthora ramorum* Within the SFPUC Watershed in San Mateo County

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In 2008 a network of 16 plots was established within the SFPUC watershed surrounding the Crystal Springs, San Andreas and Pilarciros Reservoirs to monitor the spread and genetic diversity of *Phytophthora ramorum*. This is a unique area to study Sudden Oak Death as it has very limited public access in the central Bay Area and is a relatively old infestation within the county. The sites were randomly selected based on suitable habitat type for the pathogen and each consists of three 100 meter transects. Every 10 meters along each transect, Bay Laurel stems were surveyed for visual symptoms of *P. ramorum*, and if present six visually symptomatic leaves were sampled. These samples were embedded in selective media PARP, and those with morphologically determined growth of *P. ramorum* were genotyped using ten microsatellite markers known to be variable within the North American lineage of *P. ramorum* (NA1). PCR diagnostics assigned all samples with a score for presence or absence of the pathogen regardless of isolation success. Additionally all Coast Live Oaks present in the plot network were assessed for *P. ramorum* infection by the presence of bleeding bole cankers. In 2009 6 of the 16 plots, 2 from each major drainage were selected to run an intensively sampled small-scale study and 18 transects were added for a total of 66 transects and ~375 bay trees. The plots were surveyed three times per year for the next three years. The three sampling events each year represent a seasonal snapshot of the pathogens cyclical activity. The life cycle of *P. ramorum* causes it to undergo a period of dormancy each year and an active period in spring or "peak season" conditions. This sampling scheme was designed to provide information on the pathogens activity throughout the year at these marked locations. Each year an early season sampling was conducted in March, a peak season in June, and a late season in October. During each fall sampling Coast Live Oaks were re-assessed for infection and all cankers were sampled. In total Coast Live Oaks were assessed and sampled four times over the duration of the study. As of now it has been determined that bay trees in 14 of these 16 plots are infected with *P. ramorum.* Oak mortality has been confirmed in 9 of the 16 plots.

Here we report on the fluctuations of culture success throughout the year and microclimate factors that allow the pathogen, in some cases, to remain viable year round providing perennial sources of inoculum. There is a clear lag effect between sequential rainy seasons and oak mortality. This data set provides the opportunity to calculate the infection rate of Bay Laurels through the drought years of 2008 and 2009 into the wet conditions in 2010 and 2011, as well as the effect on Oak infection and mortality over this time frame. Additionally there are over 1500 isolates which have been genotyped and used to examine genetic diversity within the watershed, by building Minimum Spanning Networks which exemplify how genotypes present in the plot network are related to one another. By determining which genotypes are ancestral or novel and where they are located within the watershed we can monitor new introductions of the pathogen and natural disease progression within the region.

Population Dynamics of Aerial and Terrestrial Populations of *Phytophthora ramorum* in a California Watershed Under Different Climatic Conditions

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We present a study of the epidemiology of Sudden Oak Death (SOD) in California within a watershed based on temporally and spatially replicated surveys of symptoms, viability of the pathogen from symptomatic leaves, and genetic analyses using polymorphic SSR markers. Our study is one of the first to address the population dynamics of *Phytophthora ramorum* on a small scale in a relatively undisturbed area with minimal management. Intense sampling of soil and leaf populations was performed over a period of two years which spanned a climatic transition from drought in 2009 to a wetter climate in 2010. SOD symptoms on leaves of the transmissive host California bay laurel increased significantly from 15 to 39% in six survey plots between dry and wet conditions, while levels of identical symptoms caused by other foliar pathogen were highest (69%) in dry conditions, suggesting *P. ramorum* and other pathogens while occupying the same niche are favored by different climatic conditions. Although some foliar genotypes of *P. ramorum* were more abundant in wet than in dry conditions, a significant number of foliar genotypes were dominant and persistent through time. Soil and foliar populations were genetically distinct, but were not segregated in different portions of a minimum spanning network, suggesting intermixing of the two. We surmise that the genetic structure between substrates is not due to the presence of two distinct populations, but to the different ability of genotypes to adapt to the different substrates. In climatic conditions unfavorable to the pathogen genetic diversity appears to increase, and in favorable conditions fewer genotypes are more widespread. We also use spatial autocorrelation to show that foliar genotypes can spread further than soil genotypes in wet years, and that soil appears to be re-inoculated on a yearly basis.

Wildland Management

Novel Approaches to SOD Management in California Wildlands: A Case Study of Eradication and Collaboration in Redwood Valley

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In California, sudden oak death (SOD) treatment efforts have been localized, often targeting specific trees or properties. The widespread nature of SOD establishment and spread in California has mostly precluded use of broader eradication strategies, which are more applicable in isolated infestations like those in Oregon. However, the 2010 detection of a new infestation in Redwood Valley, CA – more than 50 miles from the nearest infestation, and the northernmost occurrence in the state – presented an opportunity for the first eradication effort in California. The infestation was isolated to a relatively small geographic area and was of high priority, effectively located at the gateway to Redwood National Park, Yurok and Hoopa tribal lands, Bureau of Land Management and USDA Forest Service lands, and the dense tanoak *(Notholithocarpus densiflorus)* forests of the Klamath watershed.

Given the nature of the Redwood Valley infestation and its proximity to important ecological and cultural landscapes, eradication was a reasonable strategy. However, the context for the project was complex, requiring careful collaboration from the beginning. The pathogen was initially detected through stream sampling near Orick in May 2010, many miles downstream of Redwood Valley and near the mouth of the 200,000 acre Redwood Creek watershed; only through extensive sampling and the targeted engagement of large landowners throughout the watershed was the source of the infestation narrowed to Redwood Valley. Even then, the infestation spanned a number of private properties, including small residential landholdings and large private timberlands, and necessitated the cooperation and commitment of diverse stakeholders. Likewise, the project required a prompt, creative funding strategy, and ultimately involved the support of the USDA Forest Service, the Natural Resource Conservation Service, CAL FIRE, the University of California, local contractors, and private landowners. The multi-tiered collaboration required by this project is unique for SOD management efforts in California, where treatments have previously been limited in size and scope.

The swift cooperation of landowners, funders, and other collaborators was fruitful, and crews broke ground in spring 2011 – less than 4 months after the infestation was narrowed definitively to Redwood Valley. The eradication effort focused on the complete removal of the two most infectious hosts, California bay laurel (*Umbellularia californica*) and tanoak, following the results of smaller adaptive management studies in California and Oregon. All trees and seedlings within 100 meters of known positives were removed, either by handcrews using chainsaws or by treatment with herbicides. Unfortunately, efforts were complicated by an unusually wet spring in 2011, which resulted in significant expansion of the SOD infestation and an increase in the area needing treatment. However, collaborators adapted to meet the changing needs of the project, and over 300 acres were treated by the end of the year. After contractors complete work on remaining areas this spring, the bulk of the infested area in Redwood Valley will be treated. Project monitoring will assess treatment efficacy and guide future efforts.

As this disease advances, we must develop new management approaches while gleaning fresh insight from old strategies. The Redwood Valley project, which blends a unique social and geographic context with a treatment strategy not yet used in California, lends the SOD community new tools and inspiration for disease response. It also highlights the increasing need for a comprehensive strategic response plan – one that could moderate the coordinating and funding challenges that were encountered in the Redwood Valley example and are likely to emerge in future cases.

Suppression of *Phytophthora ramorum* Infections Through Silvicultural Treatment in California's North Coast

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In 2006, three forested sites in Humboldt County infested with *P. ramorum* were subjected to different combinations of treatments designed to reduce inoculum and control spread. Treatments included the following: (1) cutting of California bay laurel (*Umbellularia californica*) and tanoak (*Notholithocarpus densiflorus)* trees, the primary sporulation-supporting hosts of *P. ramorum* in California's wildlands; (2) cutting of all bay and tanoak followed by broadcast burning to assist in reducing host saplings and seedlings; (3) the above treatments combined with removal of Pacific madrone saplings to assess the effect of removing this additional *P. ramorum* host; (4) removal of bay laurel alone by chainsaw; (5) girdling of bay laurel alone; and (6) treatment of bay laurel and tanoak by herbicide alone to kill the standing tree and control sprouting. Treatments have been monitored for 7 years, and results to date suggest that the treatments that involved the cutting of bay laurel and tanoak substantially reduced *P. ramorum* inoculum levels. However, in treatment areas where scattered bay trees were inadvertently missed because of the limited window for harvest operations owing to marbled murrelet (*Brachyramphus marmoratus*) nesting season restrictions, we have observed that a relatively minor component of residual bay laurel trees may have become infected following treatment and subsequently spread *P. ramorum* to regenerating bay and tanoak sprouts and seedlings. The data suggest that pathogen reestablishment was driven by both incomplete treatment application and spread from adjacent, but untreated stands. We will discuss how new infections relate to distance from these residual trees and other bay laurel trees just outside the treatment areas, as well as how altered canopy structure and microclimate induced by treatments contribute to their effectiveness.

Is Stump Sprout Control Necessary to Effectively Control *Phytophthora ramorum* in California's Wildlands?

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In California, wildland hosts that support sporulation of *P. ramorum*, such as California bay laurel (*Umbellularia californica*) and tanoak (*Notholithocarpus densiflorus*) also develop prolific basal sprouts following mortality, injury or tree harvest. Assessing long-term silvicultural treatment effectiveness for *P. ramorum* control is complicated by this stimulation of basal sprouting following tree removals. To better design *P. ramorum* treatments, we need to know how sprouts regenerating from cut host tree stumps are involved in local persistence of *P. ramorum*. These sprouts could act as reservoirs to maintain inoculum levels as forests regenerate and/or serve as points of re- reinvasion from vegetation surrounding the treatment area. This is a critical issue in future management, especially since many landowners are averse to using herbicides for sprout control.

Following host removal treatments of infested stands in California in 2004 and 2006, we observed that stump sprouts were pathogen-free for a period of several years, despite that inoculum sources were still known to be in the immediate vicinity. However, some of these stump sprouts in these treated stands did become infected after this period. This suggests either that younger sprouts are less likely to become infected, or that climate was perhaps not as suitable for the pathogen during the initial period of low recovery.

To help clarify these issues and determine whether manual removal of sprouts after host tree removal treatments is necessary to control pathogen persistence and reestablishment, in 2011 we created different sprout cohorts alongside each other in post-treatment areas. With results available to date, we will discuss whether the age of sprouts influence their disease susceptibility. We are also monitoring canopy structure and several microclimate variables at sprout locations, allowing us to model effects of these variables on sprout infection and determine which conditions best suppress reinvasion into treated stands. This new research informs our understanding of the relative roles that inoculum reduction, canopy structure, microclimate, sprout age, and interannual climate variation play in the success of silvicultural treatments targeted at *P. ramorum* suppression.

