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June 20 to 23, 2016, San Francisco, California, USA

California bay laurel, *Umbellularia californica*

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Susan J. Frankel and Katharine M. Harrell

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Abstract

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The Sudden Oak Death Sixth Science Symposium provided a forum for current research on sudden oak death, caused by the exotic quarantine pathogen *Phytophthora ramorum*. More than 50 submissions describing papers or posters on the following sudden oak death/*P. ramorum* topics are included: biology, genetics, nursery and wildland management, monitoring, and ecology. Abstracts are also provided from a special session on *Phytophthoras* in California native plant nurseries and restoration sites.

Keywords: *Phytophthora ramorum*, invasive species, tanoak, *Notholithocarpus densiflorus*, coast live oak, *Quercus agrifolia*

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Posters

Impacts and Status of *Phytophthora ramorum*

Pacific starflower, *Lysimachia latifolia*

Slowing Spread of Sudden Oak Death in Oregon Forests, 2001-2015[1]

Alan Kanaskie,[2] Randy Wiese,[2] Danny Norlander,[2] Jon Laine,[2] Sarah Navarro,[2] Ellen Michaels Goheen,[3] Ron Rhatigan,[3] Everett Hansen,[4] Wendy Sutton,[4] Paul Reeser,[4] Nik Grunwald,[4] Zhian Kamvar,[4] and Nancy Osterbauer[5]

Abstract

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 and mandatory destruction of infected and nearby host plants. Eradication treatments eliminated disease from most infested sites, but the disease continued to spread slowly, mostly in a northward direction.

Following a sharp increase in disease in 2010 and 2011, a result of leaving many infestations untreated, the program shifted goals from complete eradication to slowing spread. In 2012 the quarantine regulations were changed by establishing a Generally Infested Area (GIA) in which eradication on was no longer required by law. Since then, eradication treatments (cutting and burning host plants) have been focused on new infestations that occur outside of the GIA. All new infestations outside the GIA are cut and burned, but the size of the treatment area varies with available funds and location of the site.

Since 2001 the area under quarantine has expanded seven times: from 22 km^2 (9 mi^2) in 2001 to 1,333 km^2 (515 mi^2) in 2015, which is approximately 31% of the total area of Curry County. The GIA has expanded four times and currently stands at 151 km^2 (58 mi^2). Within this area, hundreds of thousands of tanoaks have died in the since 2012, creating a high risk for wildfire and damage from falling trees. From the initial infestations of 2001, the disease has been found a maximum distance of 28 km (17.5 mi) to the north, 12 km (7.5 mi) to the northeast along the Chetco River, and 11 km (7 mi) to the southeast along the Winchuck River.

In early 2015 the EU1 genetic lineage of *P. ramorum* was detected on a single tanoak tree located approximately 1 mile north of a small private nursery (now closed) near the Pistol River. Genotype comparison of the tanoak and nursery isolates suggests the nursery as the probable source for the forest infestation. This is the first report of the EU1 lineage in US forests. All host plants within approximately 130 m of the infected tree were cut and burned in 2015. Two post-treatment assays of soil and vegetation on the infested site failed to detect *P. ramorum*.

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Lessons from 15 Years of Monitoring Sudden Oak Death and Forest Dynamics in California Forests[1]

Margaret Metz,[2] J. Morgan Varner,[3] Ross Meentemeyer,[4] Kerri Frangioso,[5] and David Rizzo[5]

Abstract

Monitoring host composition and disease impacts began 15 years ago in what would become a network of permanent forest monitoring plots throughout the known and predicted range of *Phytophthora ramorum* in California coastal forests. Stretching ~500 miles from Big Sur to the Oregon border, the network captures variation in interactions among the pathogen, its potential hosts, and the environment across wide gradients in climate, topography, land use, and host species abundance. Within each plot, standardized methodology has been used to track forest dynamics and disease impacts of individual trees and at the forest stand level. From early plot censuses of this and other similar networks, information was gained about how forest diversity and stand composition mediate disease risk, how the presence of the pathogen alters competitive dynamics and mortality rates among hosts, and how disease impacts alter rates of coarse woody debris accumulation. The full ecological impact of most other destructive pathogens is difficult to quantify because of a lack of baseline data at the earliest stages of an invasion and through multiple subsequent decades of forest change.

Investment in the establishment and repeat censuses of these plots has facilitated the tracking of disease dynamics at a number of temporal and spatial scales. The importance of interannual climate variation was observed as an overarching driver of disease dynamics and tree mortality, and we have seen the impact of this variation differ across host communities and climatic gradients. Monitoring included at least two multi-year periods of above average rainfall with consequent short-term increases in sudden oak death (SOD) prevalence. Waves of associated tree mortality have followed at a lag of 2-5 years after the appearance of symptoms, when environmental conditions may appear out of sync with those favorable for disease spread. Monitoring has also included two multi-year drought periods associated with lower rates of pathogen expansion, but potential increases in tree mortality. When wildfires in 2008 occurred for the first time in SOD-impacted forests, data collected in the Big Sur section of the monitoring network facilitated capitalizing on the natural experiment of comparing the individual, joint, and potentially interacting effects of disease and fire. Findings included learning that disease-fire interactions are complex and dependent on changing fuel characteristics as the disease progresses in a stand. Additionally, unexpected synergies were identified that spilled over to affect species not normally impacted by either disturbance alone. Since then, at least three other fires have occurred in parts of the monitoring network, adding to the growing understanding of forest change under novel disturbance regimes.

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With continued monitoring of forest regeneration and pathogen range expansion or contraction, there is hope to develop a fuller understanding of disease dynamics and environmental change on the multi-year or multi-decadal time scales appropriate to turnover in long-lived hosts. Monitoring across the diverse assemblage of host communities in the pathogen's range permits an understanding of how and whether disease dynamics at the advancing front of the pathogen differ from areas of long-term pathogen establishment.

Phytophthora ramorum: Update on the Impact and Wider Consequences of the Epidemic in Britain[1]

J.F. Webber[2]

Abstract

Many new *Phytophthora* pathogens have arrived in the UK via the plant trade in recent decades, but arguably *Phytophthora ramorum* has been one of the most significant introductions to affect trees. From 2002 onwards during the early stages of the epidemic, the first impacts of *P. ramorum* were seen in ornamental plant nurseries, then affecting valuable heritage plants in gardens important to the tourist trade, and eventually in broadleaf dominated woodlands (Brasier and others 2004). In the latter, a few tree species, such as European beech (*Fagus sylvatica*) and sweet chestnut (*Castanea sativa*), were occasionally affected, although native European oaks proved to be of low susceptibility in contrast to native North American oak species. However, rhododendron, in the form of valued ornamental cultivars in gardens or the invasive non-native shrub *Rhododendron ponticum*, was the common host across all environments. It also proved essential to the epidemic because it sustained pathogen sporulation, whereas the bole cankered tree hosts did not. As a harmful organism listed within the European Union EC Plant Health Directive (2000/29/EC), phytosanitary measures required eradication or disease containment achieved through the removal of sporulating hosts. Therefore, control measures against *P. ramorum* focused on rhododendron removal, and the range of tree bole hosts affected fit with the American 'model,' with the worst affected species in the *Fagaceae* family.

However, in 2009 an unexpected change occurred in the disease dynamic in Britain. Across southwest England, there were several findings of *P. ramorum*-infected plantation-grown Japanese larch (*Larix kaempferi*), but they were not close to infected rhododendron (Webber and others 2010). Widespread mortality was also observed as larch stems were girdled and the infected phloem tissue underwent a series of chemical changes incited by *P. ramorum* colonization. Not only Japanese larch but also European (*L. decidua*) and hybrid larch (*L.* x *eurolepis*) were also found affected by the ramorum-induced dieback. Moreover, *P. ramorum* was found to sporulate profusely on infected larch needles, particularly those of Japanese larch, with the numbers of spores far exceeding those produced from rhododendron foliage and California bay laurel (Harris and Webber 2016).

As larch was the third most commonly grown conifer species (after spruce and pine) and *P. ramorum* was sporadically established throughout western Britain on rhododendron, the host jump from rhododendron allowed the epidemic to develop on larch almost simultaneously across climatically suitable areas on a landscape scale. Thousands of hectares of near contiguous larch plantations were affected in some regions, and regular aerial surveillance by helicopter was put in place from 2010 onwards as part of the measures to detect and contain the spread. Between 2009 and 2016, almost 20,000 hectares (~50,000 acres) of larch throughout the UK were affected by the disease, with millions of trees felled to contain disease outbreaks. Large management zones have been set up in southern Wales and southwest Scotland (Forestry Commission 2014), and satellite outbreaks are managed on a site by site basis. The most conducive climate for disease development is in the western British Isles, due to mild winters and high

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rainfall. Years with above average rainfall, such as in 2012, can result in striking increases in disease development associated with an upsurge in larch mortality the following year.

As a sporulating host, removal of infected larch is critical to managing the epidemic, slowing disease spread, and allowing remaining larch stocks to stay healthy for as long as possible so they can be harvested without a "biosecurity penalty." The latter alludes to the loss of valuable bark bi-products through infection as well as the need to use a network of inspected sawmills licensed to process the lumber from infected larch stands. Where felling is not an option, the use of herbicide injection is also being explored with the aim of achieving a rapid kill of infected trees, reducing the potential of infected foliage to release spores into the environment. If the disease is not controlled in infected larch stands, neighboring plants and trees also frequently become infected. For example, other conifer species including Douglas-fir (*Pseudotsuga menziesii*), grand fir (*Abies grandis*), noble fir (*A. procera*), and western hemlock (*Tsuga heterophylla*) can suffer significant stem and branch infections and show symptoms of crown dieback, although only when growing in close proximity to infected larch.

A further, and more recent complication in the invasion of *P. ramorum* across the UK, is the discovery of the novel EU2 lineage (Van Poucke and others 2012) in addition to the widespread EU1 lineage. So far, the EU2 has only been found in the UK where it is limited to Northern Ireland and southwest Scotland (King and others 2015). Its distribution is, however, expanding, and in parts of southwest Scotland, the distributions of the EU1 and EU2 are now close to overlapping (fig. 1). The intensive sampling of larch across Britain to test for the presence of both EU1 and EU2 lineages of *P. ramorum* has also provided surprising insights into how frequently other Phytophthoras are associated with larch and other conifer species, such as *P. pseudosyringae* and *P. gonapodyides*. Like *P. ramorum*, both of these *Phytophthora* species produce aerial cankers on branches of mature larch trees. This raises the possibility that they may also infect and sporulate on larch needles as *P. ramorum* does, thereby providing the inoculum for aerial bark infections.

The continuing epidemic has provided a graphic example of the highly unpredictable outcome of a pathogen introduction and heighted public awareness of the environmental and economic costs that can result from such events. The changing nature of the epidemic is further emphasized by recent observations made in some remnant ancient semi-natural woodlands which suggest that ramorum disease may now be cycling on sweet chestnut, in the absence of larch or rhododendron. Sweet chestnut can apparently act as both bole host and sporulating host, although levels of sporulation by *P. ramorum* on sweet chestnut leaves are lower than those on larch needles (Harris and Webber 2016). Current management strategies concentrate on the clearance of infected larch, but if infected sweet chestnut can provide alternative disease foci, their management needs to be reconsidered in light of their sporulation potential.

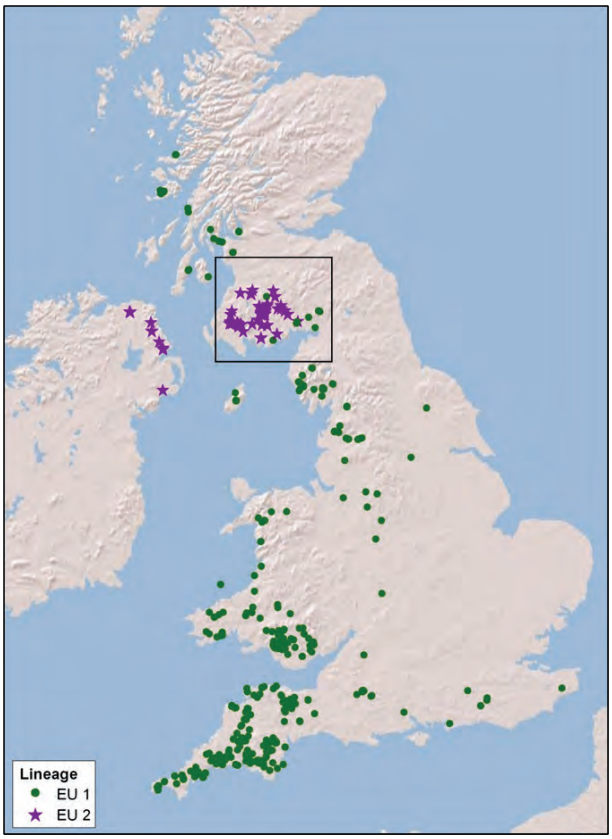

Figure 1—Map showing the distribution of EU1 (green dots) and EU2 (purple stars) lineages of *P. ramorum* infecting larch in the UK at the end of 2015.

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Sudden Oak Death and Landscape Ecology

California maidenhair fern, *Adiantum jordanii*

Resilience of Diversity-Disease Risk Interactions following Wildfire Disturbance[1]

Devon A. Gaydos,[2] Krishna Pacifici,[2] Ross K. Meentemeyer,[2] and David. M Rizzo[3]

Abstract

The potential for biodiversity to mitigate risk of infectious diseases in ecological communities – known as the diversity-disease risk hypothesis – is fundamental to understanding links between landscape change and environmental health of forests affected by sudden oak death (SOD). Previous research of the *Phytophthora ramorum* pathosystem found evidence for a dilution effect, where areas of high woody plant diversity were found to have significantly lower disease prevalence. However, little is known regarding the resilience of biodiversity effects subject to ecological disturbances. We investigate how this relationship changes following the dramatic restructuring of biodiversity by wildfire.

Previous work on this topic was centered in Big Sur, California, an ecoregion prone to complex disturbance interactions. In 2008, shortly after this study was conducted, the Basin Complex and Chalk fires burned nearly half of our disease monitoring plots, creating a natural biodiversity manipulation experiment. Using pre- and post-fire data collected over a period from 2006-2014, we compare how the diversity-disease relationship changes through time in disturbed and undisturbed plots. As a landscape epidemiology approach, we also account for the potentially confounding effects of host density and landscape heterogeneity by including variables for bay laurel (*Umbellularia californica*) density, tanoak (*Notholithocarpus densiflorus*) density, potential solar insolation, precipitation during the wet season, surrounding forest cover, and forest type (mixed-evergreen or redwood-tanoak).

We examine the diversity-disease risk relationship using three hierarchical models of varying complexity: (1) a binomial generalized linear model (GLM), (2) a zero-inflated binomial GLM, and (3) a zero-inflated binomial generalized linear mixed model (GLMM) with a spatial random effect. Our results indicate that the dilution effect was retained in both burned and unburned plots, suggesting that biodiversity effects are resilient to wildfire disturbances. These results provide valuable insights on how SOD and wildfire disturbance interact to affect landscape health, an ever more pressing need as SOD spread and natural disturbance regimes continue to be altered by anthropogenic change.

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Effects of Diversity, Topography, and Interannual Climate Variability on Pathogen Spillover[1]

Whalen W. Dillon,[2] Ross K. Meentemeyer,[2] and David M. Rizzo[3]

Abstract

Our knowledge of sudden oak death (SOD) disease dynamics indicate that without bay laurel (*Umbellularia californica*) there is seldom oak (*Quercus*) infection. This requirement of an alternate host species for disease transmission to oak species is an example of pathogen spillover. We developed a path analysis to test specific hypothesized relationships between physical and ecological factors affecting pathogen spillover. Path analysis enables simultaneous examination of direct and indirect effects from multiple factors, which can enhance our understanding of the multiple influences on pathogen spillover in SOD. We rooted our path model with the topographic wetness index, indicating potential soil wetness and moisture persistence, and examined the direct and indirect effects of species diversity, temperature, precipitation, and bay laurel density on potential inoculum load and infection of oak species.

We applied 10 years of data from a long-term SOD-monitoring plot network in southeastern Sonoma County, CA. Each of the 200 15-m by 15-m plots was equipped with a temperature logger and plots were visited once per year from 2004 to 2012, and in 2014 to assess *Phytophthora ramorum*/SOD host species for disease symptoms and download temperature data. We inspected oak species for canker symptoms and indexed potential inoculum load by counting symptomatic leaves on each bay laurel stem for 60 seconds. We recorded the abundance of all tree species rooted in each plot during visits in 2005 and 2014 to quantify community diversity. Rainfall was measured at 15 rain gauges installed throughout the study area during this period.

We conducted a piecewise assessment of the path model, enabling us to account for the repeated measures structure of these data. Results from our path model of disease observations aggregated to the plot level revealed that diversity mediates the potential for pathogen spillover through a relatively strong direct negative effect on oak infection. Potential inoculum load on bay laurel had a direct positive effect on oak infection, with its overall influence moderated by temperature, topography, and diversity. Temperature and rainfall had relatively weaker influences on pathogen spillover compared to diversity and inoculum load. The net negative effect of diversity on oak infection is consistent with the dilution effect found in other studies of SOD. Topographic wetness had significant direct influence on diversity and inoculum load, where higher values of the wetness index tended to have lower values for diversity, but higher values for inoculum load. This is consistent with areas where moisture is likely to accumulate and persist providing a more favorable environment for *P. ramorum* sporulation.

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Collaboratively Managing Sudden Oak Death Using Tangible Geospatial Modeling[1]

Ross K. Meentemeyer,[2] Francesco Tonini,[2] Douglas Shoemaker,[2] Richard C. Cobb,[2] Brendan A. Harmon,[2] Vaclav Petras,[2] Anna Petrasova,[2] and Helena Mitasova[2]

Abstract

Failure to build consensus amongst stakeholders has been a primary obstacle barring progress in developing and implementing strategies to manage sudden oak death (SOD). Consensus as to the goals of *in situ* management of SOD has rarely been reached, because stakeholders' visions of success vary widely and often compete with each other across the complex landscape of forest resources, ownership types, and overlapping jurisdiction in which this epidemic unfolds. Investments in research on the pathology of *P. ramorum* have yielded dynamic spread models identifying and ranking communities at risk. However, unresolved questions regarding the efficacy and costs of proposed management, as well as the degree and location of collective action needed to affect change, has worked against deploying management treatments. The lack of shared and articulated goals, uncertainty in effect, and misunderstanding as to the roles of individuals and institutions in controlling the disease has left much of the region vulnerable to ongoing forest loss.

We introduce Tangible Landscape, a participatory modeling tool designed to explore "wicked" socio-ecological natural resource dilemmas by providing a "smart" workbench for consensus and collaborative solution building. Tangible Landscape allows stakeholders and decision makers to gather around a geographically realistic model and explore scenarios with instant feedback as to impacts of their decisions. Coupling a scanner, a projector, and a GIS, Tangible Landscape 1) builds participant understanding of complex environmental systems and the models that simulate them using 3D visualizations; 2) allows participants to iteratively test personal management strategies by computationally "steering" simulation models using an intuitive, tangible interface; 3) provides data-driven, near real-time spatio-temporal projections of management outcomes including costs; and 4) promotes co-learning amongst participants who are also testing their own strategies.

We explored the potential of Tangible Landscape to develop a SOD management strategy for Upper Sonoma Valley, CA, an area hard-hit by the disease, using actors playing local stakeholders. This deployment uses a host-driven pathogen spread and host mortality simulation model derived from published SOD data and models. From the point of historical detection of SOD (2000) in the Valley, we challenged our role players to limit both the extent of spread and oak (*Quercus*) mortality by 2010 given limitations to the area they could treat. For this case, treatments were limited to culling of bay laurel (*U. californica*), and represented by placing props on a physical terrain model. The scanner reads these representations of effort, updates a combined GIS and disease simulation model, and provides the estimated cost of treatment per ha. The results are projected back onto the physical model and different management scenarios are compared in near real time.

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We found Tangible Landscape provided the degree of information density and realism needed for role players to 1) quickly learn the salient details of a complex epidemiological spread model 2) allow decision making to be geographically and contextually informed, 3) develop and test management alternatives, and 4) incorporate near-real time feedbacks into adaptive strategies. In all, Tangible Landscape constituted a powerful shared environment fostering co-learning and co-management among participants.

How Well Has the Spread of Sudden Oak Death Been Predicted by the Models in Northern California?[1]

Y. Valachovic[2], R. Cobb[3], and B. Twieg[2]

Abstract

Since *Phytophthora ramorum* established in the wildlands of California during the 1990s, the disease has spread rapidly throughout the state's coastal and adjacent counties, likely by a combination of human-aided events (e.g., nursery plant introductions) and natural dispersal. While human-aided events are almost impossible to predict, dedicated efforts have been made to model the natural spread of the disease throughout California. These models have been built upon aerial survey mortality data and/or extensive plot-level data gathered for infested and non-infested locations throughout the range of the disease in California and beyond, plus broader vegetation and weather data that dictate the pathogen's ability to infect hosts and further disperse. Some of these models have also sought to predict disease spread and intensity under various management scenarios.

While remaining cautious about model predictions, we find that the geographically explicit modeling efforts to date have been useful in predicting coarse scale patterns. This holds true even for the initial spread models developed in 2008 for the North Coast, where there is a rigorous dataset that supports actual disease spread. In retrospect, models that were run on a statewide scale (Meentemeyer and others 2004, 2008) predicted disease arrival and intensity well in some areas, but tended to overestimate disease spread and intensity further away from the infestation location(s) included when the model was first developed. In contrast, models with more limited geographic scope have, thus far, predicted spread rates surprisingly well. This is particularly true for an area (12 km X 85 km) of Humboldt County modeled by Filipe and others (2012) that accurately predicted the spread of the disease northward from the southern part of the county to within the proximity of the Van Duzen River by 2010. Another model that focused on the Mattole watershed slightly overestimated westward spread around Ettersburg, although the disease has been confirmed spreading north around a nearby infestation, where cryptic infection to the west may exist (Filipe and others 2013).

In reviewing the available *P. ramorum*-related modelling, we conclude that the modelling efforts to date have made significant advancements, but for land managers they should be viewed with the following limitations: 1) disease models can't predict new introductions or movement of ornamental plants; 2) while models exist for stand-level predictions (Cobb and others 2012), the lack of fine-scale data vegetation layers limits their application (see Twieg and others, this Proceedings for an example of stand-level model application); 3) available vegetation mapping

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data layers that work well at the state scale (e.g. CalVeg) are not currently sufficient to provide site-level information and are not designed to give accurate host species-specific distributions for California bay laurel (*Umbellularia californica* Hook. & Arn.) or tanoak (*Notholithocarpus densiflorus* Hook. & Arn.); and 4) models are based on past weather trends and cannot predict future weather conditions and lack understanding of fine-scale climate variations. Of the models available, the recently developed SODDr statistical model is getting closer to being suitable for informing management decision making (Cobb and others 2012, Ross 2012), but is limited by data availability and requires a modicum of programming skills to parameterize and run. However, land managers should be aware that what is available to date can provide a prediction of probable pathogen establishment at broad spatial scales and shows very clearly the scope and scale of potential pathogen invasion and disease emergence. We look forward to future modeling products, such as the Tangible Landscapes approach (see Meentemeyer and others, this Proceedings) that can help forest managers tasked with difficult operational decisions.

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Sudden Oak Death Management

Coast redwood, *Sequoia sempervirens*

Lessons Learned from the USDA Forest Service, Pacific Southwest Region, Sudden Oak Death Management Program[1]

Phil Cannon,[2] Susan J. Frankel,[3] and Pete Angwin[2]

Abstract

Over the past 15 years, the USDA Forest Service, Pacific Southwest Region, State and Private Forestry (S&PF) has provided grants which have supported over 200 projects to address sudden oak death (SOD) in California. To date, over $10 million has been provided by US taxpayers, plus an additional $8 million of non-federal matching funds. These funds have provided the infrastructure for California's SOD response: supporting laboratory diagnostics, monitoring, management, and education and outreach. Grantees have included CAL FIRE, five California universities, UC Cooperative Extension in four counties, a county agricultural department, two National Parks, two timber companies, the US Department of Interior Bureau of Land Management, three National Forests, four Native American Tribes, and a privately run forest pathology consulting company.

State and Private Forestry's responsibility is to provide technical and financial assistance to improve management of forest pests and diseases, it is not mandated to support research (the mandate of USDA Forest Service Research and Development). Nevertheless, each year when proposals are submitted in response to the annual "Request for SOD Management Proposals," lessons learned from previous projects, including applied research on the SOD pathogen (*Phytophthora ramorum*), have been relied on for proposal selection since current knowledge is a key factor in the probability for success and relevance of a proposal.

Pathogen biology and disease epidemiology were evolving quite rapidly between 2000 and 2010 and many management-oriented projects uncovered new information, such as evidence that *P. ramorum* is the primary pathogen involved in SOD and long-distance spread via windblown spores is possible. Early projects also discovered evidence to show that California bay laurel (*Umbellularia californica*) leaves can become infected and support substantial pathogen sporulation. S&PF CA SOD projects also led to improvements in techniques and strategies for pathogen monitoring. Stream baiting was developed and is effective for early detection. S&PF aerial surveillance, conducted each year for CA forests at risk for SOD, developed digital sketch maps to identify locations where trees (primarily tanoak, *Notholithocarpus densiflorus*) are symptomatic, identifying polygons for ground checking to determine if *P. ramorum* is present. Approximately 30 field verification projects have been conducted to date. Collectively, several monitoring techniques (stream baiting, aerial surveying, and field verification) have tracked the progression of SOD in California, facilitating management activities and spread model validation.

The key characteristic of the overall S&PF SOD program in California is service and support for land managers facing novel challenges presented by SOD. There is much work to be done. Currently *P.*

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ramorum is threatening Tribal lands in northern California (Hoopa, Karuk, and Yurok). As tanoak holds special cultural significance for these tribes, sudden oak death's impacts on these stands are multidimensional and represent a special concern.

Conditions 10 Years After Sudden Oak Death Suppression Treatments in Humboldt County, California[1]

Y. Valachovic[2], C. Lee[3], D. Stark[2] and B. Twieg[2]

Abstract

In 2006, three isolated sudden oak death- (SOD) infested locations within Humboldt County were selected for silvicultural treatments that targeted the removal and/or reduction of tanoak (*Notholithocarpus densiflorus* Hook. & Arn.) and California bay laurel (*Umbellularia californica* Hook. & Arn), the main hosts supporting sporulation of *Phytophthora ramorum* (Valachovic and others 2010). In these treatment areas, subsequent rates of infection on re-sprouting tanoak and bay laurel varied widely, but were very low where bay laurel was either absent or in low densities at the time of treatment. Additionally, infection rates were substantially lower in treated areas than in adjacent untreated ones (Valachovic and others 2013b). Ten years after the completion of these treatments, we examined some of the other effects at the treatment sites (fig. 1), particularly effects on fuel loading, tree regeneration, and shrub establishment (fig. 2), and reflect upon lessons learned.

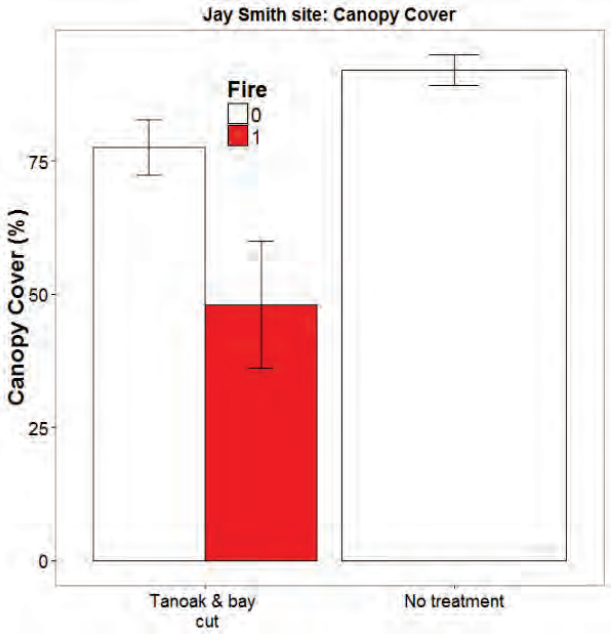

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Figure 1—Canopy cover differences as related to treatment type at the Jay Smith site. Treatments included removal of tanoak and California bay laurel with (1) and without (0) the addition of prescribed fire.

The treatments have resulted in differences in fuel loads and host tree regeneration between burned and unburned treatments, as well as between these treatments and an herbicide treatment targeting both California bay laurel and tanoak. The long-term effects following the cutting of tanoak and bay laurel host trees, plus prescribed fire, included fire-related mortality of large diameter Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) trees—probably conditioned by pre-existing site factors that gave these trees increased susceptibility to fire damage, insect and pathogen attack, and drought stress. This treatment also resulted in subsequent high densities of regenerating native and non-native shrub species (fig. 2). In contrast, in the unburned treatment areas, the treatment did not cause Douglas-fir mortality and gaps were re-populated mostly by re-sprouting tanoak and/or bay laurel. However, we note that regenerating bay laurel and tanoak stump sprouts have become infected by *P. ramorum* at fairly high rates across one of the sites, suggesting that this species may support re-invasion by the pathogen even after treatment. The density of bay laurel stump sprout clumps, saplings, and trees surrounding a given stump sprout clump is significantly related to its infection rate, and infection rates were much lower on 2-yr-old sprouts (cut manually 5 years after treatment) than 7-yr-old ones (fig. 3).

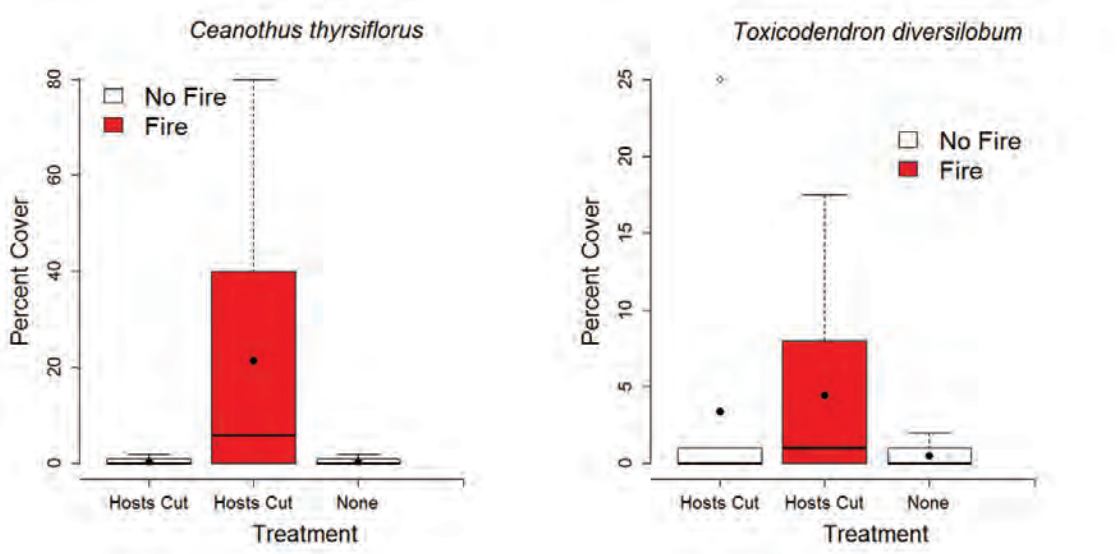

Figure 2—Changes in canopy cover, plus the addition of the prescribed fire at the Jay Smith site, stimulated *Ceanothus thyrsiflorus* and *Toxicodendron diversilobum*. Boxplots with mean shown as black dot.

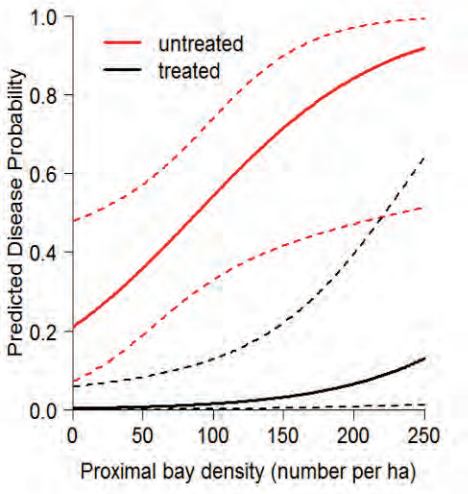

Figure 3- The relationship between probability of a sprout clump becoming infected and density of proximal bay laurel individuals (stump sprout clump, sapling, or trees). Black lines represent the sprouting bay stumps from the 2006 treatments that were cut again in 2011; red lines represent sprouting bay stumps with no further treatment since the 2006 treatment. Dashed lines represent 95% confidence intervals around the predicted probability of disease.

Fine woody debris, coarse woody debris, and duff and litter depth (measured by Brown's Transects) showed some differences among treatments. While coarse woody debris was not significantly different among treatments, the amount of coarse woody debris of broadleaf species (tanoak, bay, and madrone) at the Jay Smith site in 2016 was significantly related to the basal area cut in the treatments ($p=0.00013$; $R^2=0.64$; fig. 4a). Duff depth in the treatment with prescribed fire after cutting of hosts at Jay Smith was still significantly lower in 2016 than the other two treatments (Analysis of Variance with Bonferroni-adjusted comparisons; $p < 0.0001$; fig. 4b); a similar pattern was seen with litter depth (data not shown).

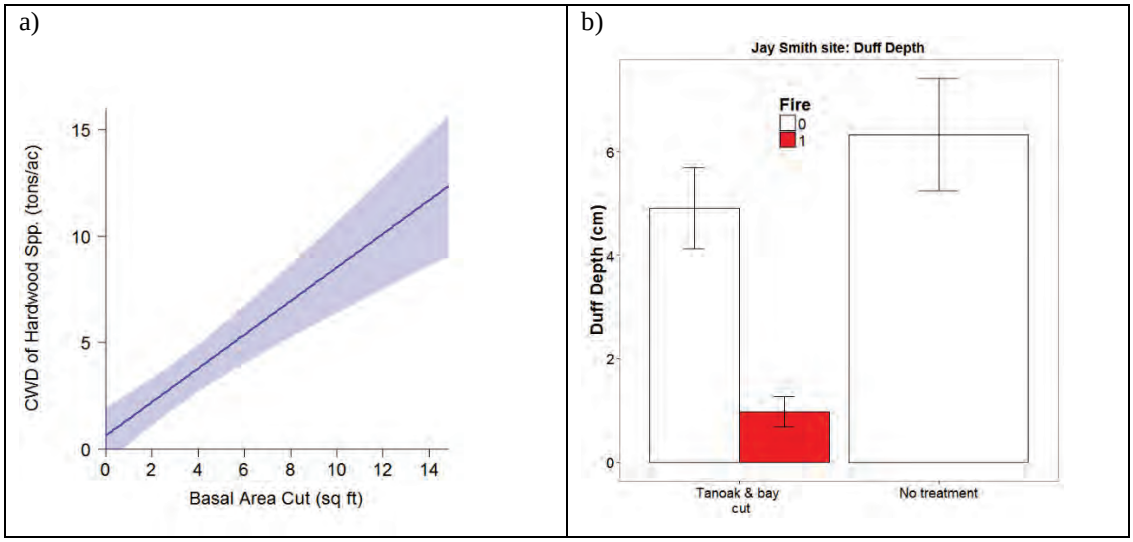

Figure 4—a) Coarse woody debris of hardwood species vs. basal area cut in treatment (with 95% confidence interval); b) duff depth by treatment.

The results of these studies are useful to inform future disease suppression treatments. Managers should be aware of the need for follow-up treatment to manage potential impacts triggered by disease suppression efforts (Valachovic and others 2013a). Future research using multiple rounds of fire to provide such follow-up treatments is needed. Additionally, results highlight the necessity to carefully evaluate potential tree-stressing environmental factors that could be exacerbated by disease management treatments. Treatments for sudden oak death, as with any treatment for pest management, should be conducted within a context of long-term forest development and forest management goals.

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Reducing CO_2 Emissions by Managing for Sudden Oak Death…Is It Possible?[1]

B. Twieg,[2] Y. Valachovic,[2] R. Cobb,[3] and D. Stark[2]

Abstract

Forest CO_2 emissions, which have recently become a more regular concern in forest management, can radically increase following pest and disease outbreaks. We inventoried trees in a stand adjacent to an infested area in northern Humboldt County, California, and used a stand-level dynamic disease model to forecast *Phythophthora ramorum*-caused tanoak mortality with and without a proposed treatment to mitigate disease spread and impacts. Using forest growth simulations employing model-predicted mortality rates, a proposed treatment that removes California bay laurel and reduces tanoak stem density results in a substantial increase in the forecasted basal area of tanoak retained after 100 years, along with an increase of up to about 100% in the amount of CO_2 emission equivalents stored as carbon in live trees. While the magnitude of treatment benefit varies depending on how parameters are applied in the disease model and growth simulator, all of the scenarios we attempted resulted in net benefits to retention of larger tanoak and carbon storage.

Introduction

The California Air Resources Board (CARB), under State Assembly Bill 32 (the Global Warming Solutions Act of 2006), is tasked with assuring that California achieves a reduction of greenhouse gas (GHG) emissions to 1990 levels by the year 2020. This equates to reducing emissions to 431 million metric tons CO_2 equivalent ($MMTCO_2e$) by 1990 and represents a reduction of 15% as compared to a "business as usual" scenario. According to CARB's 2016 Edition of the GHG Emission Inventory (Released June 2016), California has already reduced its annual emissions from 479.8 in 2006 to 441.5 $MMTCO_2e$ as of 2014. Understanding that forests in California have the capability to store huge amounts of carbon as living biomass (and potentially later as durable wood products), CARB has provided revenues from its Cap and Trade Program to fund forestry projects that forecast net GHG emission reductions. Battles *et al.* (2014) recently estimated that there are 1,050 $MMTCO_2e$ in living trees in California. Thus, even a relatively small proportional change in the amount of carbon stored in forests could have a large positive effect on GHG reduction.

In infested locations where death of mature tanoak (*Notholithocarpus densiflorus* Hook. & Arn.) boles has occurred at high rates over several years due to *Phytophthora ramorum* activity, it is common that dense tanoak re-sprouts perpetually dominate the vegetation (see Cobb, Restoration of Mount Tamalpais Forests Destroyed by the Sudden Oak Death Pathogen, this Proceedings). While the individual tanoak stems from re-sprouting stumps in continually infested forests are most likely to also die from the pathogen before getting beyond sapling stage, more sprouts replace them, and the cycle continues. The continuing presence of *P. ramorum* thus results in substantially less carbon being stored in these forests, where a community of mature tanoak trees does not re-establish.

The dynamics of *P. ramorum* and associated tree mortality in forested stands are largely dependent on the composition and structure of tree communities. With tanoak and California bay laurel (*Umbellularia californica* Hook. & Arn.) both supporting significant amounts of *P. ramorum* pathogen sporangia on

their above-ground tissues, the abundance of these hosts are centrally important in predicting disease effects in forest stands, and stands with lower densities of these hosts generally experience slower disease spread and lower mortality rates (Cobb et al. 2012). With tools to model disease-related mortality of tanoak in forest stands, along with other tools to forecast tree growth and biomass in forests, we can examine how forest management activities can alter disease impacts, and, ultimately, GHG emissions. In this project we examined currently non-infested stands adjacent to a relatively isolated infestation and used these tools to explore the potential effects on disease dynamics and carbon storage under no management and stand treatment scenarios.

Methods

The study area used for the project is a 52-acre unit along a ridge separating Lacks Creek and Stover Creek (both tributaries to Redwood Creek) in northern Humboldt County, California (T8N, R3E, HB&M). To determine initial stand composition, we measured 14 systematically located plots in 2014, using variable radius plots to estimate tree basal area and height and 1/10th-ac fixed plots to estimate density of stems 1 to 4.9 in diameter at breast height (DBH). We classified the unit up into three stand types and used USDA Forest Vegetation Simulator (FVS; v. Feb. 2016; see Crookston et al. 2005) software to summarize initial stand conditions. We then used the average conditions (weighted by stand area) in the unit to input stem density proportions by species into the Sudden Oak Death Dynamics in R (SODDr) model (Ross 2012); this implements the models created by Cobb et al. (2012) in a package for R Statistical Software. We ran the model, starting with a single initial infection location, to predict tanoak mortality over 100 years under the no-treatment scenario and under the scenario in which a treatment is conducted. The treatment consists of cutting down California bay laurel trees in the unit as well as suppressing all sprouts and thinning of tanoak stems, cutting the smallest stems first, to an average stand spacing of 15 by 15 ft. (about 200 stems per acre), with cut trees left on the ground.

Using the tanoak mortality predicted by the model, we adjusted tanoak mortality in FVS at the cycle start/end points. We also selected three different options in FVS for tanoak mortality: 1) mortality occurs uniformly in space and across all size classes of tanoak; 2) mortality occurs in large trees first (supported by Cobb et al. 2012), but is spatially uniform; and 3) mortality occurs uniformly across size classes, but is spatially concentrated at points in the stand. Under a no- treatment scenario, we assumed the disease would first infect the stand in 2017. Under scenarios with treatment, we ran simulations with the disease first infecting the stand in 2017. We also ran simulations in which the disease does not infect the stand until 2032, since the thinned stand may be less likely to become infected.

We adjusted upward tanoak sprouting rates in FVS to reflect actual rates, based on data from an adjacent, similar stand of 45 acres one year after a similar thinning treatment. Since FVS simulates sprouting of tanoak after thinning, but not after mortality, we added rates of natural regeneration corresponding to the sprouting rate per individual and to the number of individuals predicted to die in each cycle in an initial simulation. We generated carbon reports with the Fire and Fuels extension (FFE) in FVS, using the option of Jenkins et al. 2003 biomass equations, and calculated CO_2e for each of live biomass (99.7-99.9% of which is estimated to be in trees), dead material, and their sum.

Results

In 2014, there was an average of 558 stems per acre of tanoak at least 1 in diameter at breast height (4.5 ft; DBH). The thinning treatment scenario removes ~390 tanoak stems per acre at least 1 in DBH, plus all smaller tanoak stems; this produces a stand with tanoak in the 9-40 in DBH range, plus components of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco), Pacific madrone (*Arbutus menziesii*

Pursh), and giant chinquapin (*Chrysolepis chrysophylla* Dougl. ex Hook.). California bay laurel trees comprised about 2.5% of the initial stems.

The disease model predicts that tree mortality occurs at a faster rate under untreated stand conditions (fig. 1a), but the proportion of the stand stems that are tanoak is similar after 100 years whether or not the treatment is done (also see table 1). However, the thinning treatment results in a stand structure with fewer, larger tanoak trees; quadratic mean diameter under the thinning scenarios is projected to be consistently higher (data not shown). Similarly, the forecasted basal area of tanoak in the stand in 2117 is substantially higher in thinned stands than untreated ones—from 35% to 131% higher with thinning undertaken in 2017 than without (table 1). The thinning treatment is also consistently projected to have a carbon benefit. After 100 years of SOD dynamics and forest growth, the thinned stand is projected to have a maximum benefit (out of the simulated scenarios) of 23,624 metric tons of the CO_2 equivalent in live trees (fig. 1b), and a minimum benefit of 6,917 metric tons (scenario 3 in table 1). Live-tree carbon differences between disease introduction times under the thinning scenario are negligible (fig. 1b).

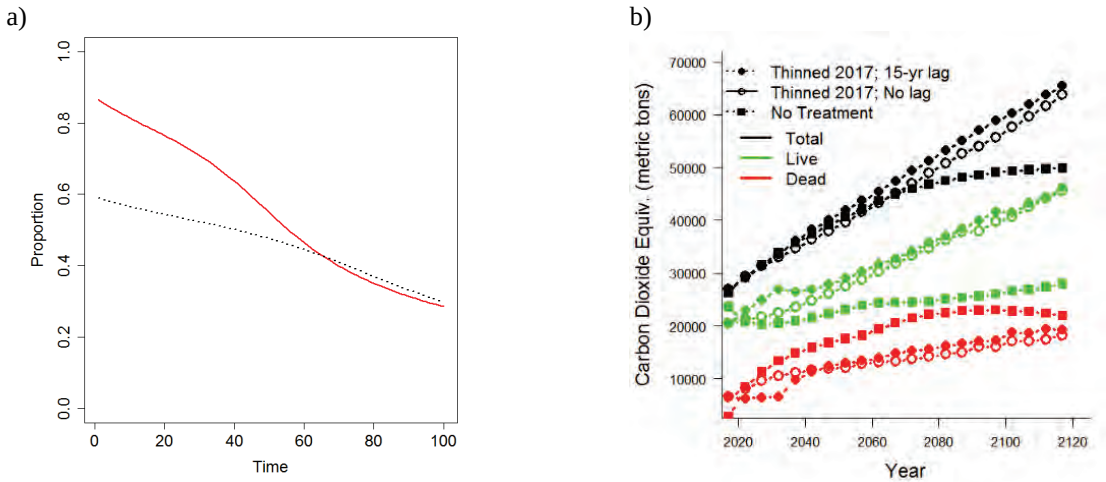

Figure 1—a) Predicted proportion of total tree stems that are tanoak in untreated (red line) and thinning treatment (black dotted line) conditions over time (in years); b) Forecasts of CO_2 equivalent present in the stand, 2017 through 2117, according to scenario 3 described in table 1.

Table 1—Stem density and basal area of tanoak forecast in 2117 under different scenarios

Scenario	Tanoak projected in year 2117	
	Stem density (trees per acre)	Basal area (square ft ac^{-1})
Mortality uniform in space and among size classes (1)		
Stand is thinned; disease arrives in 2017	189	397
No treatment; disease arrives in 2017	189	265
Mortality uniform in space; large trees killed first (2)		
Stand is thinned; disease arrives in 2017	153	343
Stand is thinned; disease arrives in 2032	161	359
No treatment; disease arrives in 2017	182	147
Mortality clustered; uniform among size classes (3)		
Stand is thinned; disease arrives in 2032	246	394
No treatment; disease arrives in 2017	192	293

Discussion

Our results suggest that removal of California bay laurel and thinning of tanoak in advance of *P. ramorum* arrival into a stand can have positive effects on retention of tanoak in larger size classes, and these effects also come with a benefit in terms of the amount of carbon retained in the stand. In this project, GHG reduction benefits were forecast under several different scenarios that account for some of the potential variability in mortality patterns. For future studies, it would be useful to include within-stand spatial heterogeneity of host distribution in the disease mortality model, Current work is underway at Humboldt State University to make the SODDr package more conducive to this. Additional attention should also be given to tanoak sprouting dynamics in FVS and regional differences. The potential avoidance of CO_2 emissions associated with similar treatments is potentially far higher than estimated here because tanoak mortality from *P. ramorum* also increases the likelihood that wildfires are less manageable and their effects more severe (Valachovic et al. 2011). Thus, management targeted toward areas vulnerable to *P. ramorum* can achieve several goals beyond reducing the spread of the disease through the landscape.

Acknowledgments

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Restoration of Mount Tamalpais Forests Destroyed by the Sudden Oak Death Pathogen[1]

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Abstract

On Mt. Tamalpais, after nearly 20 years of accumulated disease impacts, some tanoak- (*Notholithocarpus densiflorus*) dominated forests where *Phytophthora ramorum* first emerged have converted to brushy fields of tanoak resprouts. *Phytophthora ramorum* has invaded throughout the greater San Francisco Bay Area, and damaged the culturally, ecologically, and economically important forests of Mount Tamalpais, including many stands managed by the Marin Municipal Water District (MMWD) for water yield and recreation. Sustained inoculum loads have resulted in extensive tanoak mortality, in some places almost 100% of initial overstory tanoak trees have been killed by the disease. Tanoak resprouting has formed undesirable forest structure where occasional redwood (*Sequoia sempervirens*) overstory trees co-occur with dense, tanoak shrub understories. These conditions are significant management concerns from the perspectives of fuel loads, maintenance of biodiversity, aesthetics and provisioning of water resources. We instituted a series of replicated management experiments on MMWD lands to identify the most economically and ecologically efficacious actions to restore overstory trees and ecological functions.

We established 30, 1 ac treatment plots across three MMWD sites and randomly assigned treatments in blocks of five plots. In 25 plots, all understory tanoak and shrubs were masticated using a combination of an excavator with a masticating head, a skid-steer with a forestry attachment (masticator head), and hand crews. Treatments removed all hardwoods while retaining conifer (redwood, *Sequoia sempervirens*; Douglas-fir, *Pseudotsuga menziesii*) regeneration. Additional treatments will include manipulation mulch generated by mastication and replanting with *P. ramorum*-resistant species. Each plot was instrumented to measure water outflow, soil respiration, as well as estimate annual net primary productivity and nitrogen use efficiency. Mastication treatments greatly reduced fuel loads, understory density, and prevalence of sporulation supporting species. Using a set of models parameterized with field data, we found that 90% of intact tanoak overstory trees are expected to be retained by the treatment, in part because these individual trees will be isolated from inoculum sources. Soil moisture rapidly increased to field capacity during the onset of winter rains and significant outflow to deep soil layers was observed. Long-term efficacy of these treatments for the goal of restoring carbon sequestration and sustaining water yield are dependent on the success of efforts to reestablish other overstory trees which are not susceptible to *P. ramorum*. These are the first landscape scale restoration treatments implemented in SOD damaged areas, restoration in other habitats is planned to learn more about how to restore ecological benefits in the thousands of acres significantly degraded by SOD in the region.

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Managing Sudden Oak Death on Federal Lands in Southwest Oregon: Triumphs and Challenges[1]

Ellen Michaels Goheen[2]

Abstract

Since 2001, approximately 5,350 acres of tanoak forests in Curry County, Oregon have been treated to eradicate *Phytophthora ramorum* and slow the spread of sudden oak death. Over 1,300 of these acres are on lands administered by the USDI Bureau of Land Management (BLM CB), Coos Bay District and the USDA Forest Service, Rogue River-Siskiyou National Forest (USFS RRS). Treatments include using herbicides to reduce tanoak sprouting, cutting, piling and burning known infected tanoaks, and cutting, piling, and burning exposed tanoaks and other selected hosts in a buffer area around known infected trees.

Affected sites have ranged from highly accessible and heavily used hiking trails to remote, relatively inaccessible and rugged terrain. Treatments have occurred in many different land allocations including Inventoried Roadless Area, Late Successional Reserve, and Wild and Scenic River Corridor. Over the years the BLM CB and USFS RRS have made great progress to streamline the procedural side of treating sudden oak death. The tools being used include multi-year contracts with designated contractors and programmatic consultation with federal regulatory agencies. Positions dedicated to sudden oak death management have been created and knowledgeable people are in those positions. But still, access, fire restrictions, timing of funding, and timing treatments to avoid disturbing nesting northern spotted owls and marbled murrelets pose challenges to managing sudden oak death as rapidly as possible.

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Ecology: Forest Change Over Time

Coast redwood, *Sequoia sempervirens*

Long-Term Monitoring of Sudden Oak Death in Marin County and the East Bay Hills[1]

Brice A. McPherson,[2] Greg Biging,[2] Maggi Kelly,[2] and David L. Wood[2]

Abstract

Prior to 2000 the etiology, effects on host trees, and possible consequences for northern California's forests of the syndrome known as sudden oak death were unknown. We designed a plot-based study to address these issues and to set a baseline for future evaluations.

In March-April 2000 we established a total of 20 plots in two forested areas in Marin County: China Camp State Park [CCSP] (10) and Marin Municipal Water District [MMWD] (10). The host species monitored were coast live oak [CLO] (*Quercus agrifolia*) and California black oak [CBO] (*Q. kelloggii*) in both sites and tanoak [TO] (*Notholithocarpus densiflorus*) in MMWD. We employed symptom-based monitoring on every stem >2-cm DBH (1.37-m), twice per year through 2007, then once per year through 2015. Symptom categories were bleeding; bleeding plus ambrosia and bark beetle attacks; bleeding plus beetles plus *Annulohypoxylon thouarsianum* sporocarps; and death. Trees that died without these symptoms were classified separately. Between 2000 and 2015, asymptomatic CLOs (n = 683) decreased from 68.8% to 42.9% and mortality increased from 6.7% to 40.5%. For CBO (n = 52), asymptomatic trees declined from 82.7% to 47% and mortality increased from 1.9% to 40.8%. For TO (n = 132), the asymptomatic trees decreased from 62.9% to 21.9% and mortality increased from 6.1% to 62.3%. The percentages of symptomatic trees declined from 22.4% to 8.7% for CLO and 31% to 15.9% for TO.