The Current State of Knowledge on Operational Sanitation Measures to Lower Risk of *Phytophthora ramorum* Spread and the Need for Further Study

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We are working to evaluate risks associated with human spread of the sudden oak death (SOD) pathogen, *Phytophthora ramorum*, to currently uninfected areas in California. Port Orford Cedar Root Disease (POC, caused by *Phytophthora lateralis*) has brought awareness that pathogens can be moved in forest settings by materials adherent to vehicles and equipment. Discovery of this human vector prompted new disease control measures with notable social and economic costs, such as road closures, vehicle and equipment washing requirements, and standards for sanitizing drafted water.

Despite knowledge that there are distinct biological differences between *P. ramorum* and *P. lateralis*, POC-derived sanitation measures have been routinely recommended for SOD. While we know that *P. lateralis* has spread largely via soil-borne inoculum, the most often implicated spread mechanisms for *P. ramorum* do not include this pathway. However, infested soil has been linked to *P. ramorum* spread and persistence in nursery settings, and an infested soil- leaf litter- California bay laurel seedling pathway has also been experimentally shown as viable. Some epidemiology research suggests past spread in California via recreational activities, and *P. ramorum* has been isolated from boots and bike tires at several locations. Still, we have little understanding of the potential of infested soil and plant debris to spread the pathogen in forests and urban-wildland interfaces.

Here, we discuss current knowledge on sanitation issues related to *P. ramorum* and highlight areas needing further study, with consideration to drawing appropriate parallels from research on other *Pytophthora* species. We examine the consistency among recommendations for SOD-related sanitation published in several current guides, and identify where better support and/or detail is needed. We'll also share our ideas for an integrated study that focuses on potential soil and debris vectors associated with timber and fire management or arboriculture operations—operations that may present high spread risk and also bear heavy costs in heavy equipment sanitation. The goal of this study is to identify sanitation measures that present the best combinations of application ease, cost effectiveness, and minimization of spread risks. We will share results of our preliminary research in 2012, completed during the Redwood Valley control projects.

Phytophthora ramorum Managing Strategies for Disease Control, Species Conservation, and Restoration at the Stand and Watershed Scales – Insights from Epidemiological and Ecological Models

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Phytophthora ramorum continues to challenge vegetation and ecosystems in the western USA, and has continued to spread to uninvaded areas of Northern California. In this context, two main action goals emerge: shorter-term management to reduce disease damage and pathogen spread, and longer-term management for species conservation and forest restoration. How to apply the existing management tools depends on the goal, the spatial scale of the outbreak (e.g., stand with single landowner, or watershed), and practical constraints. Disease management has been applied to localized outbreaks with some success in California and Oregon, and trials of treatments, such as host removal and host protection through phosphate application, have been done at plot and stand levels. However, there is still very limited observational data on the efficacy of these treatments, both at the individual-tree level and at the community and landscape scales. Clearly, there is a need to understand better which treatments to apply for specific forest conditions and local community resources in order to design management strategies with greatest and more durable impact and least expenditure.

We use parameterized mathematical models [1, 2] representing the spatial spread of the pathogen and the competing recruitment of tree species and changes in forest composition, to assess the efficacy of management strategies at two scales. First, we consider the stand scale, typically a single-landowner plot of a few hectares, where the goals are to manage disease and retain the tanoak population. Second, we consider the watershed scale, typically an area of tens of square kilometers, with the goals of containing the pathogen outbreak and implementing forest restoration through partial replacement of hosts with conifers. In the latter case we use the Mattole watershed landscape as an example. We consider three broad types of management strategies: removal of bay laurel (curative and pre-emptive), protection of tanoak at stand scale or replacement of hosts at watershed scale, and a mixed strategy. Generally, we found that the mixed strategy offers the greatest benefits, depending on the details of implementation. In the case of stand-level bay-laurel removal, the model suggests the additional application of herbicide can improve performance dramatically by preventing stump re-sprouting; alternatively, the application of follow-up treatments is the next best option. An important determinant of the choice of management strategy is the forest composition before pathogen invasion, particularly the prevalence of tanoak and bay laurel.

1. Cobb, R.C.; Filipe, J.A.N.; Meentemeyer, R.K.; Gilligan, C.A.; and Rizzo, D.M. 2012. Ecosystem transformation by emerging infectious disease: loss of large tan oak from California forests. J. Ecol. DOI: 10.1111/j.1365-2745.2012.01960.x.

2. Filipe, J.A.N.; Cobb, R.C.; Meentemeyer, R.K.; Lee, C.A.; Valachovic, Y.S.; Cook, A.R.; Rizzo, D.M.; and Gilligan, C.A. 2012. Landscape epidemiology and control of pathogens with cryptic and long-distance dispersal: sudden oak death in northern Californian forests. PLoS Comp. Biol. **8**(1): e1002328.

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

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Phytophthora ramorum, the cause of sudden oak death, was first discovered in Oregon forests in July 2001. An aggressive eradication treatment program was immediately put into place on all lands where it was found. Eradication treatments have changed over time as we have learned more about pathogen behavior. Treatment prescriptions currently consist of cutting and burning infected and exposed host plants, and where possible, injecting herbicide into tanoaks to prevent sprouting. The effort has slowed, but not stopped, long-distance dispersal of the pathogen.

To monitor the effectiveness of eradication treatments we are revisiting treated sites and sampling soil and vegetation in fixed plots centered on stumps of known infected trees. All samples are assayed for *P. ramorum* at Oregon State University and Oregon Department of Agriculture laboratories. We established 145 plots in 2008-2009 and 143 plots in 2010. 109 of these plots were visited in both time periods.

In the sample period 2008-2009, *P. ramorum* was not recovered from soil or vegetation on 74 (51 percent) of the 145 plots sampled. Forty-seven plots (32 percent) yielded *P. ramorum* from soils only. The pathogen was present in soil and vegetation on 18 plots (12.5 percent), and on six plots (4.5 percent), *P. ramorum* was recovered from vegetation only. In the 2010 sampling, *P. ramorum* was not recovered from soil or vegetation on 90 (63 percent) of the 143 plots sampled. Thirty-six plots (25 percent) yielded *P. ramorum* from soils only, on ten plots (7 percent) the pathogen was present in soil and vegetation, and on seven plots (5 percent), *P. ramorum* was recovered from vegetation only. All positive vegetation samples were from tanoak in the 2008-2009 sampling period; most of the diseased material was collected from tanoak basal sprouts. Two *P. ramorum*-positive samples of Oregon myrtle were collected in the 2010 monitoring effort along with infected tanoak sprouts.

Phytophthora ramorum was not detected either year on 42 (39 percent) of the plots visited twice. Soil was *P. ramorum*-positive both years on 24 (22 percent) of these plots. On seven plots sampled twice (6 percent), *P. ramorum*-positive vegetation was collected in both sampling years.

Analysis continues on these data. Of particular interest is how different components of the treatment prescriptions and/or abundance and composition of post-treatment vegetation affect pathogen survival and disease development. These data are also being used to inform 2012 sampling.

Effect of Phosphonate Treatments for SOD on Tanoaks in Naturally Infested Forests

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Application of phosphonate compounds has been shown to be an effective preventative treatment for Sudden Oak Death in Oaks (*Quercus agrifolia*) and Tanoaks (*Notholithocarpus densiflorus)*. To test the effectiveness of these treatments in a natural setting, paired 400m^2 sections of mixed evergreen tanoak stands were randomly designated as either treatment or control plots and topically treated with Agrifos Systemic Fungicide. The experiment included 36 field plots, in six California counties including nearly 700 tanoak trees <8cm dbh. Both tree canopy and trunk conditions were visually assessed and scored for overall health and the presence of SOD disease symptoms each fall from 2006 until the present. In the fall of 2009, five injection treatment plots, located near the existing plots, were added to the experiment.

Phosphonate treatments were found to affect both tree mortality and spore production. The treatment plots had significantly lower mortality as well as reduced numbers of infected trees. Likewise, production of *P. ramorum* spores was reduced in the treatment plots. Since tanoaks can serve as a source of inoculum, once a stand is infected it may be very difficult to prevent subsequent infection of the entire stand. In general phosphonate treatments do slow down the infection rate even if they don't completely prevent it. The individual characteristics of the experimental plots also had an effect on the results. Factors such as slope, gradient, and the direction of the disease spread substantially affected disease incidence and mortality. In two cases the experimental plots were established in areas that were already infected with SOD, significantly reducing the effectiveness of the treatments. In addition the results show that disease symptoms appear to advance in a punctuated rather than gradual fashion year to year.

Long-Term Monitoring of *P. ramorum* Inoculum Identifies Spatio-Temporal Patterns of Pathogen Sporulation and Proves that Selective Bay Removal Reduces Risk of Oak Infection

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In 2005, eight 50 x 50 m plots, all with a significant component of California bay laurel where selected in the Soquel demonstration forest. Each plot contained a 5 m buffer zone around the edges, and sixteen 10 x 10 m squares. A bucket was placed at the center of each square: buckets were filled with at least 5 l of water every 2-3 weeks, and five bay laurel leaves were placed as baits in each bucket for 2-3 week periods throughout the year (or when leaf infection was ascertained to occur). In a preliminary laboratory experiment, it was determined that infection of 4-5 bait leaves corresponded to an inoculum level of at least 104 sporangia, while infection of 1-3 leaves corresponded to inoculum levels at least one order of magnitude lower than 104. In 2007, all bays were eliminated from four treatment plots, while four control plots remained untreated. Baiting occurred all year round until 2009 and then only between February and July until July 2011. In the course of the experiment, a total of 240,000 leaves from 128 baiting buckets were inspected for infection. Results indicated that:

1) Bait leaves were only infected when temperatures were approaching 19 C and in the presence of rainfall.

2) Hotspots of infection (buckets with 4-5 leaves infected corresponding to 104 sporangia) were not constant but shifted in place, indicating source trees produced high levels of inoculum at different times, and only for a limited time period.

3) Bay removal significantly reduced overall inoculum.

4) When bays were removed inside the treatment plots, no hotspots were ever found 20 m from the edges of any plot.

5) When bays were removed, significantly less hotspots were found 10 m from the edges of each plot.

6) Bay removal never completed eliminated inoculum within treatment plots.