Of the CLOs that were asymptomatic in 2000 (n = 454), 22% were dead with SOD symptoms by 2015 and 10% were symptomatic. However, another 14% were in remission, which we define as cessation of bleeding for at least three years prior to 2015 in a previously symptomatic tree (in the absence of beetle attacks). Although the long-term durability of remission is not known, our previous estimates of CLO infection levels did not recognize the remission category.

Phytophthora ramorum was not detected in Alameda County until 2001, 7 years after the first mortality was observed in Marin County. This presented an opportunity to apply our knowledge of SOD to provide the East Bay Regional Park District (EBRPD) with a scientific basis for developing management plans. We initiated landscape-scale monitoring of CLO in five EBRPD parks in 2008 to determine the extent of the epidemic in the oak-bay vegetation type and to estimate infection and mortality rates. A total of 535 10-m radius fixed plots were randomly assigned in oak-bay stands between 2008 and 2011. In three large parks, Redwood, Wildcat Canyon, and Anthony Chabot, infection levels in 2015 were between 14.3% and 19.0%. By examining only CLOs that were asymptomatic in 2011 in these three parks, we calculated infection rates between 3.6% and 4.8% per year. Based on the estimated numbers of mature (>20-cm DBH) CLOs in these parks, we can predict approximately 5,000 new infections per year per park. It is of interest that these new infections occurred during a historical drought that had been expected to reduce infection rates. We have been sampling phloem from trees that exhibit remission or have remained

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asymptomatic in sites with high infection levels to estimate the levels of resistance across the parks using chemical biomarkers (in collaboration with the Bonello lab at Ohio State University).

Novel Interactions between Wildfire and Sudden Oak Death Influence Sexual and Asexual Regeneration in Coast Redwood Forests[1]

Allison B. Simler,[2] Margaret R. Metz,[3] Ross K. Meentemeyer,[4] Kerri M. Frangioso,[5] and David M. Rizzo[5]

Abstract

Novel interactions between compounded disturbances can leave lasting ecological legacies on communities and alter regeneration trajectories. Sudden oak death (SOD), caused by *Phytophthora ramorum*, is a biotic disturbance, an emerging disease causing widespread oak and tanoak mortality in California's coastal forests. In these redwood-tanoak forests, wildfire is a keystone process, shaping stand structure and composition. Dominant tree species can resprout rapidly after being "top-killed" by fire, in addition to regenerating via seed. Yet, in SOD-impacted areas, fuels generated by disease-related tree mortality may alter fire behavior and consequent stand recovery. In this system, tree species differ in resprouting capacity, patterns of seedling regeneration, effectiveness as hosts for *P. ramorum*, and susceptibility to fire and SOD.

In 2006 and 2007, we established 280 forest plots to monitor the impacts of SOD in the Big Sur region. The 2008 Basin Complex fires burned across infested and disease-free plots in this area, generating an opportunity to investigate impacts of a novel disturbance interaction on forest regeneration. Following the fire, burned and unburned forest plots were repeatedly sampled to assess tree mortality, pathogen presence, microclimate, and regeneration, including resprout and seedling recruitment across a gradient of SOD impacts. We investigated how interactions between fire and disease influence forest regeneration trajectories, and in turn, how regeneration in host tree species impacts post-fire disease dynamics and prevalence of *P. ramorum*.

Tanoaks in mid-stage SOD-infested areas were more likely to suffer complete (belowground) post-fire mortality, likely due to altered fuels and increased fire severity in disease-impacted areas. Pre-fire tree size and stand-level interactions between SOD and fire significantly influenced the abundance of resprouting regeneration measured in tanoaks that survived top-killing. In plots impacted by fire and SOD, individual tanoak resprouting vigor was significantly greater than expected by our model. This suggests that the pathogen is not measurably hindering re-growth of tanoak (a susceptible host) in burned, disease-impacted areas, despite reinvasion of *P. ramorum*. We hypothesize that this is due to decreased host connectivity and severe microclimates in burned areas. This also suggests that tanoaks surviving a bottleneck generated by SOD and fire experience reduced competition, responding with increased resprouting.

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Successful recruitment via seedlings is less common than recruitment via vegetative reproduction in this system. Following wildfire, seedling survival was similar across infested and uninfested areas, and post-fire seedling communities were similar regardless of SOD disease history, suggesting that wildfire, as a disturbance, may have a stronger overall influence on these sexual regeneration patterns. Areas impacted by SOD show pulses in tanoak and oak seedling recruitment associated with periods of disease-related tree mortality. The presence of *P. ramorum* did not significantly reduce seedlings and saplings' likelihoods of survival, echoing previous results that suggest that smaller trees may be less readily impacted by SOD.

Patterns of regeneration also influenced post-fire recovery of *P. ramorum*. In burned areas, the pathogen was significantly more likely to be recovered in larger bay laurel sprouting clusters and in areas with more surviving canopy cover, suggesting that resprouting and microclimate may play a key role in post-fire disease reinvasion. These results suggest that systems dominated by asexual regeneration may be surprisingly resilient to compounded disturbances, and despite reinvasion, *P. ramorum's* impacts may be temporarily reduced by fire-related changes to the stand.

Vibrational Spectroscopy-Based Chemometrics to Map Host Resistance to Sudden Oak Death[1]

Pierluigi (Enrico) Bonello,[2] Anna O. Conrad,[2, 3] Luis Rodriguez Saona,[2] Brice A. McPherson,[4] and David L. Wood[4]

Abstract

A strong focus on tree germplasm that can resist threats such as non-native insects and pathogens, or a changing climate, is fundamental for successful conservation efforts. This project is predicated on the fact that genetic resistance is the cornerstone for protecting plants against pathogens and insects in environments conducive to the attacking organisms, a principle we have extensively applied to the study of coast live oak (CLO – *Quercus agrifolia*) interactions with *Phytophthora ramorum* in California wildlands. The largest obstacle to the implementation of host resistance as a tree health management tactic in forest environments, as well as for germplasm conservation, is the lack of tools for the rapid phenotyping of tree disease resistance in the field. Previously, in work conducted in Briones Regional Park, East Bay Regional Park District (EBRPD), Alameda County, California, we have demonstrated that the levels of soluble phenolics extracted from CLO trunk phloem can predict resistance to *P. ramorum* by way of a logistic regression model that included total phenolics and four individual phenolic compounds: ellagic acid, a partially characterized ellagic acid derivative, and two chromatographic peaks representing two uncharacterized phenolic compounds (McPherson and others 2014a). *In vitro* tests subsequently showed that ellagic acid was fungistatic against *P. ramorum* and total phenolics were fungicidal at physiologically relevant concentrations.

In further developments, here we show that Fourier-transform infrared (FT-IR) spectroscopy, a chemical fingerprinting technique, can also be used to identify CLO resistant to *P. ramorum* prior to infection (Conrad and others 2014). Soft independent modeling of class analogy identified spectral regions that differed between the resistant and susceptible trees in Briones Regional Park. Based on chemometrics, resistant CLOs constituted 20% of the population, which was about the same as the average estimate based on disease expression. Regions most useful for discrimination were associated with carbonyl group vibrations, which are often associated with phenolics. Additionally, the levels of two putative phenolic biomarkers of resistance, including ellagic acid and an unidentified phenolic, were predicted using partial least squares regression; > 99% of the variation was explained by this analysis. We expect that our tool will be a real game changer in sudden oak death (SOD) management, because currently there is no other technology to determine tree resistance in advance of a disease front (Conrad and others 2016). This predictive power is unprecedented for field trees, and recent work in other pathosystems, specifically whitebark pine-white pine blister rust, and Port-Orford-Cedar – *Phytophthora lateralis*, has shown that FT-IR spectroscopy can be used to predict resistance in the progeny of well-characterized families, in addition to that of the parent trees.

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We are now planning to apply these novel techniques to map resistant CLO in Redwood Regional Park, (EBRPD) (McPherson and others 2014b). The park is rather well characterized in terms of SOD incidence and distribution, based on longitudinal studies anchored on permanent plots. We expect to demonstrate that FT-IR spectroscopy can be a useful approach for managing forests impacted by SOD, for example for protecting resistant populations from fire, logging, and development, as well as in other situations where emerging or existing forest pests and diseases are of concern.

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Urban Activities Influence on *Phytophthora* Species Diversity in British Columbia, Canada[1]

Angela Dale,[2] Nicolas Feau,[2] Julien Ponchart,[3] Guillaume Bilodeau,[4] Jean Berube,[5] and R.C. Hamelin[6]

Abstract

Phytophthora de Bary, a genus of Oomycetes, is known as a plant pathogenic genus. The best-known species infect a wide range of hosts, including economically valuable angiosperm and gymnosperm tree species and important agricultural crops. Many *Phytophthora* are invasive and have been disseminated through nursery and agricultural trade. We hypothesize that such human activities would affect the diversity of these pathogens.

To test this hypothesis, we characterized and compared *Phytophthora* diversity between natural and urban environments in British Columbia. We collected soil samples from sites in urban and natural locations or at the interface of urban/natural areas around Vancouver, British Columbia and south Vancouver Island, in 2012 and 2013. DNA was extracted from 130 soil samples and DNA metabarcoding was carried out using 454 pyrosequencing of the internal transcribed spacer one (ITS1). In 2011, five waterways classified as urban and located around agricultural or residential areas were baited with mesh bags containing Rhododendron leaves. Leaves were collected bi-weekly for 10 weeks. *Phytophthora* species were isolated on specific media and barcoded using the internal transcribed spacer (ITS1 and ITS2).

In the 2012 soil DNA metabarcoding analyses, 25 putative *Phytophthora* species were recovered, eight of which are potentially new species. The most widespread species were *P. syringae* and a hybrid between *P. polonica* and an unknown species. Urban sites had the highest average species diversity at eight species and ranging from three to 12 species per site, whereas natural sites had an average of six species and ranged between four to eight species per site. Urban/natural interface sites had an average of five species and ranged from two to seven species per site. In total, 23, 14, and 11 species were found in urban, urban/natural interface, and natural locations, respectively. Interestingly, most of the unknown species were found in urban or urban/natural interface sites. Several species found only in urban sites were present in low frequency and could represent introductions via urban activities.

In the stream baiting experiment, 17 different *Phytophthora* species were found; the most widespread were *P. gonopodyides* and *P. lacustris*. Eight species were common to both DNA metabarcoding and baiting experiments; however, the frequencies varied with *P. gonopodyides* and *P. lacustris* found in low frequency in soil metabarcoding experiments and *P. polonica* in lower frequency in stream baiting. Although *P. syringae* was the most frequent species in the metabarcoding experiment in the soil, it was

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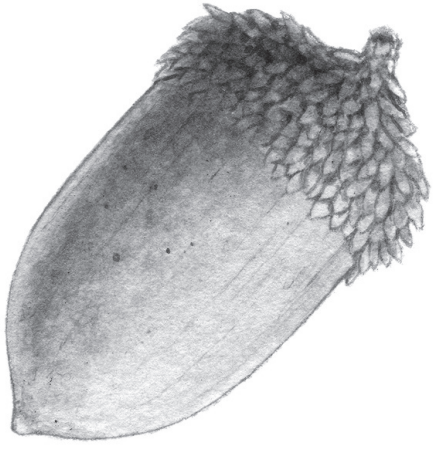
not found in the stream baiting experiment. This study suggests that urban activities influence *Phytophthora* diversity and composition. Future work should focus on establishing whether or not unknown species found in urban environments are the result of introductions and evaluating the likelihood that they could become established invasive species in natural environments.

Acute Oak Decline in the United Kingdom



Tanoak, *Notholithocarpus densiflorus*

A Polyphasic Approach to Gaining Insights Into Causes of Acute Oak Decline in Britain[1]

Sandra Denman[2]

Abstract

Acute Oak Decline (AOD) is a complex disease syndrome of native oak, making its first appearance in the 1980s in Britain. Since then increasing reports of its occurrence have raised concerns about cause and effects on these iconic trees. Symptomology studies confirmed that AOD is a distinctive condition with four diagnostic symptoms defining the condition; (a) weeping patches on trunks of affected trees, (b) cracks between the bark plates, (c) inner bark necrosis and sapwood degradation underlying the weeping patches, (d) over 95% co-occurrence of larval galleries of a native bark boring buprestid, *Agrilus biguttatus,* in the cambial tissues. A polyphasic approach encompassing landscape to molecules, to determine possible causes of the syndrome has been adopted. The microbiomes of healthy and symptomatic trees were compared using:(a) conventional methods, (b) metabarcoding of the V3-V5 fragment of the bacterial 16S rRNA gene and the fungal ITS gene region, (c) 454 pyrosequencing of total metagenomes, and (d) pyrosequencing the metatranscriptome (rRNA and mRNA) of the lesions on AOD affected trees. Microbiome analyses supported isolation data, which showed a shift from healthy to diseased trees, with members of the *Enterobacteriaceae* dominating the lesions. Two novel species, *Brenneria goodwinii* (*Bg*) and *Gibbsiella quercinecans* (*Gq*), were consistently associated with necrotic tissue, suggesting a role in lesion formation, but other species such as *Lonsdalea quercina* ssp. *britannica* and *Rahnella victoriana*, which was present in both healthy and diseased trees, may also play a role. Recognition of the involvement of multiple agents led to the hypotheses that (a) bacteria, particularly *Bg* and *Gq*, have a role in causing degradation of oak phloem and sapwood, (b) interaction between *A. biguttatus* and these bacteria lead to typical AOD symptoms, (c) AOD is a complex Decline disease dependent upon the interaction of multiple factors for disease establishment. The methods used to determine the necrogenic capability of the bacteria include: (a) host inoculation tests, (b) pathogenicity assays using wild types, knock-out mutants and visualizing green/red/yellow fluorescent transformed bacteria, (c) whole genome sequencing to determine pathogenicity and virulence genes, (d) metagenomics to characterize taxonomic and functional abundance of organisms present in healthy and diseased trees and (e) transcriptomics to extract expressed virulence genes from the polymicrobial community, and to analyze gene expression of *Bg* and *Gq in vitro* alongside phloem and sapwood, (f) metaproteomics to provide functional evidence of gene activity. In order to investigate the possible involvement of *A. biguttatus* in necrosis, larvae were reared in the laboratory and used in inoculation studies. Preliminary results of host inoculation tests show that bacteria cause necrosis in logs, but interaction between bacteria and larvae simulate all four symptoms of AOD. Pathogenicity assays indicate differing HR ability of the various bacterial species, but changes in necrogenic ability in mixed bacterial species inoculations occurred. Genome and metatranscriptome sequencing of *Bg* and *Gq* revealed encoded pathogenicity factors, with genes for plant cell wall degrading enzymes and secretion systems, furthermore transcripts aligning to these were recovered from an active lesion. Transcriptomic and proteomic work is ongoing but to date the evidence accumulated is highly supportive of a biotic cause of the disease with key roles for *A. biguttatus* and the named Enterobacterial bacteria.

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Monitoring for Pests and Diseases in Native Oak Woodlands: The Case of Acute Oak Decline in the United Kingdom[1]

Nathan Brown,[2] Stephen Parnell,[2] Frank van den Bosch,[2] Mike Jeger,[3] and Sandra Denman[4]

Abstract

In recent years, a novel form of decline has been observed in southern and central England. This syndrome has been termed acute oak decline (AOD) and affects native oak, *Quercus petraea* and *Q. robur*. Typical symptoms include bark cracks that weep dark exudates, which are caused by necrotic patches in the inner bark. Studies show bacteria are consistently isolated from lesion edges, with two species, *Gibbsiella quercinecan*s and *Brenneria goodwinii*, thought to cause tissue necrosis. *Agrilus biguttatus* (a native beetle, with apparently increasing populations) is often reported at affected sites. In fact, the small samples of inner bark taken for bacterial isolations revealed larval galleries in 36 of 38 AOD affected trees. Here, we present the findings of two monitoring exercises that were conducted to document AOD dynamics at different spatial scales. At the local scale, within stand dynamics were monitored at eight locations across England. This work was complimented by a national scale survey, which was used to investigate environmental predisposition factors.

The local scale: A 7-year study on the spatial and temporal dynamics of AOD, and occurrence of *A. biguttatus* was conducted at eight geographically separated sites, giving a first description of its epidemiology. Findings suggest affected trees occur in localized clusters rather than at random throughout the plots, pointing to a local, biotic, cause rather than wider scale environmental effect such as drought. In addition, contagion, spread between neighboring trees, was demonstrated at one heavily infected site. Lightly infected individuals have been shown to form callus over the previous year's stem symptoms and enter remission. This finding highlights the need for host predisposition, where drought may have a role, and suggests scope for resistance and management options.

AOD symptoms co-occur on the same trees as D-shaped exit holes, although many fewer oak show these external signs of the beetle. This is perhaps surprising given that galleries are consistently found below the bark, but a likely product of both a cryptic larval phase and successful host defenses. Beetle emergence is predominantly linked to a few heavily declined trees, with further stem bleeds occurring on trees in close proximity to these locations.

The national scale: Regulatory surveys to detect and establish the distribution of pests are resource intensive and costly. Advances in technology, including smartphones apps for symptom recognition and reporting, have enabled the collection of species distribution data by volunteers to occur with increasing

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frequency and accuracy. However, what data from volunteer sources tell us and how they can be best used to inform and direct official survey effort is not clear.

Here an extensive dataset on AOD gives a unique opportunity to ask how verified data received from the public can be utilized. Information on the distribution of AOD was available as (i) systematic surveys conducted by Forest Research throughout England and Wales (ii) AOD sightings reported through land owners, land managers, and members of the public (termed "self-reported" cases). Results indicate that the self-reporting data was representative of the infected area. By using the self-reported cases at the design stage the systematic survey could focus on defining the boundaries of the affected area. This maximized the use of available resources and highlights the importance of developing novel strategies tailored to volunteer programs. This example represents one of the first quantitative evaluations of the use of citizen science to estimate the distribution of a plant disease.

The survey data have been used to inform epidemiological models and to examine the relationship of AOD with climate and biogeographical variables, especially those that influence water availability.

Genetics

Tanoak, *Notholithocarpus densiflorus*

Transcriptome Analysis of Tanoak Reveals Divergent Mechanisms of Innate and Phosphite-Induced Resistance to *Phytophthora ramorum*[1]

Catherine A. Eyre,[2] Katherine J. Hayden,[2,3] Peter Croucher,[2] Shannon Schechter,[2] Jessica W. Wright,[4] and Matteo Garbelotto[2]

Abstract

Phosphite compounds have been used in the control of sudden oak death; however, their precise mode of action is not fully understood. To study the action of phosphite compounds in the context of naturally occurring host resistance, we first identified open-pollinated family groups that carried resistance, that is in which approximately 20% of offspring demonstrated a quantitatively resistant phenotype, with zero or little dieback post-inoculation. Then, multiple inoculations were performed on previously unchallenged members of these families, half of which had been treated with phosphite. Leaves were harvested and flash-frozen before and at 1 week after inoculation for RNA extraction. The remaining inoculated leaves were left intact, and the trees were followed over the course of 6 weeks to determine disease phenotype. The transcriptomes of saplings from two families were sequenced. Quantitative PCR for 80 targets in the same saplings was used for technical validation of the quantitative sequencing results, and in saplings from the two additional families for biological validation. The design and the phenotypic results allowed us to study gene expression during the disease response in phosphite-treated, resistant hosts (in which the treatment worked as expected); in phosphite-treated but susceptible hosts (in which phosphite was not effective nor was there innate resistance); and in untreated susceptible and resistant trees. As expected, we found that tanoak families differed in the presence of innate resistance and in the effectiveness of phosphite treatment. There were 9,705 genes that were differentially expressed between untreated resistant trees and untreated susceptible trees. In comparison, there were seven genes differentially expressed in the same comparison between susceptible and resistant phosphite-treated trees. Constitutive expression of some disease-related genes was linked with innate resistance: several genes were identified which had much increased expression in untreated, resistant trees prior to inoculation, but were more strongly expressed in untreated susceptible and phosphite treated trees after inoculation. These included genes associated with signaling and production of secondary compounds. Our results demonstrate the differences in mode of action of phosphite compounds from innate resistance, and an intriguing lack of difference in gene expression between phosphite-treated trees, whether diseased or apparently healthy.

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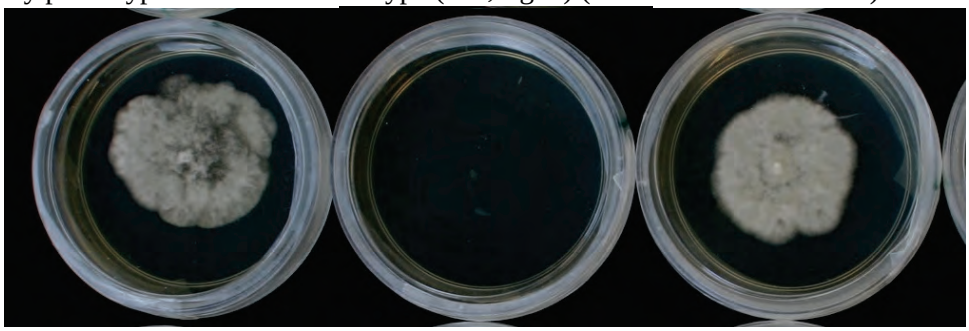
Host-Induced Genome Alterations in *Phytophthora ramorum* I. NA1 Lineage on Coast Live Oak in California II. EU1 Lineage on *Chamaecyparis lawsoniana* in UK[1]

Takao Kasuga,[2] Mai Bui,[2] Elizabeth Bernhardt,[3] Tedmund Swiecki,[3] Kamyar Aram,[4] Lien Bertier,[4] Jennifer Yuzon,[4] Liliana M. Cano,[5] Joan Webber,[6] Clive Brasier,[6] Caroline Press,[7] Niklaus Grünwald,[7] David Rizzo,[4] and Matteo Garbelotto[8]

Abstract

Rapid phenotypic diversification in clonal invasive populations is often observed, although the underlying genetic mechanisms remain elusive. Lineages of the sudden oak death pathogen *Phytophthora ramorum* are exclusively clonal, yet isolates of the NA1 lineage from oak (*Quercus* spp.) frequently exhibit host-dependent, unstable colony phenotypes called non-wild type (*nwt*, fig. 1) (Brasier and others 2006).



wt *nwt* *nwt* senesc

Figure 1—Colony types of NA1 lineage isolates of Californian *P. ramorum*. Note the variation in colony patterns and growth rates. *wt*: wild type, *nwt*: non-wild type. Sometimes *P. ramorum* isolates stop growing upon subculturing, which is termed senescence (senesc).

This phenotypic variation is seen despite the fact that population genetic and host-specificity studies negate any host-driven population subdivision. We also found comparable *nwt* phenotypes in EU1 isolates from Lawson cypress (*Chamaecyparis lawsoniana*) in the UK (fig. 2): isolates from the middle of a large ~4m long lesion on a mature tree were normal wild type (*wt*), while those from the extremities were variable *nwt* types. Previously only *wt* isolates were known in the EU1 lineage, including those from larch (*Larix*). Based on a large survey of genotypes from different hosts, we hypothesize that the

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environment in the bark of oak and Lawson cypress is responsible for the unusual phenotypic diversification in *P. ramorum*.

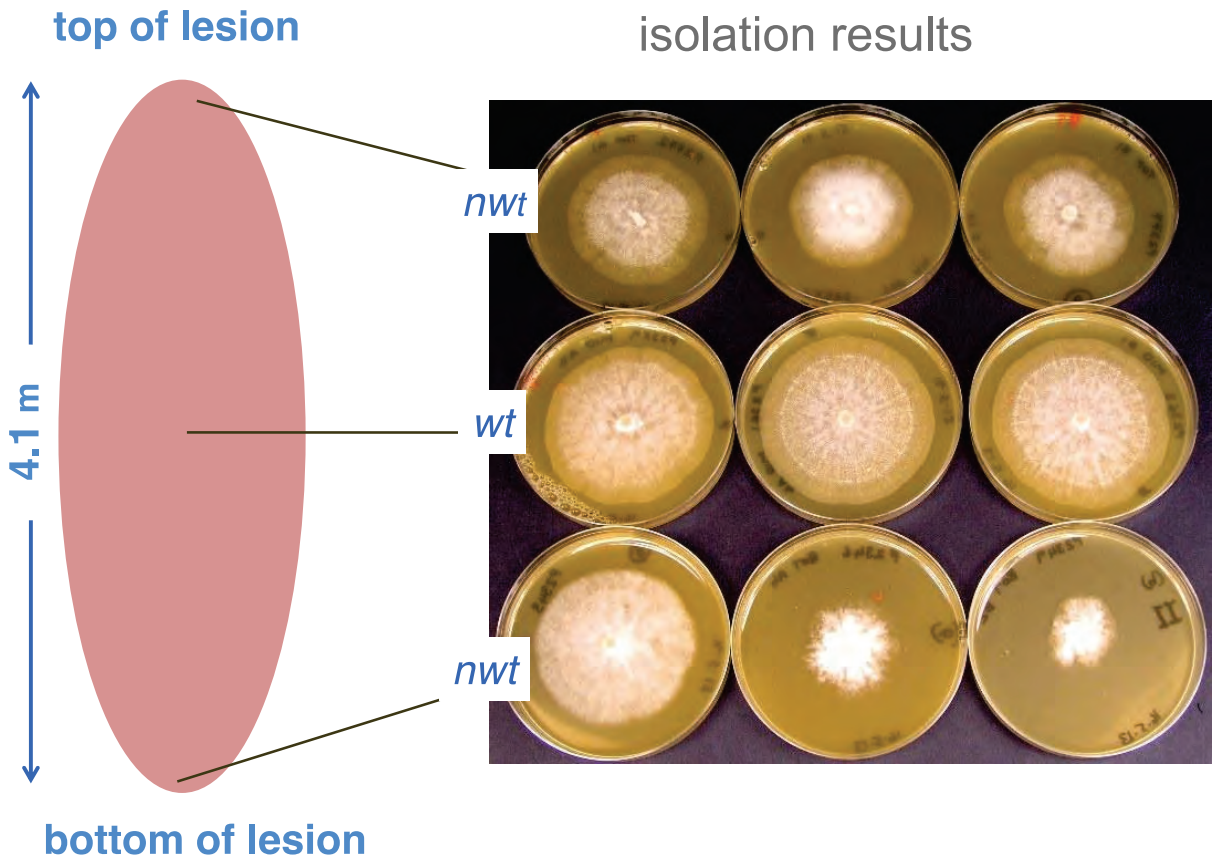

Figure 2—A single EU1 *P. ramorum* lesion developed in the stem of a 24m Lawson cypress yielded *nwt* colonies (top and bottom of lesion) as well as *wt* colonies (middle of lesion).