In 2010 and 2011, two oak inoculation experiments were performed. In both cases results showed that only suspensions of 104 sporangia could cause infection of oak stems, while inoculations using lower inoculum density were completely unsuccessful. We conclude that oak infection relies on warm temperatures and rainfall, and that source bay trees do not produce high levels of inoculum for long periods of time. Elimination of bay laurels 20 m around an oak will almost inevitably protect an oak from infection as inoculum density will never reach the required thresholds, however even a buffer of 10 m will significantly decrease the number of instances where the threshold of inoculum necessary for oak infection will be reached.

What are we Trying to Save?
Tanoak History, Values, and Ecology

Tanoak Dreamtime: Safeguarding a Native Nut Tree

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Despite over a century of conservation efforts and scientific forest management, a keystone nut tree common to California and southwestern Oregon is threatened by the 1995 introduction of an exotic disease. This is after decades of over harvesting of bark for industrial tanning beginning with American settlement, then of a full-scale tanoak eradication campaign by the mid-1900s. In addition to herbicide use, twentieth century fire suppression favored conifers over tanoak. This talk explores the limits and failures of governmental regulation to reverse devastating assaults on tanoak by examining the interplay of economic, ecological, and cultural factors that informed use and abuse. Concerned citizens are acting to safeguard these magnificent trees.

Tanoak as a Forest Product Resource: Past, Present, and Future

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Tanoak (*Notholithocarpus densiflorus*) has a reputation as a "difficult to work with" hardwood that has been viewed at different times in history as everything from a valuable resource for edible acorns to an annoying "weed" tree that interferes with commercial forestry management. This paper explores the complex character of the species from a wood products point of view and discusses the possibility of developing it as a valuable forest products resource.

A comprehensive review of the forest product literature reveals the mention of many uses of tanoak including: a source for leather tanning chemicals, fuel wood, lumber, railroad ties, flooring, and furniture. Early studies of the physical and mechanical properties of the wood show a similarity to many known commercial hardwood species. However, tanoak has never gained the status of a preferred timber tree for forest products. This paper reports on the cellular structure, wood density, strength, stiffness, permeability, treatability, and how the wood dries. Recommendations are provided for successful lumber manufacturing, including milling and drying. The potential value of tanoak in a wide variety of products and also as a feedstock for biochemicals and biofuels are examined. Finally the risks and benefits of utilizing a species that is a known host for *Phytophthora ramorum* are discussed from a forest management point of view. As interest grows in developing local resources that require little transportation from source to end use more opportunities for utilizing tanoak will likely surface.

The Geographic Range of Tanoak and the Effects of Interacting Disturbances on the Spatial Distribution and Structure of Tanoak Communities

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The broad geographic range of tanoak encompasses tremendous physiographic variability, diverse plant communities, and a complex set of ever-changing disturbance regimes (e.g. development, timber harvest, and wildfire), which now also includes serious threats posed by *Phytophthora ramorum*. Understanding how these factors - and the complex interactions between them - shape the spatial distribution and structure of tanoak communities is critical to developing comprehensive strategies for the conservation of tanoak populations.

We analyze two questions: 1. How do interactions between the abiotic environment (e.g. soil, climate), community composition, and disturbance influence the distribution and structure of tanoak communities? and 2. How do the ecological conditions produced by these relationships, in turn, influence risk of infection by *P. ramorum* across the geographic range of tanoak?

We assembled a large dataset for tanoak and its associated biotic communities by combining multiple networks of sudden oak death monitoring plots (n = 997) with geographically extensive FIA plot data (n = 1851) located throughout tanoak's known range in California and Oregon. Using path analysis, we construct predictive models of hypothesized causal relationships between multiple variables in the system with their direct and indirect effects on tanoak distribution and infection risk separately parameterized. These models are also applied to quantify impacts of *P. ramorum* on tanoak populations across its geographic range. Epidemiological studies that incorporate the role of interacting disturbances are rare but may increase understanding of disease dynamics and improve our ability to manage invasive forest pathogens.

Population Genetic Studies of Tanoak: An Overview of Current Knowledge and its Applications to Conservation and Restoration

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As for many California woodland species, until recently nothing was known of the genetic diversity, extent of gene flow and degree of divergence among lineages of tanoak. The catastrophic mortality caused by sudden oak death, raised questions concerning the potential for variations in degree of tolerance of the disease and resources suitable for restoration of populations that have been severely affected by the disease. Some of these questions have been addressed over the last few years by developing genetic marker systems and using them to study the genetic diversity of tanoak ranging from the level of individuals (predominant mating systems), through single populations (clonal and spatial genetic structure) to the landscape level (differentiation among populations, or regional lineages). I summarize this information here and discuss: 1) some of the implications that can be drawn for inferring ideal sampling strategies for detecting resistance/tolerance to SOD and, 2) the implications of this genetic diversity and divergence for the selection of resources for current conservation priorities and future restoration strategies.

Insect Visitors to Tanoak Flowers: An Undocumented Casualty of Sudden Oak Death?

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Because of their close association with true oaks, tanoaks are traditionally labeled as “wind pollinated”. However, this has never been tested. In contrast, it is possible in many California farmers’ markets to purchase “tanoak honey”- a concept that is incompatible with the theory of wind pollination. To establish the relationship between pollen and nectar gathering insects and tanoak flowers, a multi-year project has been initiated to observe tanoak flowers and record and identify the insects that visit them. Two years and over 100 hours of observations by citizen scientists has established that a number of insects do indeed visit tanoak flowers including bees, beetles and ants. Ongoing work is aiming at identifying these insects to species, as well as working to establish the importance of tanoak pollen and honey in their diet, and thus to understand the potential impact of sudden oak death on these insect populations.

Using Genomics to Study Tanoak's Past, Present, and Future

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As part of the Western Forest Transcriptome Survey, we used Massively Parallel Sequencing to sequence the transcriptome of tanoak (*Notholithocarpus densiflorus*) alone, and after infection by *Phytophthora ramorum*. We analyzed 43.5 Mbp of sequence from 4 experimental conditions, of which 18.8 Mbp mapped to the *P. ramorum* genome sequence. The availability of these transcriptome assemblies expands the utility of genomic tools to this species by providing a framework for expression and evolutionary analsysis. We discuss our findings vis-a-vis the host-pathogen interaction – through sequences the characterization of the active *P. ramorum* genes, as well as the 844 genes differentially expressed in infected tanoaks – as well as regarding tanoaks' relationships to other Fagaceae, and the myriad paths made available by the 'democratization of genomics.'

A Conservation Strategy for Tanoak to Protect Against Sudden Oak Death

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Widespread and rapid declines of trees by introduced pathogens have been a repeated event in the recent history of North American forests. The resulting diseases have altered forest composition, structure, and biodiversity at regional scales, especially in the eastern US where diseases, such as chestnut blight, have impacted forests at regional scales. The introduction of the sudden oak death pathogen, *Phytophthora ramorum* has caused widespread mortality of tanoak (*Notholithocarpus densiflorus*) and elicited a region-scale decline of an important overstory tree along the central California coast and in Southwest Oregon (Curry County). In the United States, tanoak is the most susceptible of all *P. ramorum* hosts with leaves, twigs and boles all subject to damage. The pathogen reproduces on tanoak leaves and twigs, so when conditions are favorable local epidemics spread rapidly and can infect almost all tanoak in a stand. These disease characteristics have led to amounts of mortality greater than 50% in some areas that continue to increase; millions of trees have died. In the Mt. Tamalpais Watershed (Marin County) and in Big Sur (Monterey County), type conversions have occurred with tanoak no longer a primary stand component. The disease reduces average tanoak tree size and can remove tanoak from the overstory. Tanoak is predicted to persist in many disease-impacted forests via sprout reproduction, but large trees will be increasingly less common or eliminated, a pattern similar to other exotic forest diseases.

Various strategies have been deployed to protect tanoak including: tanoak and bay laurel removal; Agrifos® preventive treatment, formation of a landscape barrier zone, hot spot treatment and eradication, and quarantines. While many of these actions are somewhat effective, none of the approaches has succeeded in stopping the sudden oak death pathogen from spreading and killing trees. Although the disease has been very destructive, the greatest concentration of tanoak populations occurs in Humboldt and Del Norte counties (CA) where the disease is less widespread. A rare opportunity exists to address a region-wide tree decline with a compressive, though incomplete, body of knowledge about the disease. In this talk we synthesize management techniques for a comprehensive tanoak conservation strategy and identify where additional work is needed, particularly in the development of resistant trees and improvement of treatment options for trees in urban and wildland settings.

There are an estimated 1.8 billion *N. densiflorus* in California and Oregon which comprise tanoak's native range. Tanoak is highly valued for cultural and biodiversity reasons, but the forest industry often sees this species as a competitor to more profitable conifer timber. The abundance of tanoak and its broad distribution provides abundant habitat for the pathogen but also creates opportunities for conservation by identifying stands with characteristics most conducive to retention of tanoak in the overstory. By utilizing pathogen risk models, healthy tanoak stands at very low risk for disease development can be prioritized for conservation and protected from development, especially when these techniques can be applied in tanoak populations with higher levels of resistance. Conservation of tanoak, and thus the reduction of disease impacts will be most successful with conservation planning using the knowledge gained over the past decade while research continues to develop protective treatments for tanoak and other forest trees threatened by this exotic, invasive pathogen.

Poster Abstracts

(Alphabetically by author's last name)

The Novel Interaction Between *Phytophthora ramorum* and Wildfire Elicits Elevated Ambrosia Beetle Landing Rates on Tanoak, *Notholithocarpus densiflorus*

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Several species of ambrosia and oak bark beetles (Coleoptera: Scolytidae) are preferentially attracted to *Phytophthora ramorum*-infected coast live oaks (*Quercus agrifolia)*, and these beetle attacks can greatly reduce the survival time of previously infected trees. While bark beetle attacks on burned trees in coniferous forests are well-documented, very little is known about the attraction of scolytids to burned hardwood trees in the coastal forests of California. The 2008 wildfires in the *P. ramorum*-infested forests of Big Sur provided the rare opportunity to study the interactions between wildfire, an invasive forest pathogen, and associated scolytids. In this study, we measured the landing rate of these beetles on tanoak, *Notholithocarpus densiflorus*, the predominant species impacted by *P. ramorum* in the Big Sur region, to determine if two forest disturbances, *P. ramorum* and fire, interact to create an increased attraction to scolytids in coastal California forests.

To evaluate landing rates, beetles were sampled from forest plots in the Big Sur region during the fall of 2009 and the spring of 2010, approximately 1 and 1.5 yr, respectively, following the wildfires. Within each plot, the presence or absence of both *P. ramorum* and fire disturbance were paired so that a fully crossed two-factor design was achieved. This yielded four disturbance treatment combinations: 1) *P. ramorum* and fire disturbance absent = *no disturbance*, 2) *P. ramorum* disturbance present and fire disturbance absent = *P. ramorum disturbance*, 3) *P. ramorum* disturbance absent and fire disturbance present = *fire disturbance*, and 4) *P. ramorum* and fire disturbance present = *mixed disturbance*. The complete design included three replicates (plots) per disturbance treatment type for a total of 12 plots, and beetles were sampled by using 14 x 20 cm yellow sticky cards attached to three tanoaks per plot. Following the quantification of beetles trapped per plot, a two-factor analysis of variance (ANOVA) was used to compare the effect of *P. ramorum* and fire disturbance on scolytid landing rates, as well as the effect of the interaction between the two disturbances on landing rates.

The vast majority of scolytids were trapped on tanoaks in *mixed disturbance* plots --81% in 2009 and 79% in 2010 -- and ambrosia beetles were the most abundant of the scolytids trapped. In 2009, the year in which 75% of the total scolytids were trapped, fire and *P. ramorum* disturbance were each significant effects in the ANOVA model, but the interaction effect was not significant. In 2010, fire disturbance was a significant effect, but neither *P. ramorum* disturbance nor the interaction effects were significant. While the landing rates of ambrosia beetles are not necessarily equivalent to their actual rates of colonization, increased landing rates on tanoaks in the plots with multiple disturbances suggest that tanoaks in those areas were particularly attractive to ambrosia beetles. We hypothesize that specific host volatiles may have attracted ambrosia beetles to specific tanoaks. Furthermore, greater quantities of moribund and recently-killed trees in forests affected by both disturbances likely led to greater population densities of ambrosia beetles in those areas.

New Technologies to Detect and Monitor *Phytophthora ramorum* in Plant, Soil, and Water Samples

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The focus of our research efforts have been to develop methods to quickly indentify plants, soil, and water samples infested with *Phytophthora* species and to rapidly confirm the findings using novel isothermal DNA technologies suitable for field use. These efforts have lead to development of a rapid Immunostrip® that reliably detects virtually all strains of *Phytophthora* in plant samples, soil, and water samples within minutes (data will be presented) after which a rapid molecular method was developed to accurately confirm the results (data will be presented). This detection paradigm allows for accurate monitoring of the location and spread of *Phytophthora ramorum* giving field officers the necessary tools required for mitigation actions with confidence.

Episodic Abiotic Stress and *Phytophthora ramorum* Blight in Rhododendron: Impacts on Root Infection, Symptom Expression and Chemical Management

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Of concern for disease management and certification programs in nursery ornamentals is that roots, when colonized by *Phytophthora ramorum*, may serve as a potential reservoir of inoculum. An additional complication is that the above ground portion of plants with root infections may be asymptomatic. Our central hypothesis is that mild abiotic stresses can compromise basic host resistance to trigger systemic development of disease from soilborne infections. Corollary to this is that these stresses may also influence the efficacy of chemical management.

Growth chamber and outdoor nursery experiments examined the influence of abiotic stresses on root infection and systemic disease development. Three *P. ramorum* susceptible cultivars of *Rhododendron* sp. – 'Cunningham's White', 'Gomer Waterer', and 'Roseum Elegans' – were examined for their responses to chilling, water logging, water deficit, or salinity. For growth chamber experiments, standard conditions were a 16 h photoperiod with a temperature cycle of 22°C (day) and 15°C (night). For chilling experiments, inoculated plants were transferred to a 20°C /4°C day/night temperature regime (12 h cycle), 16 h photoperiod for 5 days, followed by return to standard growth conditions until evaluation. Water logging, water deficit, and salinity were imposed by standard or previously published methods. In addition, three *P. ramorum* isolates, all from Marin County, CA, were used. An outdoor trial also was established in June 2011 in the nursery at the NORS-DUC facility in San Rafael, CA. In this trial, the interaction of salt and fungicide treatments (Subdue Maxx® or Aliette®) was examined in relation to root colonization and ramorum blight development. Plants were inoculated by adding a *P. ramorum*-infested V-8 broth/vermiculite-medium to the soil. Six months after initiation, plants were evaluated for symptom expression and root samples were collected and plated on PARPH medium for detection of *P. ramorum.*

There was no evidence for disease predisposition in Rhododendron by chilling. In contrast, brief episodes of salt or drought stress predisposed plants to enhance subsequent ramorum blight development, although this effect varied with isolate and appeared to be related to differences in virulence as determined by lesion development on Rhododendron leaves. In the nursery, Subdue Maxx and Aliette partially suppressed *P. ramorum* root colonization, even in plants experiencing an episode of salt stress. However, in all treatments, roots were heavily colonized, although plants appeared healthy on visual inspection and did not show any above-ground symptoms of ramorum blight. These results indicate that Rhododendron plants can sustain extensive and perhaps prolonged colonization of roots by some strains of *P. ramorum* without apparent stem or foliar symptoms, and raise concerns about the adequacy of current practices for monitoring *P. ramorum* in the nursery.

Factors Influencing *Phytophthora ramorum* Infectivity on *Umbellularia californica* and Testing of a Defoliation-Based Control Method

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The primary foliar host for *Phytophthora ramorum* is *Umbellularia californica* (bay laurel), a tree species that serves as a reservoir for infections in California woodlands. We are investigating environmental and pathogen-mediated influences on the incidence and severity of *P. ramorum* infection of *U. californica* as well as developing non-destructive means for controlling *P. ramorum* in woodlands.

The distribution and abundance of *P. ramorum* in California is typically assessed by counting symptomatic hosts and confirmed by culturing the pathogen from field-collected samples. We hypothesize that the probability of a successful culture depends on the local environmental conditions where the field samples are collected. In 2010, an extensive culturing study was conducted within a previously established plot network in Sonoma County, where *P. ramorum* has been studied since 2003. We collected symptomatic leaf tissue for 424 trees in 153 plots randomly distributed within a 275 sq km region. *P. ramorum* was successfully cultured from 138 trees (32.5%) collected from 71 plots (46.4%). Culture success was greatest in the southwest portion of the study area and lowest in the northeast. Culture success was positively related to topographic moisture index and field count of symptomatic leaves at the site and negatively related to average maximum temperature at the site. These data show that culture success could be used as an indicator of inoculum load and they suggest that caution should be used in interpreting failure to culture from field samples.

Additionally, we are developing a live plant model to assess the validity of the commonly used detached leaf method for predicting interactions that occur between *P. ramorum* and foliar hosts. Specifically, we assessed infectivity of detached leaves and attached leaves from the same *U. californica* trees in a growth chamber and compared this to infectivity of detached leaves in an incubator. After seven days, lesions were scored. Preliminary results suggest that the detached leaf assay is a good indicator of infectivity of live trees.

Finally, we are examining controlled defoliation as an alternative to the current practice of managing *P. ramorum* through the destruction and removal of *U. californica* trees and within fifteen to thirty meters of symptomatic plants. Twenty-four *U. californica* seedlings were placed in six exclosures under infected canopies in Fairfield Osborn Preserve in February 2011. Two trees in each exclosure were sprayed in May 2011 with Ethephon, which releases ethylene upon decomposition, thereby inducing leaf abscission. Lesions were counted in May prior to treatment and post treatment in August 2011. Leaves on the trees that received treatment developed significantly fewer lesions compared to the control group.

Methods for Assessing *Phytophthora ramorum* Chlamydospore Germination

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Chlamydospores of *Phytophthora ramorum* are believed to contribute to the long-term survival of this pathogen. Understanding the factors that influence chlamydospore germination is necessary for determining their role in pathogen biology and epidemiology. Germination is difficult to accurately assess when chlamydospores are attached to remnants of supporting hyphae. We developed two approaches for closely observing chlamydospores and rigorously quantifying the frequency of germination *in vitro*.

The first method utilized a combination of time-lapse photography and microscopy. Chlamydospore preparations were grown on microscope slides dipped in CMA PAR agar. Slides were observed at 12-hr intervals for 36 hrs with brightfield microscopy. Using this method it was easy to distinguish emerging germ tubes from subtending hyphae, allowing us to accurately assess germination.

The second method for assessing chlamydospores germination was based on visual observation and marking of individual chlamydospores on agar media in Petri plates. A method combining blending, sonication, and filtration of inoculum grown in liquid culture was developed to separate chlamydospores from hyphae of *Phytophthora ramorum*. The resulting 'clean' chlamydospores were then mixed with a 0.2% agar solution. An aliquot of this was spread as evenly as possible with a glass rod onto a PARP plate. Using a fine tipped marker under a dissecting scope, a small dot was made next to each separate chlamydospore. The plates were scanned on a flat bed scanner and saved as jpeg files. The open source image processing package FIJI (an ImageJ package) was used to count the marked chlamydospores. Plates were checked for germination at 3, 5, and 7 days after inoculation. Only colonies originating from marked chlamydospores were counted and labeled with a different color pen for each date. Each time the plates were scanned and assessed with FIJI. By using this method, we could be certain that the colony growth we observed was only from germinating chlamydospores, and not from hyphal fragments.

This method was used successfully to study how passage through a banana slug affected germination of *P. ramorum* chlamydospores. We found that our control plates had an average chlamydospore germination incidence of 12%, while after being eaten by slugs the average chlamydospores germination incidence was 23%. Germination frequencies of approximately 5% were observed with the time lapse photography. These rates are considerably lower than the 40 to 47% reported by Tooley et al. (2008).

Biological Control of Tanoak and Bay Laurel Resprouts Using the Fungus *Chondrostereum purpureum*

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In southwest Oregon, an aggressive program of cutting and burning host plants in an effort to eradicate *P. ramorum* was instigated. It was soon apparent that tanoak resprouts were highly susceptible to *P. ramorum* and that infected sprouts hamper eradication efforts by maintaining inoculum on site. In Fall 2009, our research team established field trials near Brookings, Oregon to assess the bioherbicidal efficacy of the fungus *Chondrostereum purpureum* on tanoak to inhibit resprouts. Treatment of stumps with *C. purpureum* has been shown to be effective for suppression of resprouting on several species, most notably red alder (*Alnus rubra*). Chontrol™ is not registered for use in California, so indigenous isolates of *C. purpureum* are being obtained and formulated for use on bay laurel in this state.

Laboratory testing of three California isolates of *C. purpureum* indicate that the fungus can colonize bay laurel stems, and further testing under natural conditions is underway. Early results of field testing showed that *C. purpureum* was able to colonize the stumps of tanoak following treatment. *C. purpureum* was found occurring naturally on tanoak logs and stumps at some other sites we visited in Brookings. Chontrol™ formulations appear to have some effect on reducing resprouting in tanoak, but the most effective and rapid treatment is the hack and squirt method of applying the herbicide imazapyr. Over time, applications of Chontrol™ may be a more permanent solution as the stumps become decayed.