We conducted a series of passage experiments to test this hypothesis (Kasuga and others 2016). When *P. ramorum* NA1 isolates from foliar host California bay laurel (*Umbellularia californica*) were inoculated and re-isolated from mature canyon live oaks (*Q. chrysolepis*), both *wt* and *nwt* phenotypes were obtained. In the first inoculation experiment, 40%-60% of re-isolates displayed *nwt* phenotypes. In the second inoculation experiment, all re-isolates initially showed *wt* phenotype; however, some *wt* re-isolates from oak converted to *nwt* phenotypes during *in vitro* growth. In contrast, no such phenotypic changes were observed when isolates from California bay laurel and *Camellia* were inoculated and re-isolated from the foliar hosts California bay laurel, *Viburnum*, and *Rhododendron*.

High-throughput sequencing-based analyses identified major genomic alterations in NA1 *nwt* isolates from oaks included partial aneuploidy and copy-neutral loss of heterozygosity. Chromosomal breakpoints were found to be located at or near transposons, linking transposon de-repression caused by the chemical environment of oaks to structural genomic changes. Similarly, two EU1 *nwt* isolates and one *wt* isolate from *C. lawsoniana* exhibited large chromosomal regions with copy number variations and loss of heterozygosity, consistent with the isolates being copy number heterokaryons. Such genomic alterations were not identified in NA1 isolates from foliar hosts. In support of the sequence-based analysis, flow cytometry showed that in contrast to foliar isolates, nuclear contents of NA1 *nwt* isolates from oaks were variable and unstable.

To conclude, this work demonstrated that major genome alterations of *P. ramorum* could be induced in a host species-dependent manner (Fig. 3).

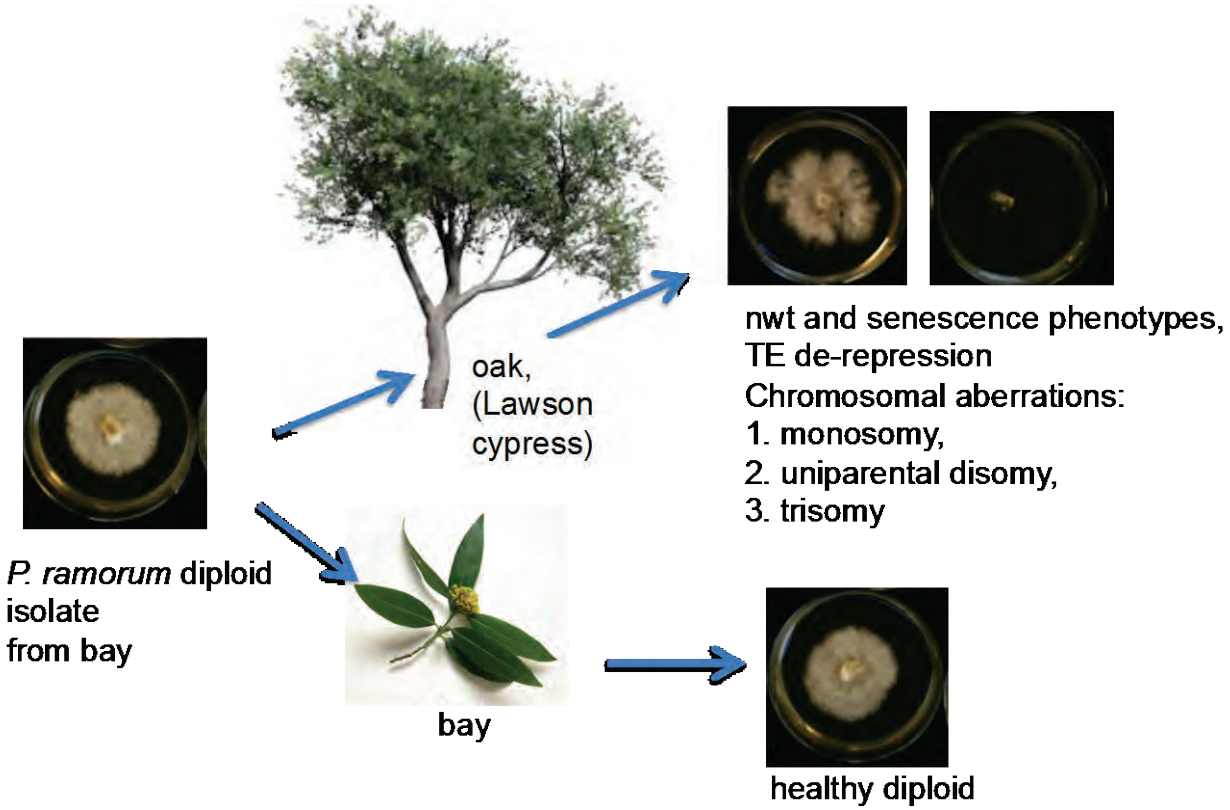

Figure 3—*P. ramorum* isolates from oaks are more likely to display the *nwt* colony phenotype than those from California bay laurel. We found that *P. ramorum* in oaks, and probably also in Lawson cypress, undergoes genome alterations that result in the *nwt* colony phenotype. *Nwt* phenotype is associated with de-repression of transposable elements.

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Analysis of Populations of the Sudden Oak Death Pathogen in Oregon Forests[1]

Zhian N. Kamvar,[2] Everett M. Hansen,[2] Alan M. Kanaskie,[3] Meredith M. Larsen,[4] and Niklaus J. Grünwald[4]

Abstract

Sudden oak death, caused by the oomycete *Phytophthora ramorum*, was first discovered in California toward the end of the 20th century and subsequently emerged on tanoak forests in Oregon before its first detection in 2001 by aerial surveys. The Oregon Department of Forestry has since monitored the epidemic and sampled symptomatic tanoak trees from 2001 to the present. Populations sampled over this period were genotyped using microsatellites and studied to infer the population genetic history (Kamvar and others 2015). To date, only the NA1 clonal lineage is established in this region, although three lineages exist on the North American West Coast. The original introduction into the Joe Hall area eventually spread to several regions: mostly north but also east and southwest. A new introduction into Hunter Creek appears to correspond to a second introduction not clustering with the early introduction. Our data are best explained by both introductions originating from nursery populations in California or Oregon and resulting from two distinct introduction events. Continued vigilance and eradication of nursery populations of *P. ramorum* are important to avoid further emergence and potential introduction of other clonal lineages.

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Biological Differences Between the Evolutionary Lineages Within *Phytophthora ramorum* and *Phytophthora lateralis.* Should the Lineages Be Formally Taxonomically Designated?[1]

Clive Brasier[2]

Abstract

It is now generally accepted that the four evolutionary lineages of *Phytophthora ramorum* (informally designated NA1, NA2, EU1, and EU2) are relatively anciently divergent populations, recently introduced into Europe and North America from different, unknown geographic locations; that recombinants between them are genetically unstable and probably unfit to survive, indicating reproductive isolation; and that they differ in growth rates and aggressiveness. EU1 lineage, for example, is on average faster growing in culture and more pathogenic on *Quercus rubra* bark than the NA1 lineage. Our recent studies show that all four lineages can be readily discriminated in simple G x E stress tests on colony and growth behavior alone. Furthermore, the EU2 lineage is, on average, more aggressive than EU1 on larch stems, but EU1 may produce more sporangia than EU2 on larch needles.

Among *Phytophthora* tree pathogens, multiple evolutionary lineages are not unique to *P. ramorum*. Our studies have revealed four lineages within *P. ramorum's* closest known relative - *P. lateralis* (informally designated TWK, TWJ, PNW, and UK). They have distinctive colony types; exhibit considerable differences in growth rate; and differ in sporangial size and shape, chlamydospore size, and in aggressiveness on *C. lawsoniana.* Overall, these differences may be greater than those seen among the *P. ramorum* lineages. Other Phytophthoras exhibiting multiple lineages include *P. cinnamomi* and possibly also *P. austrocedri* and *P. kernoviae.*

Within *P ramorum* or within *P lateralis*, the lineages tend to share characteristics, such as their basic spore morphology, a common host range, a common breeding system (potentially outcrossing heterothallic and inbreeding homothallic, respectively), and phylogenetic relatedness. These are broadly consistent with being conspecific and sharing a common ancestor. Equally, the morphological, behavioral, and genetic differences between the lineages suggest they have become adapted (via selection, drift, and reproductive isolation) to somewhat different biogeographic environments. Some evolutionary biologists might consider them equivalent to sibling or cryptic species. Taxonomists may view their phenotypic differences as sufficiently large to warrant formal taxonomic recognition. In *P. lateralis*, for example, the differences between the lineages are probably as large as those between some described *Phytophthora* species.

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Should such lineages in *Phytophthora*, therefore, be formally recognized as subspecies or even as species? ‘Lineage’ is an informal term. It has no formal taxonomic status. In the author’s view, while the term ‘lineage’ was helpful during earlier ‘fact finding’ stages of scientific understanding, it no longer adequately conveys the extent of the biological differences between the ‘lineages.’ In view of their pathological differences, it can certainly be argued that a more formal designation would be advantageous for plant health regulation and communication. The rest of the world might do well, for example, to regulate definitively against the importation of the *P. ramorum* EU2 lineage from the UK. By consolidating the biological realities, formal designation could also help remove some popular misconceptions regarding the status of the lineages, for example, that they are roughly equivalent only to genotypes.

Nurseries and Managed Landscapes

Pacific starflower, *Lysimachia latifolia*

Managing *Phytophthora ramorum* at Bloedel Reserve[1]

Darren Strenge,[2] Marianne Elliott,[3] Gary Chastagner,[3] Casey Sclar,[4] and Daniel Stern[4]

Abstract

Bloedel Reserve is a 150-acre botanical garden and nature preserve on the north end of Bainbridge Island in Washington on the Puget Sound. The grounds encompass undeveloped forest, pastures, a bird marsh, woodland plantings, and intensely maintained gardens within the limits of the City of Bainbridge Island. The garden is part of the Sentinel Plant Network, a partnership between the American Public Gardens Association and the National Plant Diagnostic Network that engages public garden professionals, volunteers, and visitors in the early detection of serious plant pests and diseases. With funding from the USDA Animal and Plant Health Inspection Service, the Sentinel Plant Network has trained hundreds of front-line garden staff on best practices for monitoring plant collections and host plants for signs and symptoms of regionally significant threats as well as the proper way to collect and submit diagnostic samples. The program also provides member gardens with a wide variety educational outreach and training materials for use in engaging communities about the impact of serious plant pests and diseases and the importance of early detection and rapid response. Since participating in their first Sentinel Plant Network training in 2011, staff at Bloedel Reserve have used the program's resources to intensify their monitoring efforts.

In early March of 2015, garden staff submitted a sample of a diseased *Pieris* exhibiting lower crown dieback and extensive leaf spotting to Washington State University (WSU) for diagnosis. Molecular tests confirmed infection by *Phytophthora ramorum.* Further sampling by the USDA, Washington State Department of Agriculture (WSDA), and WSU revealed additional infections on *Mahonia*, *Rhododendron*, *Viburnum*, *Gaultheria*, *Vinca*, *Vaccinium*, and *Camellia*. Most infections were in the Rhododendron Glen at the north end of the grounds while two *Camellia* infections were on the centrally located Camellia Trail.

After confirmation of the *P. ramorum* infection, eradication efforts began following recommendations from the USDA, WSDA, and WSU. All plants within an approximate 2-meter radius eradication zone were removed and destroyed per USDA requirements. Where appropriate, drainage was installed and trail grades were modified to prevent *P. ramorum* contaminated water from flowing over trails. Wood chips high in *Thuja plicata* material were applied as mulch. Visitor and worker access to eradication zones was severely limited and subjected to strict sanitation procedures. Bloedel Reserve has always asked visitors to stay on trails and not take plants, rocks, sticks, or other items home with them. Since pathogen confirmation, this policy has since been more intensely advertised and enforced. Standard operating procedures for maintenance practices were developed to deter introduction and spread of new infections.

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Quarantine and sanitation procedures were defined to guide propagation, nursery, and maintenance activities.

From July through September (2015), WSU performed post-removal soil steaming in eradication zones. Soil samples taken immediately following steaming were free of *Phytophthora* species. One month later, leaf litter and soil at one site was found with multiple non-*ramorum Phytophthora* species. This was likely due to disturbance of the soil while installing drainage post-steaming. Beginning in early winter (2016), biological and chemical controls were implemented. The bio-control fungus *Trichoderma atroviride* was applied via hand-pump backpack sprayer and lightly raked into the ground. A 2-inch aged dairy manure/wood chip mulch layer was applied immediately after application. Rotating foliar applications of mefenoxam, dimethomorph, and cyazofamid were used in an effort to deter further spread of *P. ramorum*. Applications will at least continue through the rainy season of 2016.

Eradication zones will be replanted with genera not on the USDA *P. ramorum* host list. All new plants will be subject to a minimum of 8 weeks of quarantine regardless of their susceptibility to the pathogen. Planting and maintenance activities within eradication zones will adhere to strict sanitation procedures on the assumption that *P. ramorum* might still be present. *P. ramorum* is likely to persist at Bloedel Reserve into the foreseeable future. Our efforts focus on containing the spread and impact of the disease and preventing its movement off of the grounds.

Hot Spots of *Phytophthora* in Commercial Nurseries[1]

Corina Junker,[2] Patrick Goff,[2] Stefan Wagner,[2] and Sabine Werres[2]

Abstract

Studies have shown that nurseries are an important source for the spread of *Phytophthora.* Most surveys and studies focusing on the epidemiology of these pathogens in nurseries are based on sampling of symptomatic plants or on samples like water of different sources used for irrigation. There is little knowledge, however, on the survival and occurrence of *Phytophthora* in the different steps of commercial plant production. This information is necessary for the development of good management strategies for *Phytophthora* in nurseries.

Therefore, within a 3-year project, detailed samples were taken at different stages of the propagation and cultivation of plants and on different sample materials like substrates, residues, wind-carried leaves, and water/sediment. The samples were taken in two nurseries between August 2011 and May 2014 every 2 months. The results will be presented. They should help nursery managers to get a better understanding on "survival places" of *Phytophthora* species in their nursery and to develop better sanitary procedures. Furthermore, the results could help improve sample design for surveys and monitoring.

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Pilot Program (Proof of Concept) to Mitigate *Phytophthora ramorum* at an Infested Nursery Based On a Systems Approach[1]

Gary Chastagner[2] and Marianne Elliott[2]

Abstract

The primary purpose of this program was to demonstrate proof of concept of certain mitigation approaches at a repeat *P. ramorum*-positive nursery site in Washington. Approaches included steam treatment of infested soil areas; creating a gravel “sandwich” above steam-treated and potentially infested soil surfaces; improving drainage systems; required sanitation of pots, flats, and other surfaces; monitoring host plants; and training of staff to identify potential problems and symptoms associated with the life cycle of *P. ramorum*. Various critical control points (CCPs) were sampled for *Phytophthora* spp. and the results shared with the nursery and regulatory agencies to demonstrate the effectiveness of their practices. The hypothesis was these specific mitigations will effectively prevent future infestations of nursery stock produced at this site.

The nursery was positive for *P. ramorum* in 2010, 2012, 2014, and 2015. In 2014, *P. ramorum* was detected on *Camellia sinensis* 'Sochi,' *Vaccinium ovatum*, and *Gaultheria shallon* during the spring inspection. After mitigations, the nursery was found positive again during the 2015 spring inspection for *P. ramorum*. Positive plants were all *Vaccinium parvifolium*. It is likely that *P. ramorum* moved onto some new plants from a holdover plant or from elsewhere in the nursery where mitigations were not performed. All isolates taken from plants, soil, leaf debris, and standing water in 2014 and 2015 were the NA2 genotype. This nursery is one of two nurseries in WA that has been positive for *P. ramorum* since 2011 and ships host material interstate, placing it under regulations in the revised USDA *P. ramorum* Federal Order.

The CCP team met with the owner and several employees to discuss a new set of mitigations to be implemented twice annually. The nursery voluntarily hired an employee whose responsibilities included ensuring that the standard operating procedures (SOPs) were being followed. Employee training sessions were also conducted to provide updated information and a "refresher course" on SOPs. Each year, the nursery was provided with an updated list of the *Phytophthora* species present at the nursery, where they were found, and their hosts. This helped personnel understand where practices could be improved as well as the risk to their plant material. Other data collected at the nursery included effectiveness of soil and pot steaming; monitoring of water flow beneath the gravel "sandwich" in production areas; and a comparison of Phytophthoras isolated from dirty pots, pots soaked in water, and disinfected pots. Collection of this data is beneficial to all parties since the nursery and regulatory agencies can see the impacts of specific practices, and this information can be included in a publication to be made available to a wider audience.

There has been a vast improvement in the practices of this nursery as a result of the required mitigations to continue interstate plant shipping. Having research results that show the effectiveness of their updated

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SOP has also been very helpful. Much time, money, and resources were committed to this project in an effort to slow the spread of the *P. ramorum* NA2 lineage from WA to elsewhere in the US.

Incubation of *Phytophthora ramorum*-Infested Leaf Debris in Soil Affects Survival, Sporulation Capacity, and Subsequent Risk of Epidemic Development within Nurseries[1]

Ebba K. Peterson,[2] Niklaus J. Grünwald,[3] and Jennifer L. Parke[2]

Abstract

Soilborne inoculum (infested leaf debris which has become incorporated into the soil) may be an important contributor to the persistence of the sudden oak death pathogen *Phytophthora ramorum* in recurrently positive nurseries. To initiate new epidemics, soilborne inoculum must not only be able to survive over time, but also be capable of producing sporangia during times favorable to infection of plant material at the soil surface. Current research has only assessed the recovery of this pathogen after being buried in soils. Two additional aspects of the disease cycle are being investigated in a field trial at the National Ornamentals Research Site at Dominican University of California (NORS-DUC): the infection of leaf baits at the soil surface and the capacity to produce sporangia post-incubation.

Rhododendron leaf disk inoculum was buried in soil at a depth of 5 or 15 cm in June 2014. Over the course of 1 year, inoculum was recovered and placed in filtered creek water at 20°C to induce sporulation, after which it was plated on selective media to assess viability. Recovery frequency of *P. ramorum* from incubated disks remained above 60% for inoculum at both depths. Sporulation has been greater from inoculum buried at 15 cm relative to 5 cm for all recovery dates. Sporulation potential initially declined; however, a moderate increase in sporangia production was observed in the autumn and winter following the burial of inoculum.

Subplots were baited with non-infested leaf disks to assess the potential for soilborne inoculum to cause infections at the soil surface. *P. ramorum* was recovered from baits placed above inoculum introduced to soil in June, albeit rarely. Recovery of *P. ramorum* from baits placed atop columns containing inoculum introduced to both depths at the beginning of each baiting period was greatest between November and January.

Incubation in soil reduced sporulation capacity in the short-term; however, sporulation capacity increased with the onset of autumn and winter. This increase corresponded to times in which the greatest recovery of *P. ramorum* was observed from leaf baits placed at the soil surface. This increase may have been attributed to the seasonally decreasing mean and maximum temperatures experienced in the soil, as validated by laboratory experiments. While inoculum incubated over the summer had reduced sporulation capacity, increases in sporulation during times of high risk of infection likely aided the development of new *P. ramorum* epidemics originating from soilborne inoculum sources.

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Relative Heat Sensitivities of Certain *Phytophthora* spp. and the Potential for Soil Solarization to Disinfest Nursery Beds in West Coast States[1]

Jennifer L. Parke,[2] Fumiaki Funahashi,[2] Clara Weidman,[2] and Ebba K. Peterson[2]

Abstract

Soilborne *Phytophthora* spp. can be important for initiating disease through movement of inoculum with surface water to roots or splashing onto foliage. Nursery beds infested with *Phytophthora* spp. can contaminate container plants set on them, causing disease year after year and posing a risk of additional spread. Persistent sources of soilborne inoculum are especially problematic for quarantine pathogens such as *P. ramorum*. Unfortunately, infested nursery beds are challenging to disinfest because of restrictions on the use of soil fumigants and the difficulty of accessing equipment for soil steaming.

Soil solarization has been shown to be an effective strategy to kill *Phytophthora* spp. in the upper layer of soil in some locations. The effectiveness of solarization treatments will vary regionally, however, due to differences in day length, solar declination angle, and local climate. To predict the effect of soil solarization, lab trials were conducted under controlled conditions to determine parameters important for inoculum survival. Inoculum survival in field trials were then evaluated where these parameters were measured and a mathematical model to predict solarization efficacy was validated.

In lab experiments, rhododendron leaf disks infested with different *Phytophthora* spp. were placed in soil or polyethylene glycol (PEG) solution and subjected to constant high temperatures. For *P. ramorum*, the time to reduce survival by 99.9% ($LD_{99.9}$) was 3.9 days at 35°C, 0.9 days at 38°C, and 15 min at 50°C. For *P. pini*, the $LD_{99.9}$ was 6.9 days at 35°C, 2.5 days at 38°C, and 40 min at 50°C. Because *P. pini* survives longer at high temperatures than *P. ramorum*, *P. pini* could serve as a conservative surrogate for *P. ramorum* in field studies conducted outside quarantine facilities. Survival time at high temperatures was greater under drier conditions as compared to wetter conditions. For example, at 35°C, *P. ramorum* survived only 3.5 days at -0.001 kPa, but 10.6 days at -6.32 MPa. A similar trend was observed for *P. pini*. These results underscore the importance of maintaining high soil moisture to maximize the efficacy of soil solarization. Survival at constant high temperature was also compared to survival during intermittent heat. Despite equivalent total exposure time at high temperature, intermittent heat was found to be less damaging to *Phytophthora* inoculum than constant heat. Thus, reduced survival of *Phytophthora* at high temperature is not based solely on response to cumulative heat (Funahashi, 2015).

Soil solarization field trials with buried inoculum were conducted from 2012-2014 with *P. ramorum*, *P. pini*, and *P. chlamydospora* in San Rafael, California; *P. pini* and *P. chlamydospora* in Corvallis, Oregon; and *P. chlamydospora*, *P. plurivora*, and *P. gonapodyides* in Puyallup, Washington. Rhododendron leaf inoculum of each pathogen was buried at 0, 5, 15, and (occasionally) 30 cm. Solarization reduced

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inoculum survival most effectively in the upper soil layers, where soil temperatures were highest. For example, in a trial conducted at the National Ornamentals Research Site at Dominican University of California (NORS-DUC) in San Rafael, CA July 16-Aug. 13, 2013, inoculum at the surface and 5 cm depth was killed within 2 days, whereas inoculum buried at 15 cm survived 15 days of solarization. In another trial at NORS-DUC with *P. ramorum, P. pini,* and *P. chlamydospora,* all inocula except *P. chlamydospora* at the 15 cm depth were killed by 2 weeks of solarization. Soil temperatures during solarization were greater with anti-condensation plastic film as compared to regular plastic film. The presence of a crushed rock layer (2.5-7.5 cm thick) atop the soil significantly increased the maximum and average temperature at all depths (0-15 cm) relative to soil without the rock layer, indicating the feasibility of implementing soil solarization to disinfest container nurseries of *Phytophthora* species in the upper layers of soil.

A mathematical model was established to predict the survival of *P. ramorum* and *P. pini* during solarization. Inclusion of the factors soil temperature, soil matric potential, and constant vs. intermittent heat described solarization effectiveness in field trials. Survival of each pathogen in field trials largely agreed with survival as predicted from the model (Funahashi 2015).

To evaluate the potential for solarization efficacy within West Coast states, soil temperatures during soil solarization were measured in 43 nursery sites in CA, OR, and WA during the summers of 2013 and 2014. Soil temperatures were recorded during 4 weeks of solarization at 4 depths: 0 cm (surface), 5 cm, 10 cm, and 15 cm. Air temperature, wind speed, and solar radiation data were obtained from nearby weather stations. Solarization was successful in attaining temperatures lethal to *Phytophthora* spp. at most sites. Exceptions included sites in northwest WA, the coastal fog belt, or sites solarized in late summer.

With soil temperature data from the sites, a predictive model was developed that was the basis for a web-based forecasting tool created by L. Coop and D. Upper at the Integrated Center for Plant Protection at Oregon State University. The forecasting tool is intended for use by nursery managers in CA, OR, and WA to predict the length of time required to kill *P. ramorum* or *P. pini* at various soil depths. User inputs include target species (*P. ramorum* or *P. pini*), start date, location, thickness of a crushed rock layer, and choice of year for predicting the current year's weather. The model assumes use of clear, anti-condensation plastic film, a minimum treatment area of 2.5 m x 2.5 m, soil moisture at field capacity when covered with plastic, and full sun exposure. The initial version of the web-based forecasting tool is available at http://uspest.org/soil/solarize. Additional modules are being developed for application to raised bed agricultural systems and other target pathogens and weed species.

In summary, soil solarization appears to be a promising technique for disinfesting the upper layer of soil in West Coast container nurseries, particularly for relatively heat sensitive species such as *P. ramorum.*

Acknowledgments

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Phytophthora Detections in Native Plant Nurseries and Restoration Sites

California bay laurel, *Umbellularia californica*

An Update on *Phytophthora* Species in California Native Plant Nurseries and Restoration Areas[1]

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Abstract

In 2012, *Phytophthora tentaculata* was detected for the first time in North America on the roots and crowns of declining sticky monkey flower plants (*Diplacus aurantiacus*) in a Monterey County, CA native plant nursery. At the time, *P. tentaculata* was listed among the top five exotic *Phytophthora* species of concern to the US due to its potential economic and environmental impacts. In 2014, *P. tentaculata* was detected on toyon (*Heteromeles arbutifolia*) and again on sticky monkey flower plants that had been outplanted at a restoration site in CA. These plants originated from a different CA nursery than the original detection, where coffeeberry plants (*Frangula californica*) were also found to be infected. In response to the concerns of spreading exotic *Phytophthora* species to the wildlands through native plant nursery stock, the California Department of Food and Agriculture lab tested more than 1,200 samples for *Phytophthora* spp. from Jan. 2014 to Jan. 2016. Samples were collected from native plant nurseries and wildlands and tested by immunoassay, culturing, baiting, and PCR using *Phytophthora*-specific primers. In addition to *P. tentaculata*, at least 25 other species of *Phytophthora* were detected from the roots of native plants or were baited from the root zones of outplanted material. One or more *Phytophthora* spp. was detected from 25% of the samples submitted. *P. cactorum* was the most commonly detected *Phytophthora* species in the study and was confirmed from 15 different native plant genera. *P. tentaculata, P. cactorum, P. cambivora, P. lacustris*, and the *P. cryptogea* complex comprised 67% of the total *Phytophthora* detections. At least 10 different *Phytophthora* species were detected from symptomatic *D. aurantiacus* roots; prior to this work, not one *Phytophthora* species was known to infect this host. In total, at least 70 new *Phytophthora* native plant associations were identified. Native plant nursery stock is planted into environments which have few, if any, native *Phytophthora* species. The inadvertent spread of exotic *Phytophthora* species into natural systems could have long-term environmental and economic impacts. The California native plant industry has reacted to these findings by raising the standards and expectations for nursery cleanliness and is beginning the process of improving growing practices.