If a formulated product of *C. purpureum* and/or its mixture with other stem and wood decay fungi applied to tanoak and bay laurel does inhibit the trees from growing new sprouts, this *P. ramorum* inoculum reservoir would be reduced or eliminated in the ecosystem. In areas where the application of herbicides is not prudent or permitted, this biocontrol treatment would be an indispensable alternative to chemical herbicides.

Forest Succession Following Wildfire and the Sudden Oak Death Epidemic

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The Big Sur region of California's central coast has had four fires greater than 20,000 ha (49,400 ac) since 1950. However, to what extent fire shapes the ecosystems there and how they respond to such disturbances is not very well understood. Lightning-caused wildfires returned to the Big Sur region in 2008 and engulfed areas that were severely affected by *Phytophthora ramorum*, the pathogen responsible for Sudden Oak Death (SOD). *Phytophthora ramorum* can dramatically increase the amount of dead woody fuels in these forests, exacerbating fire severity. The 2008 wildfires presented the opportunity to study the interaction between SOD and fire severity and the response of Big Sur's forest to these disturbances.

We established 280 long-term ecological monitoring plots throughout the Big Sur region in 2006 and 2007. The plots are circular, 500-m^2, and were randomly distributed across the landscape. All stems 1-cm dbh and greater were identified, measured, mapped and assessed for health condition. We also quantified the number and identity of regenerating seedlings and saplings, and the percent coverage of all species in the plot. One hundred twenty two (45%) of our plots burned in 2008. We returned to 84 plots in 2009 to capture the immediate response to fire, including disease progression, mortality rates and recruitment. In 2010 and 2011 we did an extensive resampling in 20 redwood-tanoak plots and 25 mixed-evergreen plots.

The data collected documents how SOD and the 2008 fires have dramatically altered the ecosystem where our plot network was established. We have quantified both live and dead basal area shifts over the years surrounding two major interacting disturbances - Sudden Oak Death and wildfire. Recruitment of new stems mainly from basal sprouts has been impressive in the burn area. Seedling totals in 2009, especially of redwood seedlings, were often orders of magnitude greater than before the fire. The diversity of species within the plots has rapidly changed from before the fire, immediately following the fire to 3 years post fire.

The fortune of having pre- and post-fire data for a large, natural wildfire has provided an opportunity to simultaneously learn about Sudden Oak Death and forest succession in a little studied, wildfire-prone ecosystem. Our data offer a glimpse into interacting disturbances across a landscape in transition.

Recovery Incidence of *Phytophthora ramorum* from *Rhododendron* Leaf Inoculum

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The quarantine pathogen *Phytophthora ramorum* continues to be detected in ornamental nurseries each year. Recurrent infestations can occur when healthy container plants are set on ground that harbors infested plant debris and chlamydospores of the pathogen. *P. ramorum* in litter has been reported to remain infectious for long time. However, the amount of time that leaf inoculum remains infectious has not been clarified and it is important to understand this for predicting risks to nurseries and for preparing inoculum suitable for field studies of *P. ramorum* control. The objective of this study is to determine the recovery incidence of *P. ramorum* from leaves infected for different periods of time.

P. ramorum (isolate 4581) was grown on 1/3 V8 agar for at least 10 days. Agar disks from the colony edge were placed on freshly detached rhododendron 'Catawbiensis Boursault' leaves wounded with a needle. In the first experiment, leaves were incubated for four different durations with 4 replicates per time period. Ten leaf disks were cut from the edges and middle of each necrotic lesion and transferred onto PAR-PH media. Recovery from each leaf disk was recorded after 3 weeks at 20°C. In the second experiment, the enlargement of necrotic lesions was recorded every 5 days and leaf sections from different lesion ages were compared after 30 days. Recovery incidence was recorded as above. In both experiments, leaf sections were microscopically observed after clearing and staining leaves.

In the first experiment, the *P. ramorum* recovery incidence from leaf disks derived from the middle of the lesions gradually decreased from 100% for 20-day-old disks to less than 10% for 80-day-old disks. In contrast, recovery incidence from disks removed from the lesion edge was nearly 100% for lesions up to 30 days old, and recovery incidence was 50% for 80-day-old disks. In the second experiment, recovery incidence was almost 100% for all leaf sections except for those nearest the needle wounds which showed lower recovery rates. Leaves were usually completely necrotic around 40 days; therefore, lesion edge disks after 40 days were cut from leaf edges. Sporangia and chlamydospore production changed during lesion expansion. These results suggest that lesion age affects the recoverability of *P. ramorum* because of differences in the development and viability of hyphae, sporangia, and chlamydospores.

Reducing the Spread of *Phytophthora ramorum* on the Redwood Nature Trail, Rogue River-Siskiyou National Forest, Curry County Oregon: A Case Study

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In late August 2009, an eight-inch diameter tanoak adjacent to a popular hiking trail on the Rogue River-Siskiyou National Forest was found infected by *Phytophthora ramorum*. The trail was immediately closed to the public. An eradication treatment consisting of injected herbicide and cutting, piling, and burning tanoaks and other selected hosts in a 300-foot radius around the infected tanoak was prescribed and completed by early winter.

Close to 1600 feet of trail lies within or on the boundary of the treatment area while approximately 200 feet of trail pass through the heart of the infested zone. Knowing the potential for *P. ramorum* to persist in soils after treatment, options to reduce the risk of human-assisted spread of the pathogen via infested trail soil were discussed. Closing the trail permanently was not considered a viable option. A previous study suggested that, due to their antimicrobial activity, western redcedar heartwood chips placed on trails could help limit the number of *P. ramorum* spores in soils and the potential for new infections from splash dispersal. As a result, a 4-inch thick layer of western redcedar heartwood chips was placed on the trailhead and through the center of the treated area. The trail was reopened to public use after the chip treatment was completed.

In October 2009, after herbicide treatment, but prior to cutting and burning, soil samples were collected at 11 locations on the trail in the vicinity of the infected tree and near the trailhead. Samples were collected at these same locations in February 2010 after the eradication treatment was completed and again in May and July 2010. The western redcedar heartwood chips were applied immediately after the July soil collection. Soil samples were collected again in November 2010, and in March and June 2011.

Soil samples from the trail surface were wetted and baited for *P. ramorum* at Oregon State University. *Phytophthora ramorum* was recovered from at least one of the 11 samples on all occasions except July 2010 and June 2011. The number of *P. ramorum*-positive soil samples from each date tested declined from 2/11, 5/11, and 6/11 before-chip treatment to just 1/11 and 1/11 samples after-chip treatment. All *P. ramorum*-positive samples were found within approximately 25 feet from the infected tree. The majority of positive soil samples were collected within what would have been the dripline of the infected tree or "down trail" from there.

Phytophthora ramorum's presence in trail soil appears to have been reduced in the year after chip treatment. Recently, additional *P. ramorum* infections have been detected near the trail and due to use, wood chip depth has also been greatly reduced, particularly where the trail narrows on steep side slopes. Additional treatments are being discussed and monitoring will continue.

Host and Habitat Index for *Phytophthora* Species in Oregon

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Phytophthora species are known as pathogens of agricultural crops or invasive pathogens destroying forests, and their prominent inclusion in various host-pathogen indices reflects this importance. It is increasingly evident, however, that *Phytophthora* species are abundant in streams in healthy forests and widespread in forest soils causing cryptic diseases, in addition to their more traditional roles as aggressive pathogens. While their ecology in non-agricultural ecosystems remains poorly understood, we now know that a numerous and diverse, nutritionally complex community of *Phytophthora* species is present in a variety of associations with forests and forest trees.

We compile existing records from all available sources of reliably identified *Phytophthora* species from forests and forest trees in Oregon, USA. The results are summarized by host and habitat. Details of specifically documented isolates, including locations and available cultures and Genbank acquisitions, as well as citations are included in the interactive paper available at ForestPhytophthora.org. We have included isolations from soil and streams in forests that are often not associated with any specific disease symptoms. Our goal is to inventory forest *Phytophthora* species, not forest diseases. On the other hand, we have included records from forest trees growing outside the forest, as Christmas trees or in the urban landscape, for example. The result is, we hope, a more accurate representation of the ecological amplitude of *Phytophthora* and a more complete record of the sources from which they may be spread.

We have attempted to compile all reliable records for this report from all sources. Most records are from three large programs or projects: the OSU Plant Disease Clinic database; the Sudden Oak Death diagnostic program; and an ongoing survey of the *Phytophthora* species associated with declining alder trees along streams in western Oregon. In addition, there are many reports from systematic surveys of *Phytophthora* species in forest tree nurseries and Christmas tree plantations. All records are based on isolations in culture, and identifications of all problematic species were confirmed with molecular sequencing methods. Older records of species that lack distinctive morphology are not included unless they have been confirmed by recent sequencing.

Thirty four *Phytophthora* species, including described but not formally named taxa, have been identified associated with 25 host species from Oregon forests or forest trees. This total includes 17 species recovered from streams, and 18 from forest soils, generally in the absence of noticeable disease on associated vegetation. The sampling that produced these lists is not systematic, however, and is very uneven. Only in tanoak forests of Curry county have the full range of habitats been sampled. Two large studies have focused on forest stream sampling, so the list from that habitat is perhaps most complete, but this work still covers only a small portion of the state. In contrast there are relatively few records of *Phytophthora* associated with root rot or bole cankers of trees in the forest apart from the invasive *P. ramorum* and *P. lateralis*. This reflects the relative health of Oregon forests despite the potential susceptibility of the trees evident from nurseries and Christmas tree plantations.

Scaling up from Greenhouse to Field Resistance in Tanoaks

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Sudden oak death has had a devastating impact on tanoaks (*Notholithocarpus densiflorus*) in California and Oregon. Tanoaks are a key component of the forests in which they are endemic, as well as one of the few species that are both killed by *P. ramorum* and contribute to its spread. Tanoaks were little studied prior to the onset of the sudden oak death epidemic, resulting in few intellectual or material resources on which to base a disease resistance research program.