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Restoration Outplantings of Nursery-Origin Californian Flora Are Heavily Infested with *Phytophthora*[1]

Tyler B. Bourret,[2] Heather K. Mehl,[2] David M. Rizzo,[2] Tedmund J. Swiecki,[3] Elizabeth A. Bernhardt,[3] and Janell M. Hillman[4]

Abstract

A survey of areas previously anthropogenically disturbed and revegetated with woody nursery-reared native Californian vegetation was conducted in Santa Clara County between August and December of 2015. Previous sampling of revegetation sites had found nursery-origin transplants to be infested with *Phytophthora* species. Samples of roots and soil were collected from underneath dead, symptomatic, and non-symptomatic transplants at 24 sites and baited with a combination of pear fruit and *Rhododendron* leaves. Strains were isolated in 18 (75%) of the sites surveyed and 55 of the 145 samples baited, resulting in 103 *Phytophthora* species by sample combinations. Thirty-one different *Phytophthora* species were identified based on analysis of ITS rDNA sequences, including novel taxa, putative hybrids, and quarantine pathogens. No strong associations between species and host plants were found. DNA was extracted directly from soil of all samples and preliminary evidence indicates oomycete ITS sequences can be reliably amplified; these will be sequenced with Illumina MiSeq and analyzed. Ongoing work includes resampling of infested sites to investigate the persistence and spread of *Phytophthora* followed by treatment efforts.

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Testing and Implementing Methods for Managing *Phytophthora* Root Diseases in California Native Habitats and Restoration Sites[1]

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Abstract

Over the past 14 years, a variety of native plant communities in northern California have been identified where introduced root-rotting *Phytophthora* species, most notably *Phytophthora cinnamomi*, *P. cambivora*, and *P. cactorum*, are causing decline and mortality of native species. In many older infested sites, the source(s) of the original *Phytophthora* introductions are not clear. Movement of contaminated soil is the most likely source in some sites that are located along roads and trails. In other cases, introductions are associated with plantings of nursery stock. In one site, a multi-species infestation (*P. cambivora, P. cactorum, P.'kelmania'*, and *P. syringae*) extending over more than 2 ha was associated with the planting of 30 to 50 or more nursery-grown *Ceanothus* plants as part of a restoration effort. Once *Phytophthora* infestations become established, they have typically spread along roads and trails and downslope with surface water flow.

Samples taken from transplanted nursery stock in a variety of other habitat restoration projects planted between about 2000 and 2014 have yielded a wide variety of *Phytophthora* species (>50 taxa). These include species not previously found at field sites in the US as well as undescribed taxa (Bourret and others, Restoration outplantings of nursery-origin Californian flora are heavily infested with *Phytophthora*, these proceedings; Rooney-Latham and others, An update on *Phytophthora* species in California native plant nurseries and restoration areas, these proceedings). Sampling conducted to date suggests that the rate of spread of introduced *Phytophthora* species from planted stock varies widely based on site conditions and the *Phytophthora* species involved. Spread appears to occur more rapidly where roots of nearby host plants extend into the planting sites and where sites are at least seasonally inundated. Furthermore, baiting results have shown that propagules of some *Phytophthora* species can survive at least 1 to several years in the absence of a live host plant (including sites with either dead or previously removed plants).

The widespread use of *Phytophthora*-infested nursery stock in habitat restoration projects poses serious risks to many native plant communities. Unabated spread of *Phytophthora* infestations in the limited habitat of susceptible rare plant species, including Ione manzanita (*Arctostaphylos myrtifolia*) (Figure 1) and pallid manzanita (*A. pallida*), may drive the remaining natural populations of these species to extinction. Even where the affected plant species are not rare (e.g., madrone, giant chinquapin, valley oak), *Phytophthora* infestations can degrade and permanently alter native vegetation. Many *Phytophthora*-affected sites no longer support the range of native species that were previously present, including keystone species.

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Land managers and resource agencies seeking to manage these introduced pathogens in native habitats have very limited options. Management options are primarily related to the extent and characteristics of the infestation and can be grouped under the following approaches: eradication, active suppression, and prevention/slowing further spread. Options can be further constrained by other habitat-specific management concerns, site accessibility, cost, and feasibility issues. The adaptive management approach provides a good framework for developing an appropriate management plan (Swiecki and Bernhardt 2013). Under this approach, managers first determine what they have by assessing plant resources, disease conditions, and the current management framework. Based on this analysis, managers identify needs and set goals and objectives to meet those needs. The next step involves selecting and implementing management actions to meet goals and objectives. Goals may need to be revised if available management strategies cannot obtain desired outcomes. Once management actions are implemented, disease and plant health outcomes are monitored and analyzed to see if goals are being met, bringing the process full circle.

Figure 1—Stand of Ione manzanita, *Arctostaphylos myrtifolia*, killed by *Phytophthora cinnamomi* in native habitat near Ione, California.

The preferred management option for all habitats is to avoid introducing *Phytophthora*. Over the long term, it is more effective, economical, and easier to prevent or avoid introductions than to attempt eradication or perpetually manage affected areas to minimize further spread. The risk of introducing *Phytophthora* through habitat restoration projects is nearly eliminated if plants are established by recruiting existing natural regeneration or via direct seeding, instead of using nursery stock. If nursery stock is used, it should be free of *Phytophthora* to the maximum extent possible. Best management practices (BMPs) for producing *Phytophthora*-free planting stock have been developed and are being adopted and implemented by agencies and native plant nurseries. Planting stock produced under these BMPs is acceptable for restoration use if no *Phytophthora* species are detected in the stock using the most sensitive testing protocol available. Testing should only be used as a final quality control check on plants that have been produced under rigorous clean production practices. Due to its limitations, testing should not be used in an attempt to find uninfected plants within an infested batch produced under inadequate phytosanitary practices.

Once *Phytophthora* has been introduced into an area, management options are limited and may not be completely effective. Eradication is the most desirable option, but is only feasible for very small areas,

such as spot infestations identified at an early stage. Recent installations of *Phytophthora*-infected nursery stock in habitat restoration areas represent sites where eradication may be possible if introduced *Phytophthora* species have not spread beyond individual planting sites. The ability of *Phytophthora* species to spread from infested sites in different types of planting situations is under investigation. For a number of *Phytophthora* species, we have found that eradication is not possible by simply removing infected host plants because of extended pathogen survival in soil.

Solarization of small areas (minimum of 1 m^2) for extended periods (1 year or more) is being tested as a means of eradicating *Phytophthora* from individual planting sites. Sites have been covered with one or two layers of clear plastic thermal anti-condensate greenhouse film (0.15 mm [6 mil] thick) that has a 4 year service life rating. At *P. cactorum*-infested planting sites solarized for 7-15 months, the pathogen could not be recovered by baiting at sun-exposed sites, but was detected at sites that received significant shading. Temperature data suggest that the pathogen was not eradicated if sites did not attain at temperature of least 35 °C at 20 cm in depth, the depth of the container stock rootball. However, a side benefit of solarization appears to be inhibition of pathogen spread from contaminated sites. If the plastic film is intact, water from precipitation only reaches the soil under the plastic via capillary movement from the surrounding wetted soil; this situation is unfavorable for zoospore release and dispersal. We are also investigating other means for spot treating infested planting sites with heat. A steam injecting soil auger (Johnson 2014) and other related methodologies are being investigated.

Active suppression of disease using systemic oomycete suppressive chemicals ("fungicides") may be possible in larger infested areas, but this tactic becomes less viable as the infested area increases. In habitat of Ione manzanita infested with *P. cinnamomi*, the advance of mortality in affected stands has been suppressed for more than 4 years by treating plants at the edge of mortality centers with a foliar spray of potassium phosphite (12.4 kg ai/h applied at 300 L/ha in alternate years). Ultra low volume (ULV) foliar applications have greater potential to be of use in this habitat because affected areas are difficult to access with the large ground equipment needed for higher volume sprays. Results of initial ULV applications (8 or 10 kg/ha at 30 L/ha) were not promising, likely due to reduced rates needed to avoid phytotoxicity. More recent tests using two ULV applications in series at least 4 weeks apart (split application) totaling 16 or 20 kg/ha at 30 L/ha are underway and initial results are promising.

In large infested areas where active suppression is not feasible, management is generally limited to slowing further spread along the margins of the infestation and preventing contamination of non-infested areas via movement of infested soil. To accomplish this, it is necessary to delineate the extent of the infested area, at least approximately. This is accomplished through a combination of soil baiting and mapping symptomatic plants, but is more difficult in mixed vegetation types that include plants with varying responses to the pathogens present. Management methods employed at affected sites have included signage, permanent trail closures, specifying wet-season closures, preferred travel directions, altering road surface materials, and specifications for crews or contractors to minimize soil movement from infested areas. These measures have the potential to reduce the rate of spread along roads and trails and the development of satellite infestations, but do not slow unassisted pathogen spread from root to root or along drainages.

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Steam, Solarization, and Tons of Prevention: The San Francisco Public Utilities Commission's Fight to Contain Phytophthoras in San Francisco Bay Area Restoration Sites[1]

Greg Lyman,[2] Jessica Appel,[2] Mia Ingolia,[2] Ellen Natesan,[2] and Joe Ortiz[2]

Abstract

To compensate for unavoidable impacts associated with critical water infrastructure capital improvement projects, the San Francisco Public Utilities Commission (SFPUC) restored over 2,050 acres of riparian, wetland, and upland habitat on watershed lands in Alameda, Santa Clara, and San Mateo Counties. Despite strict bio-sanitation protocols, plant pathogens (*Phytophthora* spp.) were detected at multiple restoration sites on SFPUC lands. SFPUC staff will provide an overview of various treatments employed to contain and treat the pathogens using multiple *in-situ* treatment techniques including soil solarization, steam injection, and microbial antagonism.

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31 Flavors to 50 Shades of Grey: Battling Phytophthoras in Native Habitats Managed by the Santa Clara Valley Water District[1]

Janet Hillman,[2] Tedmund Swiecki,[3] Elizabeth A. Bernhardt,[3] Heather K. Mehl,[4] Tyler B. Bourret,[4] and David Rizzo[4]

Abstract

The Santa Clara Valley Water District (District) is a wholesale water supplier for 1.8 million people in Santa Clara County, California. Capital, water utility, and stream maintenance projects result in extensive, long-term mitigation requirements in riparian, wetland, and upland habitats throughout the county. In 2014, several restoration sites on the valley floor and in the upper watershed were found to be contaminated by *Phytophthora* spp. Subsequently, an extensive baseline study of restoration sites planted in the last 3 years revealed 31 different species of *Phytophthora* in 16 sites, while a related study revealed at least 17 species at 13 sites, for a total of approximately 39 species across all sampled locations. Detections of *P. tentaculata* and *P. quercina*, which are ranked in the top five high-risk species to the United States by the USDA, are of particular concern. The District's response to this high level of contamination has included development of a comprehensive set of BMPs and contract specifications for work in sensitive and contaminated areas; a short term moratorium on planting nursery container stock; a complete reevaluation of restoration practices from seed collection, growing of stock to planting out; participation in regional working groups on the emerging pathogen threat; education of stakeholders, project planners, regulatory personnel, district staff and contractors; and additional testing and site remediation where feasible. Remediation of infected sites has proved challenging due to cost, site access, and dense urbanization in the lower watershed.

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The Golden Gate National Parks *Phytophthora* Response Plan[1]

Alisa Shor,[2] John Doyle,[2] Sharon Farrell,[2] Alison Forrestel,[3] Christa Conforti,[4] Lew Stringer,[4] Terri Thomas,[4] and Laura Lee Sims[5]

Abstract

In partnership with the California Native Nursery Network, the three agencies of the Golden Gate National Parks (National Park Service, Golden Gate National Parks Conservancy, and Presidio Trust) hosted the Symposium, "Responding to an Expanding Threat: Exotic *Phytophthora* Species in Native Plant Nurseries, Restoration Plantings, and Wildlands" in December of 2014. The symposium was intended to educate and inform the regional restoration and native plant nursery communities on recent findings and concerns of *Phytophthora* species that are being discovered in native plant nurseries and restoration sites at an alarming rate and empower these communities to minimize the risk of unintentionally harboring and dispersing these pathogens.

Heightened awareness of the risk and threat of *Phytophthora* had a cascading effect on the Golden Gate National Parks as the partnering agencies were called to action immediately upon detection of *Phytophthora*-infested plants in park nurseries, which operate in coordination and in support of the habitat restoration priorities of the National Park Service and the Presidio Trust. In response, a multi-agency management team of cross-departmental representatives, including nursery professionals, ecologists, restorationists, plant pathologists, and integrated pest management specialists, was convened and has continued to meet regularly since January of 2015. The team works together as proactively as possible while also problem solving at critical points in project planning, plant production, and outplanting. The management team has addressed topics including funding, data collection protocols, project delays and repercussions, unexpected *Phytophthora* contamination sources, the need to educate junior staff through upper management, and real-time ethical and management decisions in a complex working and ecological environment.

While some regional land management agencies decided to put a moratorium or heavy restrictions on the planting of container stock into restoration sites until clean plant stock can be sourced, the management team at Golden Gate National Parks decided to invest resources in testing container stock so that projects, plant production, and restoration activities could continue. The team has also placed an emphasis on improving growing practices to produce clean nursery stock and instituting best management practices (BMPs) in the field setting to minimize spread of pathogens.

In this session there will be discussion on the frontline perspective of the events and ongoing management decisions of the last 18 months in the Golden Gate National Parks in response to the elevated concerns of

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Phytophthora. An overview of the personnel and financial resources invested, operational shifts, and detections to date will be presented. Discussion will include an overview and comparison of the testing regime and results for 2014 container stock that was produced before heightened BMPs were instituted, compared to 2015 and 2016 container stock that was produced with improved growing and sanitation practices. In addition, there will be an overview of results of preliminary surveys of field and restoration sites throughout the park and the intended approach moving forward.

Posters

Coast redwood, *Sequoia sempervirens*

Phytophthora ramorum and *Phytophthora gonapodyides* Differently Colonize and Contribute to Decay of California Bay Laurel (*Umbellularia californica*) Leaf Litter in Stream Ecosystems[1]

Kamyar Aram[2] and David M. Rizzo[2]

Abstract

The prevalence of *Phytophthora* species in surface waters has earned increasing attention in the past decades, in great part as a result of "stream monitoring" programs for detection and monitoring of *Phytophthora ramorum* and other invasive species. The potential for *Phytophthora* to survive and reproduce in streams has significant implications for evaluating and managing the risk of spread of pathogenic species. Therefore, it is important to understand the ecology of both introduced and endemic species in aquatic environments.

Leaf litter is a potential substrate for the persistence and propagation of *Phytophthora* in streams. Our previous work showed that *P. gonapodyides* and related ITS clade 6 taxa are effective saprotrophs, colonizing dead leaf tissue; whereas, *P. ramorum* more effectively colonized fresh green leaves, but its colonization is limited in dead leaves. Therefore, *P. ramorum*'s prevalence on stream leaf litter may be limited by direct competition with other taxa as well as by the substrate becoming increasingly unsuitable due to decay. We conducted a field and laboratory study to determine how well *P. ramorum* and "clade 6" *Phytophthora* could colonize, persist, and sporulate on increasingly decayed leaves of California bay, the primary host for *P. ramorum* and a common riparian species in California coastal woodlands.

To determine how well these taxa naturally colonize, persist on and compete in bay leaf litter in streams, green leaves were collected from trees, incubated in two forest streams and sampled over 16 weeks. Leaves were evaluated for *Phytophthora* colonization through isolations and morphological identification. *Phytophthora ramorum* and "clade 6" taxa quickly colonized leaves in streams and persisted throughout the full duration of in-stream incubation despite loss of as much as 70% of leaf mass due to decay. Both *P. ramorum* and "clade 6" taxa could be baited from leaf samples over the entire 16 weeks, demonstrating sporulation potential despite substantial leaf biomass loss. This demonstrated that green bay leaf litter can serve as a persistent source of *P. ramorum* and other *Phytophthora* inoculum in infested streams.

While some leaf litter is green, especially in spring, much leaf fall into streams consists of senescent leaves. To determine the capacity of these taxa for using and competing for green or senescent leaves, we conducted controlled environment experiments in which green bay leaves collected from trees and recently shed, dry senescent leaves collected from the forest floor were exposed to *P. ramorum*, *P. gonapodyides*, or combined inoculum of both species in containers maintained in growth chambers,

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sampled over 16 weeks and evaluated for colonization and degree of decay. *Phytophthora ramorum* was very limited on dry senescent leaves, while *P. gonapodyides* completely colonized them. Both species contributed to early green leaf decay, but not to the decay of dry senescent leaves. In treatments combining inoculum from both taxa, *P. gonapodyides* predominantly and often exclusively colonized all leaves. This competitive advantage is not always observed under natural conditions and may relate to relative abundance of initial inoculum or other conditions of the experiment. A complementary trial was conducted using yellow senescent leaves collected directly from trees. Recovery from these leaves suggests that fresh senescent leaves function more like green leaves than dry senescent leaves as a substrate for these *Phytophthora* species.

Overall, these results indicate that while *P. ramorum* can persist on bay leaf litter in streams, competition with endemic saprotrophic taxa may limit its abundance and frequency.

Determining the Amount of Soilborne Inoculum of *Phytophthora ramorum* Within an Oregon Tanoak Forest[1]

Christina Benemann[2] and Jennifer Parke[2]

Abstract

Phytophthora ramorum continues to cause extensive mortality of tanoaks in southwestern Oregon. Effective management strategies have been developed based on our current understanding of the pathogen's epidemiology. Local dispersal can occur either by canopy throughfall ("top-down") or a ground splash ("bottom-up") pathway. Although the "top-down" aspect of the disease cycle is well understood, the importance of infested soil and leaf litter as contributing factors to the spread of disease remains unclear. To elucidate the epidemiological importance of these "bottom-up" sources of inoculum, our study aims to (i) compare the amount of inoculum washed down through the canopy to that splashed up from the soil and litter, and (ii) to detect and quantify inoculum in relation to soil depth. Over the course of the rainy season, both rainwater and soil samples were periodically collected from within the Generally Infested Area in Brookings, Oregon.

To determine if the amount of soilborne inoculum is at least as much as that derived from throughfall, a tiered bucket design was set up under infested canopies at five different locations. One bucket was set on the ground to collect throughfall and another was placed into the ground to collect throughfall and any splash-up from the surrounding soil and litter. A third bucket was baited and used to detect the presence of *P. ramorum* in real time. To determine the distribution of inoculum in the soil profile, samples were taken at 5, 10, and 15 cm depths and subjected to baiting. DNA was extracted from rainwater in the unbaited buckets and soil from the 5 cm depth. Quantification of *P. ramorum* in these samples will be attempted with qPCR, which should detect differences that will indicate how inoculum from infested canopies and soil contribute to the inoculum pool in these forests.

This information will add to the existing knowledge of disease transmission biology in natural settings, which can inform management strategies aimed at minimizing the local spread of the disease.

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Rhododendron Leaf Baiting of Coastal California Watersheds for *Phytophthora*[1]

Tyler B. Bourret,[2] Heather K. Mehl,[2] Kamyar Aram,[2] and David M. Rizzo[2]

Abstract

For more than a decade, the Rizzo lab and collaborators have monitored northern and central coastal California watersheds each spring and early summer for the presence of *Phytophthora* using submerged *Rhododendron* leaves as bait. This served as an early detection tool for the sudden oak death (SOD) pathogen, *P. ramorum*, but other species of *Phytophthora* were encountered and notable isolates occasionally saved; in recent years a more concerted effort was made to isolate species of *Phytophthora* other than *ramorum*. Twenty-three species were identified using a combination of morphological traits and ITS nrDNA sequences. Three provisional taxa, taxon mendostream (a novel member of *Phytophthora* clade 9), taxon obispostream (a member of the *P. citricola* species complex) and taxon sequoiasoil (a close relative of *P. cactorum*) are introduced based on unique ITS, mtCOX1 and mtCOX2 sequences. Due to the ongoing SOD epidemic, the exotic, invasive *P. ramorum* was the most common species isolated, followed by members of *Phytophthora* clades 3 and 6. This is consistent with studies performed in Alaska and Oregon, suggesting that many of these species are native to the area. Maps of infested watersheds are presented, spanning more than 750 km of the California coast.

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Microsatellite Analysis of the EU1 Lineage of *Phytophthora ramorum* in Washington State Nurseries, Landscapes, and Waterways[1]

Katie Coats,[2] Marianne Elliott,[2] and Gary Chastagner[2]

Abstract

Microsatellite analysis initially identified genetic variations within the NA1 clonal lineage of *Phytophthora ramorum*; however, in Washington nurseries, the genetic population of *P. ramorum* has shifted and is now dominated by two other lineages, NA2 and EU1. In this study, recently identified markers that are more variable, and therefore more informative for the EU1 lineage, were used to reveal genetic diversity within the Washington *P. ramorum* EU1 population. Data from genotyping of DNA samples provided by the Washington State Department of Agriculture (WSDA) was combined with new detailed genotype data of DNA from isolates collected by Washington State University (WSU) representatives to examine the structure of the Washington nursery EU1 population.

Fifty-eight *P. ramorum* isolates in WSU's Master Collection were previously determined to be within the EU1 lineage. Forty-five of the cultures were isolated from *Rhododendron*, six from *Viburnum*, five from *Kalmia*, and two from soil baits. Two of the isolates, one each from *Rhododendron* and *Viburnum*, were from Oregon; the remaining 56 were from Washington nurseries or trace-forward sites within Washington. The samples were analyzed with 12 previously described microsatellite (SSR) loci. One hundred nineteen EU1 DNA samples from infected plant material collected at Washington nurseries by WSDA staff were included in some analyses.

One marker locus (Ivors82) was excluded from analysis due to hypervariability in several samples between labs. Analysis with the R population genetic analysis program *poppr* showed that seven of the 11 remaining loci are uninformative within this sample set, leaving the following four informative loci: PrMS45, Ivors64, ILVOPrMS131, and ILVOPrMS145c. A total of 27 multi-locus genotype (MLG) groups were identified, revealing a great deal of variability that was not detected previously. The locus with the highest allelic diversity, ILVOPrMS131, had 16 alleles and 24 allele combinations (genotypes) in 58 isolates. Calculations using Bruvo's genetic distance inferred four major groupings containing six population clusters

Among the Washington nurseries and trace-forward sites, the highest genetic diversity was found at a nursery referred to as Nursery #43, with five samples collected in the same year having five unique genotypes. This is possibly the result of a long-term infestation, multiple introductions from other nurseries, or less likely, a single introduction event with high genetic diversity. The lowest diversity was found in the 18 isolates collected over 4 years from Nursery #41 and its trace-forward sites.

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How Do We Know if Plants in Our Nursery Have *Phytophthora*? Detection Methods and an Integrated Approach to Monitoring[1]

Christa Conforti[2]

Abstract

A *Phytophthora cactorum*-infected nursery crop of *Ceanothus thyrsiflorus* was used to evaluate three *Phytophthora* monitoring methods. The *Phytophthora* detection level of three non-destructive sampling methods was quantified and compared to the detection level of destructive sampling. Non-destructive methods were (a) composite soil/root samples, pear-baited, (b) effluent samples, pear-baited, and (c) Agdia ImmunoStrip tests run on root samples. Time and expense of each method were also tracked. The baited soil/root sample method was the least labor intensive and most cost-effective method to test a large number of plants at our nursery. It also allows for species-level identification of *Phytophthora*, and has no risk of false positives. But detection level was 60%, so we have decided to combine it with destructive sampling, for an integrated monitoring approach.

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Next Generation Sequencing of Oomycete Communities in Nursery Irrigation Water[1]

Joyce Eberhart,[2] Fumiaki Funahashi,[2] Zachary S. L. Foster,[2] and Jennifer Parke[2]

Abstract

Horticultural nurseries are under increasing pressure to reduce, remediate, and recycle irrigation water. A major constraint for reusing irrigation water is contamination by waterborne plant pathogenic *Phytophthora* and *Pythium* species. Current research is focused on helping plant nurseries monitor oomycete pathogens in their irrigation water to determine the need for water treatment, evaluate effectiveness of treatment options, and enable selection of cost-effective ways to disinfest water.

A sensitive method to identify waterborne oomycetes present at low concentrations was needed. A semi-quantitative method that would allow for determining the relative frequency of diverse, co-occurring species was also desired (Parke and others 2014). Next generation sequencing has the potential to accomplish both goals. Vannini and others (2013) developed an ITS6 and ITS7 primer set that was successful at amplifying oomycetes with the Roche 454 platform. Because Illumina has higher throughput capabilities and is becoming the standard for next generation sequencing, we investigated modifications that would allow us to use the same primers with the Illumina MiSeq platform. Each primer was designed with the appropriate Nextera XT adapter and the forward primer included a spacer designed to raise the annealing temperature to the recommended level. Primer sequences were as follows:

MiSeq ITS6: 5’ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG K GAAGGTGAAGTCGTAACAAGG 3’

MiSeq ITS7: 5’ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG AGCGTTCTTCATCGATGTGC 3’

For detection of waterborne oomycetes, water samples (1 L) were collected from various locations in a nursery and filtered through 5µm Millipore filters. DNA was extracted directly from filters using beadbeating followed by a chloroform/phenol extraction. PCR was performed using Platinum™ taq and PCR product was submitted to the Oregon State University Center for Genome Research and Biocomputing (CGRB) Core lab. The CGRB Core lab performed cleanup of the PCR product and attached the barcodes using the Nextera XT Index kit. Samples were normalized and pooled before quality controls were performed with qPCR and a Bioanalyzer. Sequencing was performed on the Illumina MiSeq on a 250 bp paired-end run.