Since 2006, a comprehensive research program centered on a common garden of open-pollinated seed families has aided the understanding of the role resistance might play in the disease dynamics and management of tanoak populations. Seedlings have been assessed for growth and resistance traits in the nursery, and have been transplanted into an infested field site in order to assess performance under natural disease pressures. Combined analysis of data from the three settings demonstrates the utility of disease resistance measured in year 2 nursery assays for predicting high fitness in the field, especially in combination with low to moderate growth over 3 years in the nursery. The resistance screens, the first understanding of how resistance and growth traits in the nursery interact to influence fitness in a natural setting, our expanded screening of resistance from seedlings originating across the geographic range, and the seed families we developed promise to be useful tools in the conservation of western US forests.

Ethanol Attracts Scolytid Beetles to *Phytophthora ramorum* Cankers on Coast Live Oak

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Successful infection of coast live oak, *Quercus agrifolia*, stems by *Phytophthora ramorum* results in the formation of a canker visible initially at the bark surface by the release of a dark red to black colored exudate referred to as "bleeding." Bark and ambrosia beetles are often attracted to diseased trees within the first year after bleeding cankers appear and bore their gallery entrance holes almost exclusively within the canker boundaries, suggesting the presence of a primary attractant. These attacks accelerate tree mortality. Ethanol concentrations were analyzed in sapwood samples collected from paired diseased and healthy trees at three study sites in California. Samples from diseased trees were taken inside and outside of the boundaries of small spot cankers and larger cankers at the stem base. Trees with large basal cankers contained 4.3 times more sapwood ethanol than trees with spot cankers. Sapwood from within cankers had the highest concentrations with 4.3 and 15.5 times more ethanol than sapwood from 1 cm or 15 to 30 cm outside the canker boundary, respectively. Paired healthy trees had the lowest sapwood ethanol levels.

Insect traps were installed at all three sites and baited with ethanol, ethanol+α-pinene, or ethanol+4-allylanisole lures. The most abundant beetle captured across all sites was the non-scolytid lead cable borer, *Scobicia declivis.* The most abundant scolytids captured were *Xyleborinus saxesenii*, *Pseudopityophthorus pubipennis*, and *Monarthrum scutellare*, with considerable variation among sites. These species have all been trapped previously on *Q. agrifolia* inoculated with *P. ramorum*. Traps baited with ethanol only captured significantly more beetles of each species in most instances.

In another experiment, a 50% aqueous ethanol solution was sealed in the sapwood of *Q. agrifolia* logs. The bark surface immediately above the ethanol infused sapwood then received one of six treatments: 1) sprayed with an antitranspirant (Moisturin®) solution to block ethanol release, 2) sprayed with (-)-α-pinene, 3) attached an ultrahigh release pouch of (-)-α-pinene, 4) sprayed with both antitranspirant and (-)-α-pinene, 5) sprayed with antitranspirant plus attached a ultrahigh release pouch of (-)-α-pinene, and 6) no treatment control. The bolts were placed at two of the study sites for eight weeks (26 May-21 July, 2011), and then the number of combined bark and ambrosia beetle attacks were counted within the area of treated bark. In control logs, the number of beetles attacking the bark above the ethanol infused sapwood was 4.4 times greater than on the opposite side of the log where ethanol was absent in the sapwood. The attachment of an ultrahigh release pouch of (-)-α-pinene was the only treatment on the bark surface that impacted the beetles, reducing their densities to 18.4% of the attacks on logs without these pouches.

Elevated ethanol concentrations in *P. ramorum* cankers on *Q. agrifolia* and the attraction of bark and ambrosia beetles commonly associated with these cankers to traps or logs baited with ethanol provides strong evidence that ethanol is the primary attractant for these insects.

Melt Curve Analysis for Determining Mitochondrial Haplotype in *Phytophthora ramorum* and Correlations with SSR Genotypes

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With a clonally reproducing species such as *P. ramorum* the ability to monitor mitochondrial haplotypes would provide an additional tool for analysis of population structures and monitoring pathogen movement. Having a technique that could assess this mitochondrial background that was both accurate and rapid would enhance the utility of this type of analysis.

Sequence comparison of the mitochondrial genomes of a NA-1 and EU-1 genotype of *P. ramorum* identified seven regions containing single nucleotide polymorphisms (SNPs) that could be used to evaluate mitochondrial haplotypes. Sequence analysis of these regions for a range of isolates, including NA-2 isolates, identified 28 polymorphic loci that grouped NA-1, NA-2 and EU-1 isolates into 4 mitochondrial haplotypes corresponding to genotype with NA-1 isolates represented by 2 haplotypes, IIa and IIb, that differed by a single A/T SNP in the 5' end of the *rpl6* gene. While sequence analysis is an accurate method for haplotype determinations, it is time consuming and expensive when trying to analyze a large number of isolates. Because of the base differences in the haplotypes the temperatures required to denature amplicons spanning these SNPs will differ, so another alternative for haplotype classification is melt curve analysis. The primers for 8 loci containing SNPs were redesigned to generate amplicons approximately 200 bp in size and melt curve analysis was evaluated on the 40 isolates that had been previously sequenced. All 8 loci correctly differentiated SNPs for each locus, and only two were needed to assign a haplotype designation. To validate the accuracy of the melt curve analysis for mt haplotype classification, 96 isolates collected from field surveys were tested blind by melt curve analysis and subsequently by sequence analysis to confirm the accuracy of classification. In all cases, even for haplotypes IIa and IIb from Oregon forest samples, the haplotypes were correctly determined. Therefore, melt curve analysis should provide a rapid, accurate and less expensive alternative to sequence analysis for haplotype classification. SSR data for the field isolates used in the validation of the technique was collected and the relationships between mitochondrial haplotype and nuclear genotype will be discussed.

Relationship Between Climate and Tree Mortality Levels on the North Coast of California Infested with Sudden Oak Death

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Phytophthora ramorum has caused extensive oak and tanoak mortality in portions of the Central and North Coasts of California. In conjunction with stream and terrestrial surveys, aerial detection surveys have played a critical role in detection and monitoring efforts associated with sudden oak death (SOD) throughout these regions. Aerial surveys conducted by the U.S. Forest Service, Forest Health Protection have consistently documented the extent and intensity of hardwood mortality across forested areas affected by SOD since 2005. The main objective of this analysis is to determine whether oak and tanoak mortality levels, within regions infested with SOD, are related to climatic conditions.

Many environmental factors influence the severity of disease epidemics. Precipitation data from weather stations and data from the Palmer Drought Severity Index (PDSI) were used during analysis. Three study areas were included: one in the Santa Cruz Mountains, including portions of San Mateo, Santa Cruz and Santa Clara Counties; one in the North San Francisco Bay Area, including portions of Marin, Sonoma and Napa Counties; and one in southern Humboldt County. All study areas were known to be infested with *P. ramorum* prior to 2005 based on PCR confirmations by UC-Davis and UC-Berkeley staff. Only locations where aerial surveys occurred every year were included during analysis. Within each study area, total acres with oak and tanoak mortality and estimated number of recently killed trees were calculated each year from 2005 to 2011.

Detected tree mortality levels varied among years but similar trends were found at two of the three study areas. Mortality levels increased from 2005 to 2007, then decreased from 2008 to 2010 and increased again in 2011 within both the North Bay Area and in southern Humboldt County. Higher levels of precipitation during the two years prior to observed mortality appeared to correspond with higher mortality. Observed mortality levels at these two North Coast locations (both number of acres with mortality and number of killed trees) were closely related to mean departure from normal precipitation of the two previous years based on linear regression with F-tests (values of $p < 0.05$, r^2 ranged 0.64 - 0.93). Observed mortality levels were also closely related to the mean PDSI value of the two previous years (values of $p < 0.05$, r^2 ranged 0.58 - 0.93). The strongest relationships between observed mortality levels and climatic data were in southern Humboldt County. However, no significant trends were found between climatic data and aerial survey data collected in the Santa Cruz Mountains Area. An exponential relationship also was detected between number of killed trees that were mapped each year and climatic data in the North Bay and southern Humboldt County Areas (after a square root transformation, values of $p < 0.04$, r^2 values ranged 0.64 - 0.75).

Although other factors influence oak and tanoak mortality in coastal regions infested with *P. ramorum*, precipitation seems to be an important predictive factor when estimating annual mortality levels in the North Bay Area and southern Humboldt County. Future climatic events such as drought, El Niño and La Niña will likely affect severity of tree mortality associated with SOD throughout coastal landscapes.

Verifying Critical Control Points for *Phytophthora* Introduction into Nurseries

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In 2007/2008, the Oregon Department of Agriculture implemented the Grower Assisted Inspection Program (GAIP) for nurseries. GAIP participants must adopt best management practices (BMP) for critical control points (CCP) where foliar *Phytophthora* species can be introduced into their nursery. These CCP were identified as used containers, irrigation water, soil substrate, potting media, and incoming plants (1). The goal of this study was to determine the presence or absence of *Phytophthora* at four CCP in GAIP nurseries 3- to 4-years after implementation of the program.

During January to March 2011, samples were collected from irrigation water, potting media, used containers, and soil substrate at 13 nurseries participating in the GAIP. Irrigation water samples (3.7 L each) were collected from each water source. Potting media samples (1,000 cm^3 each) were collected from individual media components and from finished media. Used container samples were collected by scraping potting media and debris from the insides of 25 used containers to create a composite sample of 1,000 cm^3. Soil substrate samples were collected by walking transects within each nursery and collecting 350 cm^3 subsamples at three points located equidistant along each transect to make one composite sample per transect. All samples were testing by baiting with healthy *Viburnum davidii* leaves followed by plating on PARP (3, 4). Statistical analyses were performed using analysis of variance for a completely randomized design with unequal replication and by calculating the least significant difference between means.

A total of 354 samples were collected from all CCP checked in this study, with 30.2% testing *Phytophthora*-positive. *Phytophthora* was detected in 10.3%, 30.4%, 36.4%, and 45.5% of potting media, used container, soil substrate, and irrigation water samples, respectively. *Phytophthora* incidence in irrigation water and soil substrate samples was significantly different from *Phytophthora* incidence in potting media samples, although there was no significant difference between soil substrate and used container samples ($p < 0.05$). When looking at the number of nurseries with *Phytophthora* detected at each CCP, soil substrate (92.3% of nurseries) and irrigation water (66.7%) were significantly more likely sources of potential contamination than potting media (30.8%) and used containers (33.3%)($p < 0.05$).

The results of this study underscore the importance of these CCP as sources of *Phytophthora* contamination within nurseries. Although all four CCP are important, directing efforts at irrigation water and soil substrate may provide the greatest opportunity for risk mitigation in nurseries with limited resources.