Sequence data processing was done using QIIME v. 1.8.0. Only forward reads were used for a preliminary analysis. Sequence bases with quality scores >25 were retained and chimera detection was conducted with the “usearch61” method after primer sequences were removed. Curated sequences were grouped into operational taxonomic units (OTUs) at a similarity level of 99% by uclust algorithms.

[1] A version of this poster was presented at the Sixth Sudden Oak Death Science Symposium, June 20-23, 2016, San Francisco, California.

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Oomycete reads were queried by BLAST algorithms against a reference dataset with *Phytophthora* sequences downloaded from Phytophthora-ID.org and other oomycete sequences used in Robideau and others (2011). The R programming environment (www.R-project.org) was used for downstream analysis.

To ensure that we can detect multiple species in water and accurately determine their relative abundance, we created mock communities composed of 11 *Phytophthora* and *Pythium* species. DNA extracts of cultures of each species were quantified and combined to determine if their relative abundance in the mixture would be reflected in the sequence analysis. Extracts diluted to 5 ng/µl DNA were combined in equal amounts and pooled. PCR performed with the pooled samples showed that many species had similar numbers of reads, but PCR bias may be observed for some species (fig. 1). We then pooled these extracts using 20x greater amounts of DNA from *Phytophthora syringae* and found that the number of reads for this species was approximately 16x the average number of reads for the other species (fig. 2). We also separately amplified each species and the resulting amplicons were combined at 5 ng/µl each. The relative abundance of the number of reads was mostly similar for these samples combined equally post-PCR (fig. 3).

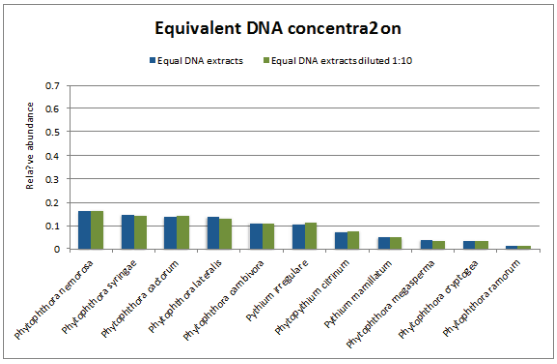

Figure 1—DNA extracts (5 ng/*µ*l) were combined in equal amounts and pooled. PCR was performed with the pooled samples and with a 1:10 dilution of the pooled samples.

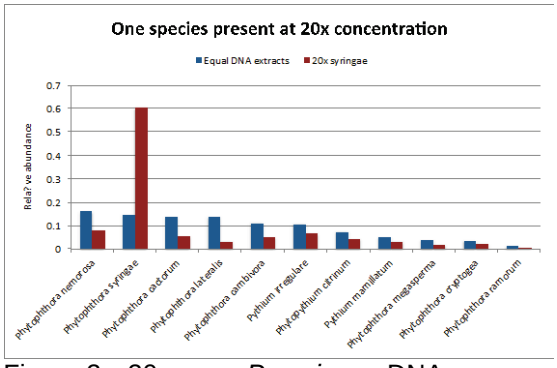

Figure 2—20x more *P. syringae* DNA was combined with the DNA of other species. The number of reads of *P. syringae* was approximately 16x the average number of reads for the other species.

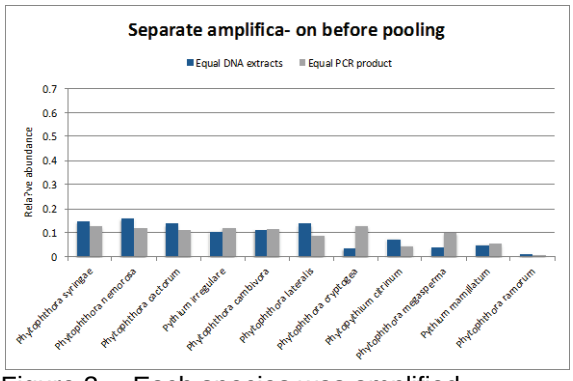

Figure 3— Each species was amplified separately and the resulting amplicons were combined at 5 ng/*µ*l each.

For a subset of the water samples, we compared the detection sensitivity for DNA extracted directly from filters with extraction from rhododendron leaf baits. Leaf baits were incubated in water samples for 3 days followed by incubation in moist chambers for an additional week. Up to five 6-mm disks were taken

from lesioned areas and stored in silica gel at room temperature (Ockels and others 2007). DNA was extracted from the leaf disks with the Synergy™ extraction kit. Amplification procedures were the same as for extractions from filters. Samples obtained using different detection methods (filtered water or baited leaves) and the mock community were used to generate a non-metric multidimensional scaling plot of oomycete OTU communities. The results show distinct community assemblages of oomycetes among the two different detection methods and the mock community (fig. 4).

Once protocols have been fully developed and validated, Illumina sequencing has the potential to be a sensitive method to detect, identify, and estimate the relative abundance of oomycete communities from water samples. This knowledge will be used to help nursery managers make informed decisions about effective water disinfestation strategies, reducing the risk of establishment of plant pathogens.

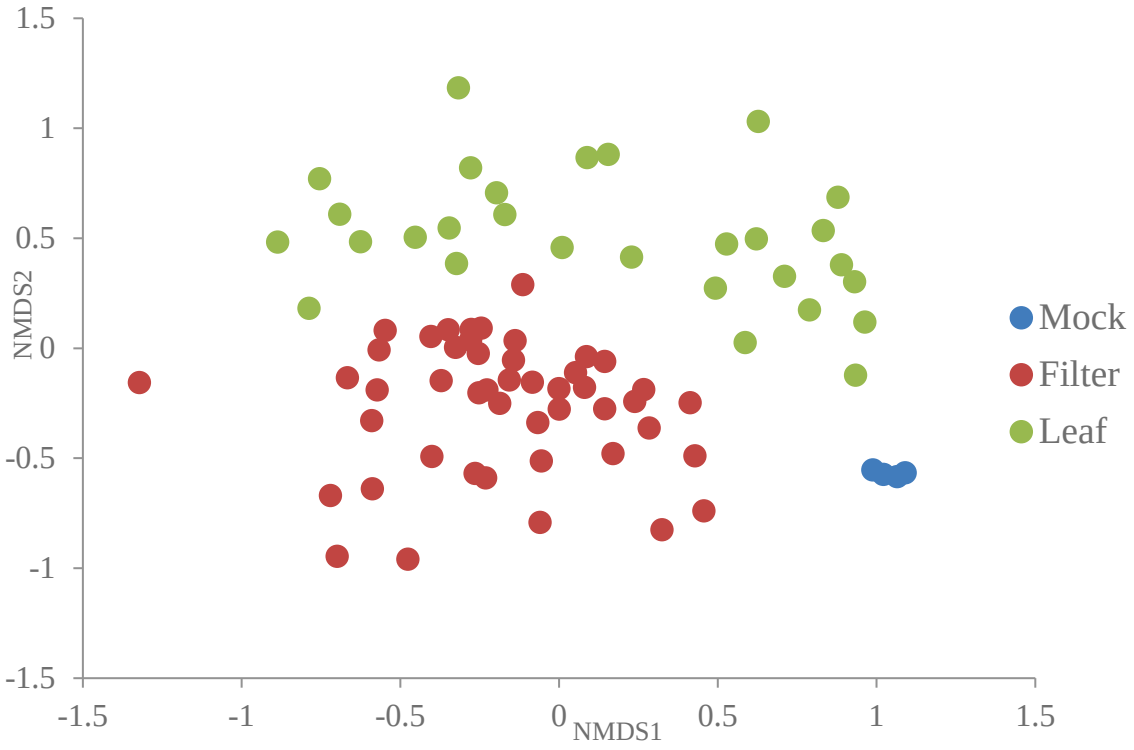

Figure 4—OTU abundance-based ordination of samples (each dot represents the oomycete community sequenced from an individual sample).

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Monitoring Streams and Stormwater Ponds for Early Detection of Oomycete Plant Pathogens in Western Washington, a Citizen Science Project[1]

Marianne Elliott[2], Lucy Rollins[2], and Gary Chastagner[2]

Abstract

Sudden Oak Death (SOD) is the common name for a disease caused by *Phytophthora ramorum* (oomycetes), an invasive plant pathogen of regulatory concern. The nursery, timber, forest specialty product, and Christmas tree industries in Washington are at risk because of the spread of *P. ramorum* within nurseries and from nurseries into waterways and the landscape. This study was initiated in 2010 in order to monitor for early detection of *P. ramorum* in western Washington streams and ponds. Since *P. ramorum*, to date, has been documented to be established in only a few WA streams, this survey provides a baseline description of other oomycete species present in western WA water bodies in urban, rural, and wildland areas.

In 2013, two bait samples positive for *P. ramorum* were collected from the Dungeness River near Sequim, WA. Further sampling of streams in this watershed in 2014 did not yield information about the source of inoculum contaminating the Dungeness. The site where the positive sample was found had no apparent direct water connection with a *P. ramorum*-positive nursery and the source of inoculum is unknown. In spring 2015, Washington State University volunteers did intensive sampling of 11 streams in the watershed. No *P. ramorum* was found at any of the sites sampled. It is possible that *P. ramorum* is no longer present or is at undetectable levels in the Dungeness River watershed. During this study, the first detections in Washington State of *P. bilorbang* and *Halophytophthora fluviatilis* were reported. Additionally, a potential new species of *Phytophthora* related to *P. pseudosyringae* that was recovered is being analyzed by a student volunteer.

Another goal of this project is to identify *Phytophthora* species that may be moving from landscaped areas into stormwater retention ponds. In essence, these ponds may serve as sentinel sites for the detection of exotic *Phytophthoras* that are introduced into landscape sites via the movement of diseased nursery stock. This aspect of the project is also providing research opportunities to local high school and college students. These projects provide the students with hands-on research experience and the opportunity to learn about a group of organisms that is not covered in any detail in their biology classes. As a result, these students gain experience doing scientific research that they can use in future study, as well as adding it to their resume. This has worked out well for everyone involved, including teachers, students, and researchers.

We plan to develop this citizen science program further. In addition to monitoring for oomycete pathogens, other parameters such as nutrient loads, pesticides, water chemistry, and bacterial

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contamination could be studied in stormwater retention ponds. During this project we interacted with volunteer organizations such as Stream Keepers and Master Gardeners, as well as landowners, students, and the general public. As a result, awareness of waterborne plant pathogens and the importance of sanitation to prevent movement of these organisms has been increased with these groups.

Testing Biological Control Agents for Suppression of *Phytophthora ramorum* in Potting Mixes in a Simulated Nursery Environment[1]

Marianne Elliott[2] and Gary Chastagner[2]

Abstract

The spread of *Phytophthora ramorum* from infested areas in nurseries and landscape sites is commonly associated with the movement of inoculum in water or from the movement of contaminated soils. It has been shown that *P. ramorum* can survive asymptomatically on roots of containerized plants and in potting media. The development and implementation of cost effective and environmentally acceptable best management practices (BMPs) to limit the spread of *P. ramorum* has been identified as a high priority need by the nursery and forestry industries as well as state and federal regulatory agencies.

Studies have shown that growing plants in suppressive composted bark or mulch is an effective way to reduce the likelihood of *Phytophthora* root rot development. The addition of inhibitory organisms to a potting mix that is conducive to their growth shows potential as a means of controlling *P. ramorum* and other diseases in the soil environment and will prevent the movement of inoculum in containerized plants. This study, at the National Ornamentals Research Site at Dominican University of California, examined whether commercially available suppressive organisms could be added to potting mixes to help suppress *P. ramorum* in a nursery setting, thus providing a BMP for nurseries and landscapers who are concerned about mitigating *P. ramorum* in soil. The effects of temperature and soil moisture on *P. ramorum* survival in containers were also examined.

There were no significant relationships between the number of times pots were positive for *P. ramorum* and biocontrol treatments or host plants. The results of both trials show that *P. ramorum* inoculum can increase in overwintering potted plants, irrespective of other treatments such as potting mix composition and treatment with biocontrol agents. Logistic regression analysis showed a significant relationship between the number of *P. ramorum* positives and potting mix type, which could be due to differences in soil moisture and maximum temperature among the treatments. Soil moisture and maximum soil temperature were negatively correlated during the warmer months, and there was a strong negative relationship between maximum daily ambient temperature and levels of *P. ramorum* in all treatments. The difference in *P. ramorum* colonization of pots among blocks in the experiment suggests that environmental factors, particularly temperature, are important to consider. The lack of control of *P. ramorum* in the soil by the biocontrol agents may be related to soil conditions such as temperature, moisture, and pH. It may also be related to the interactions with *P. ramorum* and other soil organisms under these conditions, despite their ability to control *P. ramorum* on foliage and *in vitro*.

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Development of a Predictive Model to Estimate the Effect of Soil Solarization on Survival of Soilborne Inoculum of *Phytophthora ramorum* and *Phytophthora pini*[1]

Fumiaki Funahashi[2] and Jennifer L. Parke[2]

Abstract

Soil solarization has been shown to be an effective tool to manage *Phytophthora* spp. within surface soils, but estimating the minimum time required to complete local eradication under variable weather conditions remains unknown. A mathematical model could help predict the effectiveness of solarization at different sites and soil depths. Prior research on solarization efficacy has focused on the minimum temperature and exposure time required to kill pathogens, and most mathematical models of the effects are based on cumulative temperature over time. However, two additional factors, soil water potential and diurnal temperature fluctuation, may influence pathogen survival. Our objectives were 1) to develop an accurate model to estimate conditions lethal to *Phytophthora* spp. based on results from controlled lab experiments and 2) to test the model with field collected data.

We assessed temperature, water potential, and intermittent heat effects on survival of *Phytophthora ramorum* and *P. pini* in infested leaf inoculum. Survival was assessed by plating the leaf inoculum and observing outgrowth of the pathogen. For both pathogens, survival frequency at higher temperatures was greater at lower water potentials. Survival was also greater when exposure to high temperature was interrupted by a cooler temperature. Results indicate that heat effects on pathogen survival increase gradually during heat treatment, suggesting the temperature effect is not simply cumulative. The mathematical model was tested in solarization field trials conducted at the National Ornamentals Research Site at Dominican University of California (*P. ramorum* and *P. pini*) and the Botany Farm, Corvallis, OR (*P. pini*) by comparing calculated heat units with and without solarization to the recovery of *P. ramorum* and *P. pini* from inoculum buried at 0, 5, and 15 cm. The model was improved significantly with the addition of water potential and temperature fluctuation as explanatory variables, allowing for greater accuracy in predicting soil solarization efficacy.

Using field weather station and soil temperature data, we then expanded the mathematical model to predict soil temperature regimes during soil solarization from inputs of solar radiation, air temperature, relative humidity, and average soil temperature in non-solarized sites. The model is currently being improved to estimate the efficacy of soil solarization in various locations in WA, OR, and CA.

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CALINVASIVES: A Revolutionary Tool to Monitor Invasive Threats[1]

M. Garbelotto,[2] S. Drill,[3] C. Powell,[4] and J. Malpas[4]

Abstract

CALinvasives is a web-based relational database and content management system (CMS) cataloging the statewide distribution of invasive pathogens and pests and the plant hosts they impact. The database has been developed as a collaboration between the Forest Pathology and Mycology Laboratory at UC Berkeley and Calflora. CALinvasives will combine information on the dispersal potential of pathogen and pest threats, the current location of each, the hosts threatened, and their distribution, making it possible to assess the risk posed by each existing and emerging threat to California's native habitats. The database will be generated with the cooperation and assistance of Calflora, and will be interconnected with their existing database. Plant species listed in Calflora will include cross-links to the CALinvasives database with a description of the threat, photographs, and its range overlapping the host. From the pathogen/pest page, the user will be able to access the interactive map with records of pathogen by host, and links to pest/pathogen the interactive map with records of pathogen by host, and links to pest/pathogen mitigation and treatment resources. Calinvasives will provide information on the geographical distribution of emergent pathogens and pests in real time, linking the user to existing resources to identify likely management approaches and possible mitigation strategies.

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Validation of the Bait Test with Rhododendron Leaves for *Phytophthora* Diagnosis[1]

Corina Junker[2] and Sabine Werres[2]

Abstract

Bait tests are very helpful for diagnosis of *Phytophthora* in for example soil, substrate, water, sediment, and rootball samples (Werres and others 2014). By attracting the motile zoospores of the *Phytophthora* species with the baits these pathogens can be separated from other organisms. Bait tests are simple and cost effective and - compared with other diagnostic techniques - they are much more independent on the varying sample quality. Furthermore, bait tests can be used for evaluating big sample quantities.

Within the preparation of laboratory accreditation according to ISO standard ISO/IEC 17025 the European Plant Protection Organization (EPPO) recommends the validation of laboratory methods ("Specific requirements for laboratories preparing accreditation for plant pest diagnostic activity" EPPO standard PM 7/98; PM 7/76). In this guideline, detailed instructions on test verification are given, like analytical sensitivity and specificity, selectively, repeatability, and reproducibility.

Within the European project "Responses of European Forests and Society to Invasive Pathogens" (RESIPATH, http://www.slu.se/resipath) the bait test with rhododendron leaves will be validated according to the EPPO guidelines. For the validation the standard protocol with detached rhododendron leaves according to Themann and and Werres (1998, 2000) will be used. First results of the different validation steps will be presented.

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Diversity of Foliar *Phytophthora* Species on *Rhododendron* in Oregon Nurseries[1]

B.J. Knaus,[2] K.A. Graham,[2] Niklaus J. Grünwald,[2] and Valerie J. Fieland[3]

Abstract

The genus *Phytophthora* contains some of the most notorious plant pathogens affecting nursery crops. Given the recent emergence of the sudden oak death pathogen *Phytophthora ramorum*, particularly in association with *Rhododendron* spp., characterization of *Phytophthora* communities associated with this host in nursery environments is prudent. Many taxa may present symptoms similar to *P. ramorum* but we do not necessarily know their identity, frequency, and importance. Here, we present a survey of *Phytophthora* taxa observed from seven nurseries in the U.S. state of Oregon. Incidence and diversity of *Phytophthora* communities differed significantly among nurseries and among seasons within nursery (Knaus and others 2015). The taxa *P. syringae* and *P. plurivora* were widespread and detected at most of the nurseries sampled. Nine other taxa were also detected but were found either in a single nursery or were shared among only a few nurseries. Characterization of the *Phytophthora* communities present in nurseries is an important step toward understanding the ecology of these organisms as well as an aid to nursery managers in determining what risks may be present when symptomatic plants are observed. This study builds on an increasing literature, which characterizes *Phytophthora* community structure in nurseries.

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Development of Reagents for Immunoassay of *Phytophthora ramorum* in Nursery Water Samples[1]

Douglas G. Luster,[2] Timothy Widmer,[2] and Michael McMahon,[2] and C. André Lévesque[3]

Abstract

Current regulations under the August 6, 2014 *USDA APHIS Official Regulatory Protocol (Confirmed Nursery Protocol: Version 8.2) for Nurseries Containing Plants Infected with Phytophthora ramorum* mandates the sampling of water in affected nurseries to demonstrate they are free of *P. ramorum*. Currently, detection of *P. ramorum* in these samples requires baiting periods followed by pathogen growth and/or expensive nucleic acid molecular detection methods. A rapid detection tool would greatly reduce the time, cost, and effort needed to demonstrate that nurseries are free of *P. ramorum*. To meet this need, we are currently developing rapid antibody detection tools for *P. ramorum* in nurseries, focusing on detection in irrigation water, surface water, and irrigation sources.

Recently published information has revealed extracellular *Phytophthora* proteins with hyper-variable domains that represent promising targets for development of species-specific diagnostic immunoreagents. Sequence alignments across numerous *Phytophthora* species have identified *P. ramorum*-specific antigenic domains within these proteins, providing an opportunity to target these domains for generation of antibodies for detection of zoospores, sporangia, and possibly chlamydospores. Using this information, we have made polyclonal antibodies against recombinant peptides that are reactive against *P. ramorum* zoospores and sporangia, respectively, in Western Blot and ELISA assays, as well as live cell immunofluorescence experiments. Sensitivity and specificity testing is in progress against a number of *Phytophthora spp.* that have been reported to be identified in nursery and surface water samples from across the U.S. Peptide antigens that generate the required specificity for *P. ramorum* in polyclonal antibody testing will be used for monoclonal antibody production for sandwich ELISA and immunostrip assays.

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Sentinel Plant Monitoring of *Phytophthora ramorum* at a Research Nursery Over a Six-Year-Period Indicates Limited Aerial Pathogen Spread[1]

Tomas Pastalka,[2] Karen Suslow,[2] and Wolfgang Schweigkofler[2]

Abstract

The National Ornamentals Research Site at Dominican University of California (NORS-DUC) is a research nursery that was established in 2009 to study invasive plant pathogens like *Phytophthora ramorum*, causal agent of sudden oak death and ramorum blight. In order to fulfill federal and state regulations, the possible movement of pathogens from the research site must be monitored using a sentinel plant system with host plants of *P. ramorum* (*Rhododendron*, *Camellia*, and *Viburnum*).

Symptoms on the sentinel plants are studied using culturing, immunoassays, and sequence analysis. Symptom development follows a clear seasonal pattern with a peak in the rainy season (December to March) and very few infections in the dry season. To date, *P. ramorum* has not been detected on sentinel plants, indicating limited aerial spread of the pathogen under the suboptimal climatic and environmental conditions for the pathogen in San Rafael (warmer and drier than areas closer to the coast). Infection studies in the research nursery corroborate this conclusion and indicate that other dispersal strategies (e.g. through water circulation or standing water) might play a more crucial role in certain environments, such as in nursery settings. However, *P. ramorum* infections did occur on host plants (California bay laurel, *Umbellularia californica*) in the proximity of the research site during the rainy season. *P. ramorum*-like symptoms on sentinel plants were associated with a number of Oomycetes, including *P. hibernalis*, *P. syringae*, *P. multivora*, *P. cf. fallax*, and an isolate closely related to *P. boehmeriae* as well as an ascomycetous fungus closely related to *Neofusicoccum cryptoaustrale*. *P. fallax* has previously only been isolated from crown dieback symptoms of eucalyptus in New Zealand and Australia and *N. cryptoaustrale* from eucalyptus in South Africa. Host ranges of the *P. cf. fallax* and *Neofusicoccum sp.* isolates detected in CA are unknown. These plant pathogens might represent new invasive species in the USA**.**

Aerial spread of *P. ramorum* in nurseries might be reduced using buffer plants. A NORS-DUC study was conducted to identify ornamental plants that are not susceptible to *P. ramorum* infection so growers may select these buffer plants to break up large, contiguous blocks of high-risk plants and thus reduce the area of destruction should the pathogen be found on their property. Three potential buffer plants were tested - *Buxus sempervirens*, *Nandina domestica*, *Liriope muscari* - two sun plants and one shade plant, respectively, and all heavily traded in the industry. Buffer plants were arranged around infected rhododendron plants during winter 2014/15 and symptom expression was evaluated in June of 2015. *P. ramorum* was not detected on buffer plants. The experiment was repeated 2015/16 under El Niño-conditions, and *P. ramorum* was isolated numerous times from *N. domestica* and *L. muscari*. To our knowledge, this is the first time that *P. ramorum* was found infecting those two plant species.

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Management of Foliar Infection of Rhododendron by *Phytophthora ramorum* With Film Forming Polymers and Surfactants[1]

Ebba K. Peterson,[2] Eric Larson,[2] and Jennifer L. Parke[2]

Abstract

Phytophthora ramorum, causal agent of sudden oak death (SOD) and ramorum leaf blight, remains a persistent problem of regulatory concern within the horticultural industry. Damages to nurseries have been realized as a result of enforced quarantine and sanitation efforts designed to prevent the spread and establishment of this invasive pathogen. Additionally, the introduction of *P. ramorum* to heritage gardens provides a treatment challenge when the goal to prevent local spread conflicts with the preservation of plant collections. There is a need for the development for alternative treatments that may prevent *P. ramorum* infection, particularly of foliar tissues from which sporangia are produced.

Film-forming polymers (FFPs, also commonly marketed as anti-transpirants) and surfactants are promising treatments to prevent *P. ramorum* infection of rhododendron. Five FFPs (Anti-Stress 2000, Moisturin, Nature Shield, Nu-Film P, and Vapor Gard) and three surfactants (Tergitol NP-7, Zonix, and an unregistered AGAE product) were screened with detached leaf assays utilizing rhododendron cv. Roseum Elegans. Anti-Stress, Nu-Film, and a Zonix and Nu-Film combination were retained for additional experiments testing for utility at managing *P. ramorum* on horticultural rhododendron.

To test durability of protection of leaves from infection, potted 1-gal. rhododendrons cv. Roseum Elegans were sprayed with Zonix, Anti-Stress, or Nu-Film and maintained with overhead irrigation in a container yard for 4 weeks. Plant leaves from each treatment were removed at weekly intervals and challenged in detached leaf assays. A second trial included an additional treatment of Zonix combined with Nu-Film. Overall, the Zonix treatments provided the least amount of protection and Nu-Film and the combination treatment provided the most protection; however, all treatments declined in efficacy over the 4 weeks, especially in Trial 2.

The capacity for these treatments to protect rhododendron plants from foliar *Phytophthora* infections was then tested from two different sources of inoculum naturally occurring in nurseries: (a) exposure to infested surface waters and (b) aerial spread from infected plants. For (a), potted 2-gal rhododendron plants were sprayed with DI-water, Anti-Stress, Nu-Film, or a Nu-Film and Zonix mixture. Fourteen branches per treatment were removed and dipped in a *P. ramorum* zoospore suspension for 4 minutes. Branches were incubated for 7 days at 68 °F, and leaf lesion area was measured.