Comparison of the Recovery of *Phytophthora ramorum* from Tanoak and Bay Laurel, and the Potential Recovery of Inoculum in Fog

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Due to the relative ease with which tanoak may become infected and contribute to the local establishment of *P. ramorum*, the Oregon SOD eradication program has focused its efforts upon the aggressive treatment of tanoak over all other host species. Despite its importance in the epidemiology of SOD in California, because of the apparent lack of infection, bay laurel has been retained at some eradicated sites. Many have since been found positive for *P. ramorum.* This affords us the opportunity to simultaneously compare rates of infection and sporulation of *P. ramorum* from these two important hosts in Oregon, where bay laurel is not thought to be as strong a contributor to the spread of SOD.

To monitor spore production from bay laurel, bait leaves of rhododendron and tanoak were placed in plastic bags secured in screened, 4 L buckets set underneath bay trees retained at previously eradicated areas. Bait leaves have been removed and plated in *Phytophthora*-selective media every two weeks since the buckets were first deployed in February, 2011. This method is a new extension of the ongoing study by which the SOD eradication program has monitored the sporulation of *P. ramorum*; for the comparison of sporulation from bay to tanoak, we selected all baits placed underneath untreated, infected tanoak during the same time period. The recovery of *P. ramorum* from foliage was assessed at a single, untreated SOD site in which both bay and tanoak were present. Between May and September 2011 we sampled 20 to 25 symptomatic tanoak sprouts from random trees in two week intervals. On the last collection period we also gathered symptomatic bay leaves in the understory of infected tanoak to determine the extent by which *Phytophthora* spp. were infecting California bay laurel at that location.

While the collection of inoculum in baited buckets has proven to be a useful measure of detection during rain, we also sought to assess the feasibility of monitoring sporulation in precipitation resulting from fog. Three fog traps were constructed as follows: a screen of shade cloth was suspended in the opening between two Douglas-fir or alder canopies approximately 15 to 20 m above the ground and greater than 20 m away from the nearest tanoak canopy. Condensed fog ran into a trough connected to a covered, baited bucket by ½ inch plastic tubing. Assessment for collection of *P. ramorum* or other *Phytophthora* spp. ensued as with the rain buckets.

Sporulation by *P. ramorum* was detected in all seasons provided there was measurable rain. Recovery of *P. ramorum* from baited buckets and foliage was greater from tanoak than bay laurel during the entire observation period. No other *Phytophthora* spp. were recovered from tanoak foliage or from buckets placed underneath tanoak canopies at untreated sites during this study period. In contrast, greater species diversity was recovered from buckets placed underneath bay laurel, and from bay laurel leaves. *P. ramorum* was recovered from one fog trap, although the recovery rate of culturable species was low.

The Effects of Salinity on *Phytophthora ramorum* Viability and Infectivity

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Phytophthora ramorum, a threat to Eastern U.S. forests, has been found in waterways outside the boundaries of infested ornamental nurseries outside of California and Oregon. Very little is known about what factors are conducive to its survival and sporulation in water. Water collected from various sources with different salinity was used to better understand what effect salinity has on the life cycle of *P. ramorum* and its ability to infect tissue. Water samples, collected from natural bodies of water in May 2010 that had measured conductivity values of 5.6, 30.5, 32.3, and 35.3 mS, were added to cups containing *P. ramorum*-infested sand (1,000 chlamydospores/cm^3). Rhododendron leaf disks were placed on the water surface for 1 week at 20 C and then plated on a *Phytophthora*-selective medium (PARPH+V8). Very few leaf disks (≤ 3%) were infected at the three highest conductivity levels while 100% infection occurred at the lowest level (5.6 mS). Similarly, Rhododendron leaf disks were placed on the surface of different salt solutions (conductivities of 10.3, 26.5, 36.0, 57.2, and 67.9 mS) added to *P. ramorum*–infested sand at two chlamydospore levels (100 and 1,000/cm^3) for 1 week and plated on PARPH+V8. The percentage of leaf disks infected exposed to 100 chlamydospores/cm^3 were 61.1, 23.1, 3.3, 0, and 0%, respective of the above conductivity values, while the percentage of infection at 1,000 chlamydospores/cm^3 was 100, 70.0, 55.6, 2.2, and 0%, respectively. This research demonstrates that *P. ramorum* can form infective propagules that infect plant tissue at high salt concentrations gaining an insight as to the survival and factors affecting infectivity of *P. ramorum.*

Stream Baiting in South Louisiana for *Phytophthora ramorum*

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The use of stream monitoring is an important method for early detection of *Phytophthora ramorum*. Five different waterway locations representing different ecosystems and potential *P. ramorum* inoculum sources across South Louisiana were monitored for *P. ramorum* using bait bags containing whole *Rhododendron* 'Cunningham's White' leaves from December to January 2011. After 1 week, the leaves were retrieved and 30 leaf disks per bait bag (11-mm-diam) were taken from necrotic areas of the exposed leaves and placed on a *Phytophthora*-selective agar medium (PARPH+V8) or 2% water agar and incubated in the dark at 20 ºC. Plates were monitored for mycelial growth and suspected *Pythium* and *Phytophthora* species were transferred individually to V8 agar to obtain pure cultures. The pure cultures were identified using internal transcribed spacer polymerase chain reaction (ITS PCR). Thirty-four cultures containing ten different Oomycete species were positively identified from all locations, including: *Phytophthora* spp. (2.9%), *P. cryptogea* (11.8%), *P.* taxon *sylvatica* (11.8%), *Pythium* spp. (14.7%), *Py. aphanidermatum* (2.9%), *Py. diclinum* (14.7%), *Py. litorale* (29.4%), *Py. sterilum* (2.9%), *Py. tumidum* (5.9%), and *Py. undulatum* (2.9%). The Amite River was the only stream baiting study area to contain *Phytophthora* species. *Phytophthora ramorum* was not found.

Germination of *Phytophthora ramorum* Chlamydospores: A Comparison of Separation Method and Chlamydospore Age

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Chlamydospores are thick-walled, resistant structures produced by many *Phytophthora* species. These structures can be a key component in the ecology and epidemiology of *Phytophthora* species by ensuring pathogen survival during adverse conditions. While *P. ramorum* characteristically produces large amounts of chlamydospores in vitro, the role of these propagules in the disease cycle in both natural and managed systems remains unclear. Germination is difficult to observe and quantify if chlamydospores are not free of mycelium. Moreover, the low frequency of germination commonly reported suggests that endogenous and exogenous requirements for germination may not have been met. Finally, findings of high germination frequency by some researchers have not been universally reproducible. Given the potential importance of chlamydospores to the life cycle of *P. ramorum*, there is a need for comparison and refinement of methods regarding chlamydospore germination.

An experiment was conducted utilizing a 2x2x4 factorial design with the factors chlamydospore age, chlamydospore isolation method, and nutrient medium. Cultures were grown on 10% clarified V8-juice agar plates to establish colonies either two weeks or four months old. Sporangia production was reduced by using Parafilm to seal plates. Inoculum from each colony age was sampled by removing 7-mm agar plugs from a ring midway between the center and margin of the colony or by scraping the colony surface with a rubber policeperson to produce a suspension with final concentration 300 chlamydospores per mL water. One plug or 2 mL of suspension of each colony age were separately added to plates containing 5 mL of one of four aqueous treatments: autoclaved, filtered (20-µm filter disc) seawater; PARP broth; nonsterile, filtered (20-µm filter disc) creek water; and autoclaved, deionized water. Each treatment was replicated five times. Germination incidence was quantified at seven days by counting the proportion of germinated to non-germinated chlamydospores (out of 100) for each replicate.

Chlamydospore germination frequency in each of the treatments ranged from 0-70%. Preliminary results suggest that chlamydospore age, isolation method, and nutrient media significantly affect chlamydospore germination.

Effect of Herbicides on Production of Inoculum and Root Colonization of Plants Infected with *Phytophthora ramorum*

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In Oregon, efforts to eradicate *Phytophthora ramorum* from forested areas have included use of herbicides to kill infected plants. Use of herbicides on disease-infected plants leads to various outcomes, from decreased spread of disease to greater spread of disease, depending on the plant-pathogen system being examined. In this study, *Viburnum* cuttings, *Rhododendron* cuttings and *Quercus prinus* seedlings were treated with herbicides at standard application rates for woody shrubs 4 days after their roots had been infected with *P. ramorum*. The amount of inoculum in runoff samples over time was studied using a quantitative assay analyzed as a mixed model regression, and the percent colonization of roots at the end of each experiment was analyzed by a General Linear Model. In preliminary experiments, the effect of 2, 4-D amine, glyphosate , and triclopyr were studied in samples taken every three days over a period of 19 days, which was sufficient time to observe physiological impairment of treated plants. In those experiments, herbicide had no effect on the amount of inoculum produced from roots, or on percent root colonization. In studies lasting 35 days, long enough for herbicide-treated plants to completely die and nontreated plants to become well infected, weekly samples were taken, with 3 replicates per herbicide. Root-infected *Viburnum* cuttings treated with glyphosate gave off significantly more inoculum than untreated cuttings (at days 14, 21, and 28, $p \leq 0.007$), but there were significantly more colonized roots on cuttings that had not been treated with herbicide ($p \leq 0.001$). Triclopyr-treated *Viburnum* cuttings gave off slightly more inoculum than nontreated plants on days 28 and 35 ($p \leq 0.03$), but root colonization was not affected; imazapyr had no significant effect on inoculum production, but reduced root colonization ($p \leq 0.009$). When glyphosate was applied to root-infected *Q. prinus* seedlings, the herbicide-treated seedlings gave off more inoculum than nontreated ones on days 14, 21, and 28 ($p \leq 0.007$), but no difference in root colonization was seen. In a similar experiment using infected cuttings of *Rhododendron* 'Cunningham's White', herbicide-treated cuttings gave off less inoculum at some sampling times than untreated plants on days 14, 21, and 28 ($p \leq 0.009$), but no effect on root colonization was observed. These results suggest that while herbicide treatment had some effect on the behavior of *P. ramorum*, generally increasing inoculum production while decreasing root colonization, it did not have effects of ecological significance on either.

Effect of Environmental Conditions and Lesion Age on Sporulation of *Phytophthora ramorum* on California Bay, Rhododendron, and Camellia

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The objective of our research was to determine the environmental conditions and lesion age favorable for *Phytophthora ramorum* sporulation under field conditions. For two years, new camellia, rhododendron, California bay (*Umbellularia californica*) nursery stock were seasonally inoculated (every 3 months) on foliage. They were covered overhead to prevent rainfall from falling on the plants, but otherwise the plants were completely open to the natural environment. Consistent leaf wetness periods were produced with overhead misting systems and controlling sensors to simulate rainfall, fog, dew, or other conditions that might be supportive of sporulation. For each season, these wetness conditions began when leaf lesions were 3, 6 and 9 weeks old and, at each of these time points, the wetness conditions were maintained for 8 days. Sporulation was evaluated by washing leaf lesions just before the wet period began (day 0), and at 1, 2, 4 and 8 days during the wet period. Leaf wetness and temperature were measured near the plants.

Sporulation rate remained relatively high even at the lowest maximum daily temperatures measured (8 ^{o}C) but the rate fell quickly when maximum daily temperatures exceeded 33 ^{o}C in all species regardless of other measured conditions. For all species, the highest sporulation rate was seen at the end of 2, 4, or 8 days of artificial misting, with the highest rate at day 4. California bay sporulated significantly after the first day but camellia and rhododendron required 2 days of misting before significant sporulation could be detected. When the actual consecutive hours of leaf wetness above 90% were evaluated, then there was a significant logarithmic linear increase of sporulation as leaf wetness hours increased. Lesion size did not explain sporulation well for rhododendron and bay, and was not significant for camellia. Lesion size was therefore taken out of the final explanatory model. However, lesion age was a much stronger predictor of sporulation. Sporulation increased as lesion age increased from 3 weeks to 9 weeks for bay, from 3 weeks to 6 or 9 weeks for camellia, but decreased from 3 weeks to 6 or 9 weeks for rhododendron. The fitting for the final models were good. For Rhododendron, California bay, and camellia, the fitted covariates explained 45.7%, 60.9%, and 59.9% of the deviance respectively.

With readily available electronic environmental sensors and dataloggers (as those used in this study), nursery operators could monitor environmental conditions (temperature and leaf wetness), and in conditions with high sporulation risk, avoid certain cultural practices such as irrigation, plant handling, or pruning that might increase the chances of sporulation and infection. Preventative fungicides could be applied. The results could help improve existing risk models for Sudden Oak Death in California forests when environmental parameters conducive to sporulation of California bay are incorporated.

Effect of Fungicides and Biocontrol Agents on Inoculum Production and Persistence of *Phytophthora ramorum* on Nursery Hosts

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Once *Phytophthora ramorum* is introduced into a nursery on a host, its local spread and establishment is primarily dependent on sporangia and zoospore production. Nursery operators commonly use fungicides to prevent the establishment of Phytophthora diseases, although current research only supports the use of fungicides for preventing infection. It is still unknown, however, what effect fungicide treatments have on sporulation, spread, and persistence of the pathogen on established infections. With this additional knowledge, fungicide treatments could be more effectively used to prevent the spread and establishment of the pathogen in nursery operations. The goal of this study was to evaluate the activity of foliar applied fungicides and biocontrol agents to inhibit sporulation and reduce pathogen persistence in ornamental hosts.

The experiment was established, at the National Ornamentals Research Site at the Dominican University of California (NORSDUC), San Rafael on Nov 3, 2010 and at Felton, CA on Nov 23, 2010. Nursery stock of camellia and rhododendron were inoculated at both research sites. Each plant in 4 blocks of each species were inoculated with 10 inoculum plugs with a locally derived isolate of *P. ramorum*. Treatments were applied approximately 4 weeks after inoculation, and included: *Bacillus subtilus* (Cease®), mandipropamid (Micora®), *Trichoderma atroviride* (Plant Helper), *Reynoutria sachalinensis* extract (Regalia® SC), mefenoxam (Subdue Maxx®), dimethomorph (Stature® SC), mancozeb (Dithane® 75DF), fluopicolide (Adorn®), pyraclostrobin (Insignia®), mono- and di-potassium salts of phosphorus acid (Alude®), and cyazofamid (Segway®).

P. ramorum sporulation in the field and in flooded disk assays was evaluated from December, 2010 to April, 2011. Pathogen viability and persistence in leaf lesions was evaluated from December, 2010 to June, 2011. The lesions in the Felton experiment failed to sporulate at detectable levels, and *P. ramorum* was not recoverable from the majority of the lesions in culture, possibly because a low-viability isolate was used for inoculations. In the San Rafael experiment, the fungicides cyazofamid and mefenaxom reduced sporulation in the first few weeks of lesion development. Pathogen persistence (measured by colony growth in semi-selective media from lesion isolations) was also similarly affected. Results of ongoing trials that include new active ingredients will also be presented.

Infection of Five *Phytophthora ramorum* Hosts in Response to Increasing Inoculum Levels

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The objective of this work was to establish inoculum density relationships between *P. ramorum* and selected hosts based on whole plant inoculations. Knowledge of levels of initial inoculum needed to generate epidemics is needed for disease prediction and development of pest risk assessments. Sporangia of six *P. ramorum* isolates representing the NA1 and EU1 lineages were produced by incubatingV8-juice agar plugs in 1% soil extract for 48 h, and adjusting the suspensions to 0, 50, 100, 500, 1000, 2000, and 3000 sporangia/ml. Whole plants (2- to 3-year-old) of *Quercus prinus* (chestnut oak), *Q. rubra* (Northern red oak), *Acer rubrum* (red maple), *Kalmia latifolia* (mountain laurel), and *Rhododendron* 'Cunningham's White' were dip-inoculated and incubated in a 20°C dew chamber in darkness for five days. The total number of diseased and healthy leaves was recorded and leaves were scanned. A linear model as well as a two-parameter asymptotic regression analysis through the origin were fit to the data. For all five species, the percentage of infected leaves increased from 0 to 2000 sporangia/ml and then seemed to level off. Calibration threshold estimates for obtaining 50% infected leaves based on the linear analysis ranged from 36 to 750 sporangia/ml for the five hosts. Half-life (LD50) estimates from the asymptotic regression analysis ranged from 94 to 319 sporangia/ml. Multiple regression analysis revealed statistically significant differences ($p = 0.0076$) among hosts in the increases in infection in response to increased inoculum density. Our results provide estimates of initial inoculum levels necessary to cause disease on these five *P. ramorum* hosts, and will be useful in disease prediction and for development of pest risk assessments. Spore concentrations occurring in nature have rarely been determined experimentally, so it is not known whether the level of spores determined experimentally to result in a given level of disease is a common occurrence in native ecosystems.

First Results with a Lab-on-a-Chip System for a Fast *Phytophthora* Diagnosis

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For *Phytophthora* species that are quarantine or regulated organisms highly specific and sensitive diagnostic tools are recommended for surveys and monitoring. Furthermore these diagnostic techniques should give results within short time and should be not too expensive. The techniques currently used for routine diagnosis of *Phytophthora* species in plant tissue are mainly molecular techniques (conventional and real-time PCR) and direct isolation. That means samples must be brought to a diagnostic lab with specific equipment. That takes time and means financial losses for the commercial nursery industry because they have to stop plant sale until the (negative) result is available. Furthermore with PCR only a single *Phytophthora* species can be detected per run. Therefore techniques that can be used directly in the field and that can detect different *Phytophthora* species within one run would be much better diagnostic tools.

Within a collaboration of industry and academics a chip based technical platform could be developed that miniaturized hybridization and PCR on a chip (Julich et al. 2011). In a following project the chip technology will be improved and different techniques for sample preparation will be tested. First results on the specificity of the developed probes tested with *in vitro* samples will be presented.

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Reference

Julich, S.; Riedel, M.; Kielpinski, M.; Urban, M.; Kretschmer, R.; Wagner, S.; Fritzsche, W.; Henkel, T.; Möller, R.; Werres, S. 2011. Development of a lab-on-a-chip device for diagnosis of plant pathogens. Biosensors and Bioelectronics 26 (10):4070-4075.

Survey of Eastern U.S. Native *Rhododendron* spp. for Antagonistic Endophytes Towards *Phytophthora ramorum*

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Rhododendron maximum and *R. catawbiense* are two species that are native to the Eastern U.S. They can be found throughout the Appalachian Mountain range and during bloom are very important tourist attractions. *Phytophthora ramorum* is known to be pathogenic to both species although no symptoms have been observed in wild habitats in the Eastern U.S. Endophytic fungi are known to have a symbiotic relationship with their host, including protection against pathogens. It was the purpose of this study to survey natural stands of *R. maximum* and *R. catawbiense* in the Eastern U.S. in order to understand what endophytic fungi are present and whether they have the potential to protect against *P. ramorum* infection.

In 2009, leaves of *R. maximum* and *R. catawbiense* were collected in four distinct locations in Virginia, West Virginia and Pennsylvania. At each location, three mature leaves that did not show any visible signs of necrosis were collected from 10 different plants. The leaves were stored in a cooler and taken back to the laboratory to be processed, within 1 week's time. Leaves were surface sterilized for 1 minute in 70% ethanol and rinsed three times in sterile water for 10 minutes each time. After drying, five 11-mm-diameter disks were aseptically cut from each leaf with a cork borer and plated on water agar supplemented with streptomycin (50 mg/L). The plates were stored at 20 ^{o}C. Over time, the plates were observed and fungal mycelium growing from the disks was individually transferred to half-strength potato dextrose agar (1/2PDA). Each collected isolate was screened for antagonistic activity towards *P. ramorum* by a dual culture assay. Individual plugs of the fungal isolate were transferred to a 1/2PDA plate containing a plug of *P. ramorum* isolate WSDA-1772 (NA1 mating type), which were stored at 20 ^{o}C. After 1 week, the plates were observed for any obvious growth inhibition of the *P. ramorum* colony. Three plates were prepared for each fungal isolate. The fungal isolates that showed some antagonistic activity were tested further in the same dual culture assay described above. Five plates were prepared for each repetition and there were three repetitions per fungal isolate. The antagonistic activity was quantified by calculating the antagonistic index (AI). The AI was determined by subtracting the length of the ray of the *P. ramorum* colony growing towards the fungal isolate from the average length of the three rays of the *P. ramorum* colony growing in the other directions (RM) and dividing the result by RM. Therefore, the closer the value is towards one, the higher the antagonistic activity.

A total of 631 fungal endophyte cultures were originally isolated from the leaves of the two *Rhododendron* spp. at the four locations surveyed. These isolates were grouped into 72 different types based solely on colony characteristics. Preliminary screening identified 118 cultures that demonstrated some antagonistic activity towards *P. ramorum*, which were tested in detail to determine their AI. All isolates, except one, had a statistically higher ($P<0.05$) AI value than the control. AI values ranged from 0.502 to 0.04. Location, group type, and the *Rhododendron* species were all significant factors in the AI. These results show that endophytic fungi found in leaves of wild *Rhododendron* spp. have the potential to inhibit the growth of *P. ramorum*. Further work will continue to identify these species and determine what role they may play in protecting the plant from *P. ramorum* infection.