For objective (b), potted 2-gal rhododendron plants were sprayed with DI-water, Anti-Stress, Nu-Film, or a Nu-Film and Zonix mixture. Plants were arranged in groups on pallets under shade cloth in a container yard. At this time, detached leaves infested with *P. plurivora* in the laboratory were clipped to flags and placed within the upper canopy of plants to act as a primary inoculum source. New, untreated field-

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inoculated rhododendron plants were also placed in the center of each group of test plants to act as an inoculum source. Plants and inoculum were left in the container yard until mid-May, after which we collected all symptomatic leaves and plated them in selective media to confirm infection by *P. plurivora*.

In the dip trial (objective a), plants treated with DI-water (control) had the greatest number of *P. ramorum*-positive branches and the greatest lesion area. All treatments significantly reduced average lesion area relative to controls. For aerial spread between plants (objective b), infection rates for test plants in the container yard were low. Plants treated with DI-water (control) had the greatest number of infected leaves per plant; the least amount of infection was observed on plants treated with Anti-Stress and the Nu-Film and Zonix mixtures.

Lastly, these compounds were tested to examine how they affected sporulation and lesion development when applied post-infection. Wounded leaves were inoculated with *P. ramorum* zoospores 1 day prior to application of Zonix, Anti-Stress, Nu-Film, or a Nu-Film and Zonix mixture. After 10 days, lesion sizes were measured and the numbers of sporangia produced per leaf were counted. All treatments, except for Nu-Film, significantly reduced the number of sporangia produced. None of the treatments caused a significant reduction in symptom development relative to controls.

From these studies, it appears that either an Anti-Stress 2000 or a Nu-film P and Zonix combination may prove valuable in preventing the establishment and spread of *P. ramorum* in nurseries and ornamental gardens due to reductions in infection and sporulation. These materials are already used in the nursery industry, although the anti-transpirants are not labeled for use as pesticides. The surfactant Zonix is labeled for use as a biofungicide on ornamentals and vegetable crops in most states. Nu-Film should also help prevent the washing off of Zonix from plant foliage by rainfall or overhead irrigation. In contrast to conventional chemical treatments (e.g. mefenoxam), these compounds pose a reduced risk for the development of resistant isolates and allow for the detection of infected plants while minimizing the risk of further inoculum spread.

Soil Moisture and Temperature Conditions Affect Survival and Sporulation Capacity of Rhododendron Leaf Disks Infested with *Phytophthora ramorum*[1]

Ebba K. Peterson,[2] Niklaus J. Grünwald,[3] and Jennifer L. Parke[2]

Abstract

Soilborne inoculum (infested leaf debris which has become incorporated into the soil) may be an important contributor to the persistence of the sudden oak death pathogen *Phytophthora ramorum* in recurrently positive nurseries. To initiate new epidemics, soilborne inoculum must not only be able to survive over time, but also be capable of producing sporangia during times favorable to infection of plant material at the soil surface.

To accompany field studies of the epidemiological risk of soilborne inoculum in nurseries, laboratory assays were performed investigating how incubation of inoculum at various temperature and moisture regimes affects sporulation capacity and survival of *P. ramorum*. For all experiments, wounded rhododendron leaves were infected with *P. ramorum* zoospores and then incubated for 2 to 3 weeks. Leaf disks were punched out of the lesioned areas, which were inserted into mesh sachets and placed at the various moisture and temperature treatments. Over time we recovered sachets and placed the disks in tubes containing filtered creek water at 20°C to induce sporulation. The tubes were vortexed after 1 week and the water was filtered to capture sporangia, then the leaf disks were plated on selective media to discern how incubation conditions affected survival.

To test how incubation at different temperatures and moisture levels affects sporulation, leaf disk inoculum was packed into capsules containing soil at matric potentials of 0, -40, and -400 kPa. Capsules of each moisture level were placed in growth chambers set at an average temperature of 6.7, 14, 20, or 28°C. Six capsules per moisture level per temperature were removed at 2, 6, 12, and 18 weeks post-incubation to assess for survival and sporulation potential. Recovery was high for all but the warmest and driest treatments. Sporulation remained greatest over time for disks incubated at cooler temperatures for most treatments and was greater for treatments at non-saturated moisture levels.

To test chilling effects upon sporulation, leaf disk inoculum was incubated in saturated soil at 20°C for 3 weeks, which had the effect of reducing sporulation capacity relative to the pre-incubation sporulation and controls maintained at 4°C. Inoculum was then placed at either 4 or 20°C. Disks were retrieved and assessed for sporulation capacity for up to 168 days after the exposure of a subset of this inoculum to 4°C. Maximum sporulation from the 20°C to 4°C treatment was observed 49 days post-exposure in both trials.

To determine how prior moisture and temperature conditions affect sporulation responses to chilling, at the week-18 assessment for the experiment testing the interactions between moisture and temperature, an additional set of samples for each temperature:moisture combination was placed at 4°C. After 49 days the

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samples were retrieved and assessed for sporulation and survival. Incubation at 4°C increased sporangial production for all inoculum initially incubated at 20 and 28°C, but not 6 and 14°C regardless of moisture.

The likelihood of new epidemics developing from soilborne inoculum sources will be modulated by the time of introduction and the soil environment. Exposure to moderate temperatures and moisture regimes simulating soil conditions rapidly reduces sporulation potential from leaf material infested with *P. ramorum*; however, sporulation potential increases post-exposure to cooler temperatures, especially for inoculum incubated at 20°C. This work is in agreement with field observations that the onset of cooler temperatures in autumn and winter may initiate new *P. ramorum* infections from soilborne sources.

Identification of Five New Hosts of *Phytophthora ramorum* in an Infested Forest in California[1]

S. Rooney-Latham,[2] C.L. Blomquist,[2] A. Williams,[3] E. Gunnison,[3] and T. Pastalka[4]

Abstract

Phytophthora ramorum causes stem and bole cankers (sudden oak death) and foliar and twig dieback (ramorum blight) of susceptible plants. To date, more than 100 tree, shrub and herbaceous hosts of *P. ramorum* have been identified. In March 2015, plant samples were submitted to the CDFA Plant Pest Diagnostics Lab from the Marin Municipal Water District for disease analysis. The samples were collected near Bolinas Ridge in Marin County, CA in a maritime chaparral-live oak woodland forest with a history of *P. ramorum* and *P. cinnamomi*. The collectors noticed a large amount of unusual die-back in many plant species earlier in the drought year and were concerned that the plants were infected with *P. cinnamomi* or other *Phytophthora* root pathogens. Manzanita species (*Arctostaphylos canescens, A. sensitiva, A. virgata*), chinquapin (*Chrysolepis chrysophylla*) and chaparral pea (*Pickeringia montana*) were submitted for diagnosis. Isolation from the roots onto PARP media from samples was attempted even though the roots appeared healthy. No *Phytophthora* sp. grew on isolation plates and no *Phytophthora* spp. were detected from the roots using the Agdia *Phytophthora* spp. specific immunoassay. The *Arctostaphylos* and *Pickeringia montana* samples contained foliar tissue with leaf spot, vein necrosis and stem canker symptoms which were tested separately for *Phytophthora*. *Phytophthora* spp. was detected from the leaf spots and stem cankers from the *Pickeringia montana* and some of the *Arctostaphylos* spp. by immunoassay. In addition, *P. ramorum* was detected in culture and confirmed by sequence analysis from the symptomatic foliage of these hosts.

In April 2015, a second visit was made to the site to obtain official samples for regulatory purposes. In addition to the hosts collected initially, *Arctostaphylos glandulosa* and *Rubus ursinus* (California blackberry) were collected for testing for *P. ramorum*. On some *Arctostaphylos* plants, the leaves on entire branches were completely brown while the cambium was still green. Both mature and young foliage were affected. On some manzanita plants, the older foliage seemed more broadly affected, while on others the newest foliage on the growing tips was the most symptomatic. Chaparral pea plants exhibited small leaves that were necrotic with some partially defoliated. Cankers on the plants were located near the nodes with numerous necrotic areas on the short thorny stems. *Phytophthora ramorum* grew from isolated foliar and stem blight symptoms of *Pickeringia montana, A. virgata* and *A. glandulosa. Phytophthora ramorum* DNA was also detected from foliar leaf spot symptoms of blackberry using a species specific qPCR. Isolations from blackberry were unsuccessful on PARP media. Declining chinquapin trees appeared drought stressed with dry, chlorotic, and off-colored leaves. Affected branches and root suckers were collected and dark vascular streaks were seen in the xylem tissue when the bark was removed. These symptoms were seen in fully grown trees and young seedlings. *Phytophthora*

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ramorum DNA was isolated in culture onto PARP medium from the vascular streaking in a single tree but tested positive by PCR for *P. ramorum* from multiple trees.

Although the Bolinas Ridge area in Marin County, CA is known to be infested with *P. ramorum*, five new associated hosts of *P. ramorum* were identified at this site. *Arctostaphylos virgata* is endemic to Marin County and is listed on the California Native Plant Society Inventory of Rare and Endangered Plants. The severe leaf and stem blight symptoms seen on *A. virgata* and *A. glandulosa* differ from those previously described on *A. manzanita*, but are similar to those on other ericaceous hosts. Chaparral pea (Fabaceae) is an endemic shrub to California that occurs along the coast and in the foothills of the Sierra Nevada Mountains. Difficulty in sampling its thorny stems may have contributed to it being overlooked as a host of *P. ramorum* in the past. Chinquapin has been suspected as a host of *P. ramorum*; log inoculations indicated it was susceptible. This is the first report of *P. ramorum* being isolated from symptomatic chinquapins in the wildlands. The dark vascular streaking associated with infection by *P. ramorum* suggests that *P. ramorum* may also be a wilt pathogen on some members of the *Fagaceae*. Pathogenicity experiments are ongoing and are dependent on obtaining the proper species for testing.

Host Range Determination and Fungicide Resistance Assessment of *Phytophthora lateralis* Isolates from Horticultural Nurseries in Oregon[1]

Franziska Rupp,[2] Ebba K. Peterson,[2] Joyce Eberhart,[2] and Jennifer L. Parke[2]

Abstract

Phytophthora lateralis causes root rot of Port-Orford cedar (*Chamaecyparis lawsoniana*; POC) in native forests of northwest California and southwest Oregon and in landscape plantings of horticultural *Chamaecyparis* cultivars in the western US and Europe. In spring 2015, following observations of mortality amongst plant groups in two horticultural nurseries in Oregon, *P. lateralis* was isolated from the roots of two additional conifer species: *Microbiota decussata* and *Juniperus communis*. Species identification was confirmed by sequencing the ITS region using ITS4 and ITS6 primers. To substantiate the extended host range of *P. lateralis*, we conducted Koch´s Postulates with the recovered nursery isolates on potted *Juniperus* and *Microbiota* plants.

One isolate from *Juniperus* and one from *Microbiota* were included in a greenhouse inoculation trial. We additionally included one isolate recovered from POC used in POC-resistance screening trials as a standard. Test plants included *J. squamata* cv. Blue Star, *J. communis* cv. Blueberry Delight, and *M. decussata* cv. Celtic Pride; a susceptible POC clone provided by the Dorena Genetic Resource Center was included as a positive control. We applied zoospore inoculum to the base of each plant. Plants were flooded for 3 days after inoculation, then again for 24 hours once a week. Inoculum was re-applied after 3 weeks. After 7 weeks, root segments and stem lesions were plated on *Phytophthora*-selective media. We then characterized and re-sequenced isolates to confirm the identification as *P. lateralis*.

Symptoms (foliage chlorosis, stem discoloration, root necrosis, and mortality) were observed on POC inoculated with all three *P. lateralis* isolates and on both *Juniperus* and *Microbiota*. In comparison to plants inoculated with the POC isolate, greater mortality of *Juniperus* and *Microbiota* was observed when inoculated with the nursery isolates. Nursery isolates were successfully re-isolated from POC, *Juniperus*, and *Microbiota*. Characterization of recovered isolates was completed via morphology and ITS sequencing; *P. lateralis* was the top match at 99% - 100%.

Because disease occurred in nurseries despite frequent application of the oomycete-specific fungicide Subdue Maxx (Syngenta Crop Protection), all isolates (four from *Microbiota*, seven from *Juniperus*, and the POC isolate) have been tested *in-vitro* for resistance to the active ingredient mefenoxam. Mefenoxam resistance was assessed on 10-day-old cultures growing on agar plates amended with different concentrations (100 ppm, 10 ppm, 1 ppm, 0.1 ppm) of mefenoxam. Colony growth on fungicide-amended media was compared to growth on non-amended media. No evidence for resistance has been detected at these concentrations.

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Koch´s Postulates was completed on *Juniperus* and *Microbiota*, adding two new hosts to the list of species susceptible to *P. lateralis* infection. Given observations in the inoculation trial, further research is in progress to assess differences in aggressiveness between the nursery isolates and standard POC isolates used in POC resistance screening as well as determine pathogen clonal lineage. Importantly, no evidence for the development of resistance to mefenoxam was found; however, the potential for the development of resistance in these nurseries should be considered and monitored.

Acknowledgments

We would like to thank Dr. Richard Sniezko and Erin Hooten of the USDA Forest Service Dorena Genetic Resource Center for providing susceptible POC seedlings and Paul Reeser and Wendy Sutton for assistance producing inoculum.

Thermal Inactivation of Infested Plants, Nursery Equipment, and Soil is a Management Option for the Treatment of *Phytophthora ramorum*, Causal Agent of Sudden Oak Death[1]

Wolfgang Schweigkofler,[2] Vernon Huffman,[2] Karen Suslow,[2] and Kathleen Kosta[3]

Abstract

Infected nursery plants play an important role in the spread of *Phytophthora ramorum*, the causal agent of sudden oak death and ramorum blight. In order to minimize the risk for disease transmission to new areas, nurseries are inspected regularly for *P. ramorum*, and federal regulations require the eradication of infested plants and the disinfestation of nursery soil and equipment.

The National Ornamentals Research Site at Dominican University of California (NORS-DUC) is a federally funded research nursery devoted to testing and developing environmental friendly management options for quarantine pathogens of ornamental plants. In the laboratory, the effect of wet and dry heat on the survival rate of *P. ramorum* growing on Rhododendron leaf disks is tested. Incubation at 30 °C showed little effect on the survival rate. At
40 °C, growth rates started to decrease. Incubation at 50 °C for 30 minutes (wet heat) inactivated *P. ramorum* completely, whereas dry heat was slightly less effective. At the research nursery, thermal inactivation of plant debris, soil, and nursery equipment infested by *P. ramorum* was achieved by steaming using a commercial steaming unit (SIOUX Steam-Flo SF-1) at a minimum temperature of 50 °C. Temperature increase was influenced by ambient temperature, soil depth, moisture content, and compactness. Steaming was also used to decontaminate soil in a commercial nursery in the Central Valley of California which was found positive for *P. ramorum* previously. No *Phytophthora* was detected in official samples post-treatment; consequently, the commercial nursery was released from federal quarantine.

Further studies on heat treatment and steaming in the laboratory and the research nursery on other *Phytophthora* species, such as *P. tentaculata*, indicate that the method might be suited to control a wide range of plant pathogenic oomycetes in nursery soils.

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Research on the Quarantine Pathogen *Phytophthora ramorum* at the National Ornamentals Research Site at Dominican University of California[1]

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Abstract

The National Ornamentals Research Site at Dominican University of California (NORS-DUC) was founded in the year 2009 by a Farm Bill grant to study *Phytophthora ramorum* in a sophisticated research nursery that reflects an authentic commercial nursery setting (www.dominican.edu/norsduc). NORS-DUC goals are to develop practical solutions for containment, remediation, and eradication of quarantine pathogens in nurseries and to reduce the risk of long-range spread of pests through infested nursery stock shipments. Research at NORS-DUC is conducted by a team of permanent staff as well as by *P. ramorum* experts from other institutions who can apply for grants to work at NORS-DUC. The research site offers a unique opportunity to study different aspects of ornamental diseases caused by *P. ramorum* and other quarantine organisms that cannot easily be accomplished using experiments in a laboratory.

Mission and Purpose

The National Ornamentals Research Site at Dominican University of California mission is to provide nursery growers with solutions and collaborating researchers with lab and field support to aid in their project success in the areas of horticulture and plant disease management.

- NORS-DUC is the only research site in the USA dedicated to the study of invasive quarantine pathogens of ornamental plants in an open, nursery-like environment.
- NORS-DUC is a collaboration between Dominican University of California, the United States Department of Agriculture (USDA), and the California Department of Food and Agriculture (CDFA).
- NORS-DUC invites scientists from other universities as well as public and private research centers to conduct studies on quarantine soil-borne organisms at a state-of-the-art research facility.
- NORS-DUC focuses on diseases of ornamental and forest plants, especially those caused by members of the genus *Phytophthora*, like *P. ramorum*, causal agent of Sudden Oak Death, and more recently *P. tentaculata*, a newly discovered plant pathogen in North America impacting native plant nurseries in CA.
- NORS-DUC primarily focuses on applied research, such as validation and development of best management practices; development of remediation options for soil, water, and infested plants; and development of monitoring and control strategies.
- NORS-DUC shares research results with the public through a strong outreach program as well as scientific and technical publications.
- NORS-DUC invites DUC students to participate in research activities in the lab and the research nursery.

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Recent Projects and Collaborations

During the first 5 years of NORS-DUC, researchers from UC Berkeley, UC Davis, Washington State University, Oregon State University, Ohio State University, Colorado State University, Clemson University, and several USDA laboratories conducted experiments at the NORS-DUC site. They focused on such diverse topics as: chemical, thermal, and biological control of *P. ramorum*; long-term survival and sporulation capacity of the pathogen in the soil; resistance mechanisms of host plants; genetic stability of *P. ramorum* isolates growing on host and non-host plants; effect of physiological stress on host plant susceptibility; and disease epidemiology in nurseries.

Recently, studies on new emerging invasive *Phytophthora* species, such as *P. tentaculata*, were also initiated at NORS-DUC, focusing on early detection and development of control strategies. Research at NORS-DUC resulted in the acceptance of steaming as a method for treating *P. ramorum*-infested soils by the USDA Animal and Plant Health Inspection Service (APHIS), and a strong outreach program aims to disseminate scientific results quickly and efficiently to nursery and landscape industries as well as other stakeholders.

Infrastructure

NORS-DUC contains two research sites: site SOUTH (fig. 1) is trapezoid shaped (40 x 36.5 x 47 x 21 m) and site NORTH is rectangular (65 x 16 m). The perimeters of both sites are fenced with a single entry point. Each site contains six individually fenced-in research plots (fig. 2); within each research plot exists a research bed that can be used for the experiments. Most research beds are 3.5 x 9 m and can be subdivided into two 3.5 x 4.5 m half beds. Four research beds are filled with soil for in-soil experiments (fig. 3). The other eight research beds are used for experiments with potted plants (fig. 4); one plot contains a green house (3.6 x 5.5 m). All research beds are lined with a waterproof pond liner to maintain and collect water, which then is filtered, UV-treated and checked for *P. ramorum* presence before release from the research site. A sentinel plant system is used to detect aerial pathogen spread (fig. 5). Incoming plants are held at a quarantine site for 6 weeks before moving into the research plots (fig. 6). The NORS-DUC lab is equipped for microbiological, biochemical, and molecular analysis.

Future Directions

- Study the biology, spreading pattern, and control options of new, invasive pathogens like *P. tentaculata* and other Phytophthoras.
- Develop collaborations with new partners, like the nursery industry, universities, and private companies with an interest in plant pathology, horticulture, and agriculture.
- Become a local leader in sustainable management of diseases of ornamental plants.

Information on request for proposals to work at NORS-DUC can be found at www.dominican.edu/norsduc.

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Figure 1-Aerial photo of nursery site SOUTH. Figure 2-Fenced-in research plots. Figure 3-Plot for in-soil experiments. Figure 4-Plot for research with potted plants. Figure 5-Sentinel and barrier plants on the outside perimeter of the research site. Figure 6-Quarantine site for incoming plants.

Potential Susceptibility of Canadian Flora to EU2 Lineage of *Phytophthora ramorum*[1]

S.F. Shamoun,[2] G. Sumampong,[2] D. Rioux,[3] and A. Schlenzig[4]

Abstract

A total of 33 host species commonly found in eastern (8) and western (25) Canadian landscapes and forest sites were selected for this study. Detached leaves/needles were inoculated with *Phytophthora ramorum* EU2 lineage mycelia which was isolated from stream bait near an infected larch plantation in Scotland, UK. There was a large variation in susceptibility among hosts. Among the non-conifer hosts, Pacific dogwood, manzanita, camellia, rhododendron and salal in western Canada and sumac, yellow birch, wintergreen and white ash in eastern Canada were the most susceptible to *P. ramorum* EU2 lineage. For conifer hosts, both balsam fir and white spruce in the eastern Canada and grand fir, sitka spruce, Douglas- fir and western larch in western Canada were the most affected following the inoculation.

Introduction

Phytophthora ramorum is an oomycete pathogen and a causal agent of a disease commonly referred to as sudden oak death (SOD). The pathogen causes foliar blight and shoot dieback of nursery plants, including *Rhododendron* and *Viburnum*. It is also responsible for the widespread mortality of tanoak and coast live oak in coastal California and southwestern Oregon, US, as well as, Japanese larch in the UK. There are four distinct clonal lineages of *P. ramorum*, one originally discovered in Europe, but also detected in a few nurseries, waterways, and one wildland site in western North America (EU1), a new lineage recently detected in Europe (EU2), and two lineages only present in North America (NA1 and NA2). The host range of *P. ramorum* is very broad (more than 120 host plants). Many of the host species are currently present in forested and urban areas on the West Coast of the US and Canada. To better assess the risk posed by an exotic pathogen such as *P. ramorum*, it is often a good strategy to evaluate its capacity to infect plants prevalent in the area of interest. This approach has been used with success where potential hosts were identified by artificial inoculation before being found naturally infected by *P. ramorum*. For instance, *Kalmia latifolia* was first identified as highly susceptible to *P. ramorum* under laboratory conditions [(Phytopathology 2002 (92: S81)] and was thereafter found as a host in the UK [(DEFRA. 2003. www.defra.gov.uk/planth/pra/sudd.pdf]. Similar results were published when a larch (*Larix laricina*) was found susceptible for the first time after artificial inoculations in 2008 (Phytopathology 2008 (98: S75)] before Japanese larch (*L. kaempferi*) was reported heavily infected in plantations in the UK in 2009 (Webber and Brasier 2010; Webber and others 2010) (figs. 1 and 2).

In the UK, the current distribution of the EU2 lineage is limited to southwest Scotland and Northern Ireland (NI). The EU2 lineage is almost exclusively the only lineage of *P. ramorum* in NI. The EU2

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lineage has not been found in England and Wales; only the EU1 lineage is present there (fig. 3). The natural hosts for EU2 lineage include Japanese larch, grand fir, noble fir, rhododendron, red oak, *Vaccinium* sp., *Magnolia* sp., and *Pieris* sp. (Personal communication/correspondence with Joan Webber and Alexandra Schlenzig, May 17, 2016 and May 16, 2016, respectively).

To date, we have investigated the susceptibility of selected eastern and western Canadian host plants to three lineages (NA1, NA2, and EU1) [(Elliott and others 2011; Jinek and others 2011). However, there is an urgent need to determine the susceptibility and risk assessment of the newly emerged lineage EU2 in the UK (Van Poucke and others 2012) and its potential threat to Canadian flora and impact on nursery industry and forest ecosystems.

Materials and Methods

Plant Species Tested

Table 1—Western Canada plant species

Host Name	Species
Arbutus	*Arbutus menziesii*
Garry Oak	*Quercus garryana*
Big Leaf Maple	*Acer macrophyllum*
Red Alder	*Alnus rubra*
Poplar	*Populus trichocarpa*
Pacific Dogwood	*Cornus nuttallii*
Paper Birch	*Betula papyrifera*
Camellia	*Camellia japonica*
Salal	*Gaultheria shallon*
Oregon Grape	*Mahonia nervosa*
Rhododendron	*Rhododendron caucasicum x ponticum*
Currant	*Ribes spp.*
Himalayan Blackberry	*Rubus discolor*
Manzanita	*Arctostaphylos spp.*
Bay Laurel	*Umbellularia californica*
Raspberry	*Rubus idaeus*
Blueberry	*Vaccinium corymbosum*
Grapes	*Vitus vinifera*
Western Hemlock	*Tsuga heterophylla*
Lodgepole Pine	*Pinus contorta*
Western Larch	*Larix occidentalis*
Douglas Fir	*Pseudotsuga menziesii*
Sitka Spruce	*Picea sitchensis*
Grand Fir	*Abies grandis*
Western Red Cedar	*Thuja plicata*

Table 2—Eastern Canada plant species

Host Name	Species
Yellow birch	*Betula alleghaniensis*
Sugar maple	*Acer saccharum*
Red oak	*Quercus rubra*
White ash	*Fraxinus americana*
Wintergreen	*Gaultheria procumbens*
Sumac	*Rhus typhina*
Balsam fir	*Abies balsamea*
White spruce	*Picea glauca*

Culture and Detached Leaves/Needles Inoculations

Detached leaves/needles representing eastern and western Canadian regions (tables 1 and 2) were inoculated with mycelia from a single isolate of *P. ramorum* EU2 (PFC5414) acquired under a Canadian Food Inspection Agency (CFIA) Permit #P-2013-03068 from Alexandra Schlenzig (Scottish Government, Agr, Food & Rural Comm. Directorate). The Scottish EU2 isolate was baited from a stream coming from an infected larch plantation in 2012.

Healthy-looking leaves and needles were collected, rinsed in sterile distilled water (twice) and blotted with a paper towel to remove excess moisture prior to wounding and inoculation with an agar plug. Ten leaves were wounded next to the midrib using forceps; whereas, conifer needles were cut at their base with a surface-sterilized scissor. After wounding, a 5 mm plug of *P. ramorum* EU2 lineage inoculum or blank V8 agar plug was placed mycelium side down over the wounded area on the abaxial side of the leaf or on three needles joined together at their base. After 10 days, leaves were photographed on a scanner and lesion size on each leaf caused by *P. ramorum* EU2 lineage was measured using ASSESS software (Lamari 2002) or measured with a ruler for needles. Lesion area was adjusted for lesion caused by wounding in the control (no inoculum) treatments and considered to be zero if the lesion is equal to or less than that caused by wounding.

Results and Discussion

There was a large variation in susceptibility among tested Canadian hosts to infection by the EU2 lineage of *P. ramorum* (tables 3 and 4). Among the non-conifer hosts, Pacific dogwood, manzanita, camellia, rhododendron, and salal in the west (fig. 4), and sumac, yellow birch, wintergreen, and white ash in the east were the most susceptible to *P. ramorum* EU2 (fig. 5). For conifer hosts, we found both balsam fir and white spruce in the east and grand fir, sitka spruce, Douglas-fir, and western larch in the west to be the most susceptible. These results extend the known potential host range for the *P. ramorum* EU2 lineage.

The detached, *in vitro*, leaf/needle inoculation method of Elliott and others 2011was used in the current investigation, as this method and other published SOD research work elsewhere are well established and applied as approved USDA Animal and Plant Health Inspection Service (APHIS)/CFIA protocols for testing *P. ramorum* host ranges throughout North America. The natural hosts for EU2 lineage in the UK include Japanese larch, grand fir, noble fir, rhododendron, red oak, and *Vaccinium* (Webber and others 2014).

The Canadian Forest Service mandate is to monitor the EU2 lineage situation in the UK and to work closely with the CFIA to update the existing Canadian Pest Risk Assessment (PRA) to address new, relevant *P. ramorum* information as it arises. Furthermore, most recently, a draft genome assembly for *P. ramorum* EU2 lineage was collected from outbreak sites in Scotland (Sambles and others 2015). This information will enhance our understanding of the infection biology of the pathogen. Also, will assist researchers worldwide in accelerating our knowledge and relationships between the four lineages of *P. ramorum* (i.e., NA1, NA2, EU1, and EU2), as well as in developing molecular diagnostic assays for detection and field monitoring of the EU2 lineage (Sambles and others 2015; King and others 2015). Ongoing research is focused on the evaluation of sporulation potential of the EU2 lineage of *P. ramorum* on Canadian flora. Our study confirms the potential threat of the EU2 lineage of *P. ramorum* if it becomes established in Canadian nurseries and wildlands. Furthermore, it will have a great impact on the Canadian horticultural industry, biodiversity, and sustainability of forest ecosystems.

Table 3—Leaf necrosis (% lesion ± SE) on western Canada hosts 10 days post-inoculation (n=5). The most susceptible species are shown in bold.

Host	Rep1	Rep2
1-Arbutus	11.7 ± 3.48	4.9 ± 1.53
2-Garry oak	0.7 ± 0.12	0.8 ± 0.12
3-Bigleaf maple	2.3 ± 0.50	3.1 ± 0.52
4-Red alder	2.8 ± 0.83	1.6 ± 0.37
5- Poplar	4.6 ± 1.65	2.4 ± 0.64
6-**Pacific dogwood**	85.7 ± 5.97	81.2 ± 6.06
7-Paper birch	19.4 ± 1.75	15.7 ± 4.18
8**-Camellia**	47.0 ± 14.23	31.4 ± 2.91
9-**Salal**	18.3 ± 2.35	25.1 ± 3.41
10-Oregon grape	1.2 ± 0.31	1.7 ± 0.56
11-Rhododendron	31.6 ± 4.60	20.1 ± 5.68
12-Currant	6.4 ± 1.20	9.3 ± 2.02
13-Himalayan blackberry	0.1 ± 0.05	0.0 ± 0.01
14-**Manzanita**	58.4 ± 8.55	46.9 ± 8.60
15-Bay laurel	3.3 ± 1.08	1.7 ± 0.25
16-Raspberry	10.1 ± 10.16	0.1 ± 0.03
17-Blueberry	6.3 ± 3.78	15.3 ± 2.30
18-Grapes	11.2 ± 6.52	2.3 ± 1.15
19-Western hemlock	0.0	5.4 ± 0.00
20-Lodgepole pine	0.0	8.2 ± 3.02
21-**Western larch**	51.4 ± 5.65	86.1 ± 3.26
22-**Douglas fir**	24.7 ± 13.0	4.4 ± 23.04
23-**Sitka spruce**	54.0 ± 4.58	76.6 ± 6.02
24-**Grand fir**	68.9 ± 5.35	71.5 ± 6.82
25-Western redcedar	0.2 ± 0.15	0.6 ± 0.43

Table 4—Leaf necrosis (% lesion ± SE) on eastern Canada hosts 10 days post-inoculation (n=5). The most susceptible species are shown in bold.

Host	Rep1	Rep2
26-**Yellow birch**	93.0 ± 3.81	83.7 ± 6.52
27-Sugar maple	10.6 ± 5.43	2.6 ± 2.11
28-Red oak	14.5 ± 7.55	34.5 ± 14.73
29-**White ash**	34.1 ± 17.52	12.2 ± 11.3
30**-Wintergreen**	83.3 ± 10.42	86.5 ± 10.91
31-**Sumac**	8.2 ± 0.07	41.1 ± 1.67
32-**Balsam fir**	54.0 ± 2.74	57.2 ± 9.41
33-**White spruce**	81.5 ± 0.96	83.2 ± 0.00

Conclusions

1. For non-conifer species, Pacific dogwood, manzanita, camellia, rhododendron, and salal in the west; while yellow birch, wintergreen, sumac, and white ash in the east, were the most susceptible Canadian flora to infection by the EU2 lineage.
2. For conifer hosts, we found both balsam fir and white spruce in the east and grand fir, sitka spruce, Douglas-fir, and western larch in the west to be the most susceptible to EU2 lineage infection.
3. These results extend the known potential natural host range of the EU2 lineage of *P. ramorum* which include Japanese larch, grand fir, noble fir, rhododendron, red oak, *Vaccinium* spp., *Magnolia* sp., and *Pieris* sp. in the UK.
4. Research is ongoing to evaluate the sporulation potential of the EU2 lineage on Canadian flora (i.e., to discover the "spore pump" hosts.
5. The Canadian Forest Service is closely monitoring the situation of the EU2 lineage in the UK and working with the CFIA to update the existing Canadian PRA and address new relevant *P. ramorum* information as it arises.

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Figure 1—Symptoms of sudden larch death (SLD) in Japanese larch plantations (Crown dieback). (Courtesy of the Forestry Commission, UK).

Figure 2—Cankers, resin bleed on stem and branches, and necrotic needles of Japanese larch. (Courtesy of the Forestry Commission, UK).

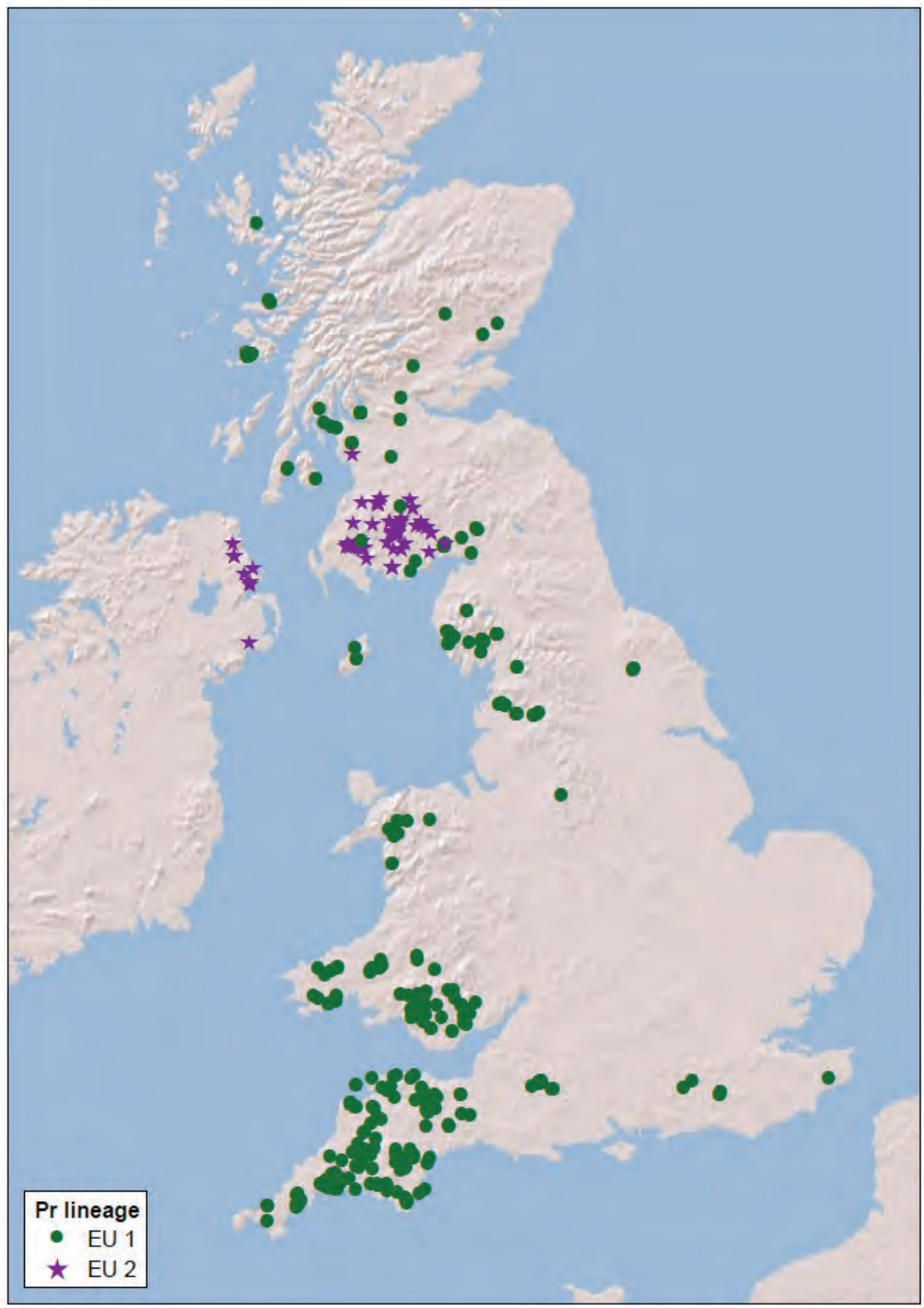

Figure 3—Distribution map of *Phytophthora ramorum* lineages EU1 and EU2 in the UK (Courtesy of Joan Webber, UK Forest Research).

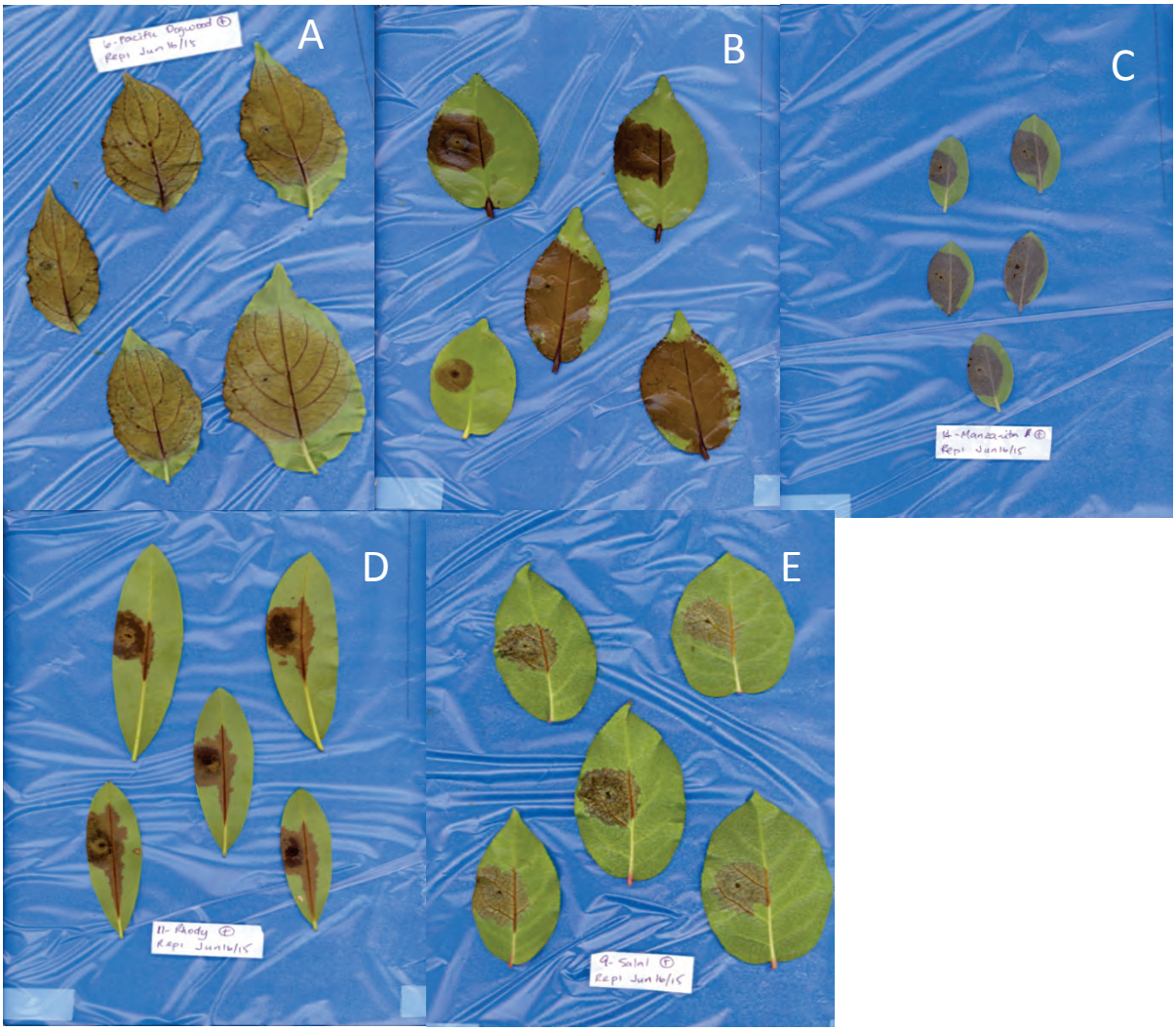

Figure 4—Lesions formed by *P. ramorum* EU2 (PFC5414) on detached leaves of highly susceptible western Canada hosts. A. Pacific dogwood; B. Camellia; C. Manzanita; D. Rhododendron; E. Salal.

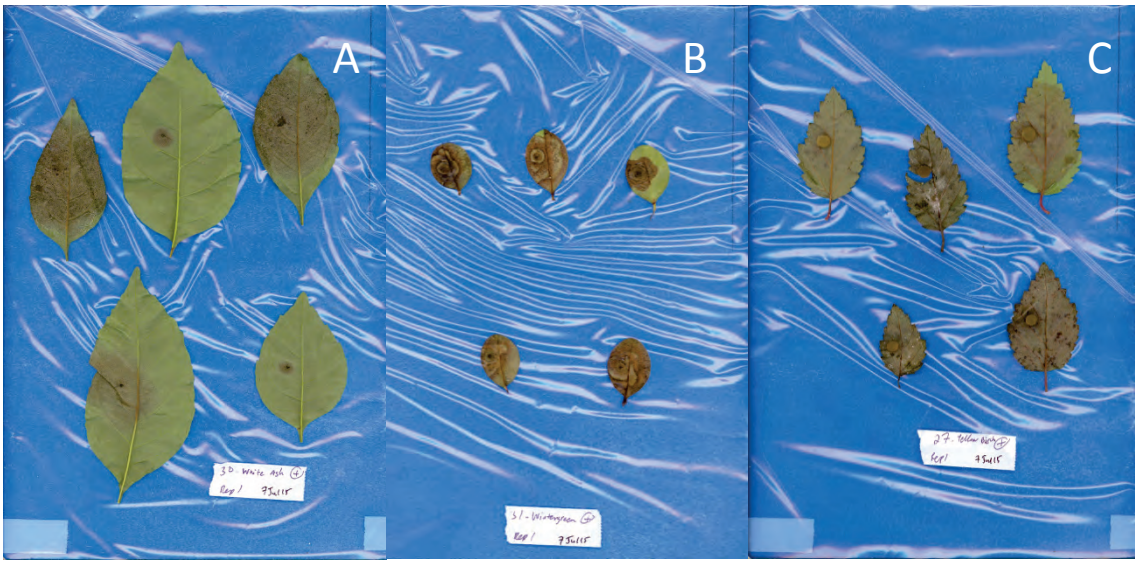

Figure 5—Lesions formed by *P. ramorum* EU2 (PFC5414) on detached leaves of susceptible eastern Canada hosts. A. White ash; B. Wintergreen; C. Yellow birch.

Interaction of *Trichoderma asperellum* With *Phytophthora ramorum* Inoculum Soil Populations and Enzyme Secretion[1]

Supriya Sharma,[2] **Wolfgang Schweigkofler**,[2] **Karen Suslow**,[2] **Timothy L. Widmer**[3]

Abstract

There is a continuing desire to investigate the potential of biological control to manage the spread of *Phytophthora ramorum*. A specific isolate of *Trichoderma asperellum* has been demonstrated to be effective in reducing *P. ramorum* soil populations to non-detectable levels. This study was conducted to investigate the interaction of different *T. asperellum* application rates with different initial soil populations of *P. ramorum* in a mock nursery setting and to investigate the impact of these interactions on important enzyme levels. Field trials were set up in the fall and spring where soil in a nursery bed was infested with *P. ramorum* chlamydospores at three different levels (< 2, 5-10, and > 15 cfu/cm^3 soil). A commercially formulated wettable powder of a *T. asperellum* isolate was applied at two different levels (10^6 and 10^7 cfu/cm^3 soil) by drenching the soil and raking into the top 3 cm of soil. Soil samples were collected every 4 weeks and baited with rhododendron leaf disks to determine the presence of *P. ramorum*. Overall results were inconsistent and difficult to make definitive conclusions. In the spring trial, only the high application rate of *T. asperellum* eliminated *P. ramorum* at all three initial soil populations.

The primary mechanism involved in the biological control of *P. ramorum* with the tested *T. asperellum* isolate appears to be direct parasitism. The enzyme laminarinase, which catalyses the hydrolysis of β-1-3 linkages in polysaccharides of D-glucose residues connected by β-1-3 linkages or mixture of β-1-3 linkages and β-1-6 linkages, is believed to be an important enzyme in cell wall degradation of *Phytophthora* spp. Studies were conducted to determine if enzyme activity was induced in the presence of *P. ramorum*. Secreted laminarinase activity was measured in liquid medium in the presence of various levels of *P. ramorum* mycelium. As a comparison, enzyme activity was also measured in the presence of *P. tentactulata* and in medium alone. After 24 and 120 h, laminarinase activity was significantly higher in the presence of *P. ramorum* mycelium compared to controls in the medium alone. Enzyme activities increased as *P. ramorum* mycelium was increased. Enzyme levels did not increase in the presence of *P. tentaculata*, regardless of the amount of mycelium present in the medium.

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Solarization of Reused Pots Is an Inexpensive and Efficient Method to Eliminate *Phytophthora cactorum* and Other Serious Soilborne *Phytophthora* spp. Found in Production Nurseries[1]

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Abstract

The reuse of plant pots by nursery growers has repeatedly been shown to be a method by which transfer of plant pathogens within a nursery will occur. More critically, this practice is an efficient pathway to infest landscape settings or habitat restoration sites by the out-planting of pre-symptomatic infected plant material. The transfer of water molds (oomycetes), such as plant pathogenic *Phytophthora* species, is a major threat to restoration projects and prevention of cross-contamination via pots should be a critical nursery operational control. Our research has established performance and efficacy criteria that demonstrate the risk can easily be managed by solarization of used pots.

In the summer of 2015, the National Ornamentals Research Site at Dominican University of CA (NORS-DUC) and the California Department of Food and Agriculture (CDFA) conducted two outdoor solarization experiments designed to verify lab-based studies of the time:temperature at which *Phytophthora cactorum*, a serious, commonly-found soilborne plant pathogen in the nursery industry, would be killed. In addition to its role as a disease agent, *P. cactorum* is also a useful surrogate for on-site validation studies of related serious pathogens, such as *P. ramorum* and *P. tentaculata*. Soil collected from each respective nursery and *P. cactorum*-infected leaf disks were combined and filled into sachets. Inoculum sachets were inserted into nested stacks of 1-gallon black pots, D-40 black tubes and Tubex tubes. In both of the outdoor experiments conducted in a hot and cool climate over the course of 3 weeks and 6 weeks, respectively, the pathogen was killed within the first week in the treatment pots (those wrapped in "clear" plastic which was purchased from a local hardware supply store). In the hot climate, the pathogen was also killed in the controls with no plastic wrapping, but in the cool climate, the pathogen was isolated weekly from the controls throughout the 6-week period. Mirror-control sachet samples of *P. cactorum*, mixed with each soil source, were maintained at the CDFA Plant Pest Diagnostic Laboratory and at NORS-DUC at ambient temperatures within a lidded container held in the dark. These controls were sampled weekly. All lab controls remained viable throughout the course of the experiment. Dataloggers recorded temperature every 30 minutes in the pots, placed at an interior position within the center stack of pots, as well as the ambient temperatures. Temperature data was correlated with the length of time required to kill the pathogen.

In the first week when *P. cactorum* was killed at both locations in the 1-gallon pot treatments, daytime high temperatures at the hot location ranged from 50-57 ^{0}C and ambient high temperatures ranged from 30-41 ^{0}C; daytime high temperatures at the cool location ranged from 39-45 ^{0}C and the ambient daytime

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highs ranged from 19-26 ^{0}C. Temperatures remained within those ranges on a daily basis for 4.5-6 hrs (hot) and 2-4 hrs (cool). *P. cactorum* was also killed in the control pots during the first week in the hot climate; temperatures in those pots ranged from 39-47 ^{0}C for 3-6 hrs on a daily basis.

The Effect of Moisture on Infection of *Rhododendron* 'Cunningham's White' and *Viburnum tinus* by Zoospores of *Phytophthora ramorum*[1]

Paul W. Tooley[2] and Marsha Browning[2]

Abstract

We performed studies to determine the effect of leaf wetness on infection of whole plants of *Rhododendron* 'Cunningham's White' and *Viburnum tinus* by zoospores of *Phytophthora ramorum*. We also evaluated the effect of a post-inoculation drying period on infectivity of the two host species with zoospore inoculum. Twelve plants of each species were spray-inoculated with 50,000 zoospores/ml of nine combined *P. ramorum* isolates, and placed in a dew chamber at 20°C in darkness. Two plants were removed from the dew chamber after 0, 1, 2, 4, and 6 h incubation, and placed on a greenhouse bench to allow the leaves to dry. After 1 h of drying, the plants were placed inside humidity tents on a greenhouse bench (average humidity 53.1%). The mean percentage of infected leaves for both host species increased gradually over the dew chamber moisture period of 1 to 6 h, reaching ca. 80% infection by 6 h. A further increase was observed up to 72 h, the final moisture period tested. There was a marked effect of a 30 min drying period between inoculation and dew chamber incubation on the percentage of leaves infected; *Rhododendron* 'Cunningham's White' sustained less than 40% infection, while *Viburnum tinus* sustained nearly 75% infection. Thus, the two host species tested responded differently to the effects of drying. Disease percentages for both host species dropped off sharply at drying periods longer than 30 min. Overall, we found that zoospores of *P. ramorum* can infect hosts in a shorter period of time than can sporangia and that zoospore infection appears to be strongly affected by the effects of post-inoculation drying. Knowledge of infectivity parameters for *P. ramorum* will lead to a better understanding of epidemic development and lead to improved recommendations for control.

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This Tree Is Not Big Enough for the Both of Us: Symptoms of *Phytophthora ramorum* on California Bay Laurel Are Lower When Insect Herbivores Are Abundant[1]

Kerry E. Wininger[2] and Nathan Rank[2]

Abstract

Leaves of California bay laurel (*Umbellularia californica*) are considered the primary natural source of inoculum for the devastating forest disease sudden oak death (*Phytophthora ramorum*), and yet this plant and the insects associated with its leaves remain understudied. This is unfortunate due to the role herbivorous insects may play in disease transmission and alterations to plant disease susceptibility. There is also a deficit of knowledge on how landscape-level variability or the effect of microclimate may influence insect presence and about systems involving both a plant's pathogens and its insect herbivores.

Two hundred woodland plots within a 275 km^2 region of Sonoma County have been assessed since 2003 for disease progression. Insect diversity and abundance on leaves of bay have been monitored since April 2014, with species appearing most often from the suborder Sternorrhyncha, which includes aphids, scale, and whiteflies. We have found a negative relationship between insect and pathogen presence on the tree level for California laurel aphid ($p = 0.04$) and one species of armored scale insect ($p = 0.004$).

We are investigating these interactions on a finer scale, including direction of correlation and across two microclimates, in 10 plots at Fairfield Osborn Preserve (December 2015 - May 2016). Both an observational and insect-removal experimental approach is being taken, charting the progression of disease and insect levels through the rainy season. We hope this may inform management strategies to slow spread and cope with this disease that threatens to unhinge native ecosystems.

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Longevity of Active *Phytophthora ramorum* in Terminal Tree Hosts following the Removal of Primary Sporulating Hosts[1]

Barnaby Wylder,[2] Mick Biddle,[2] Anna Harris,[3] and Joan Webber[3]

Abstract

The Forestry Commission-managed forest estate located in Plym, Devon (southwest England) was one of the first locations in late summer 2009 to have stands of *Larix kaempferi* (Japanese larch) confirmed as infected with *Phytophthora ramorum* (EU1 lineage). The 398 ha forest had a high proportion (>30%) of *L. kaempferi* showing catastrophic levels of *P. ramorum* infection with trees of all ages affected to some extent. Although a few infected rhododendron were present, larch was the primary sporulating host so all larch trees were removed from Plym forest between 2009-2011 as part of disease management. Prior to removal however, the spores released from infected foliage on larch trees had already initiated dieback and bole cankers in a wide range of non-sporulating coniferous and broadleaved trees that were either part of the understory or present in stands next to the infected *L. kaempferi*.

In March 2015, areas of forest adjacent to where the larch had been removed were surveyed again. Trees with symptoms of dieback and *P. ramorum* cankers could be readily identified and affected hosts included *Fagus sylvatica, Abies grandis, Pseudotsuga menziesii,* and *Tsuga heterophylla*. Some of the trees had stem cankers that were sunken and calloused although the development of infection appeared to have arrested. In others, the affected trees had apparently successfully contained the infection with callus growth completely occluding the old cankers. However, on some trees the cankers were still active, evidenced by signs of recent resinous exudation on conifer stems and black bleeding lesions on *F. sylvatica.* For a subset of these trees the cankers were almost at the point of entirely girdling the affected stems. Bark samples taken from a number of these active cankers on various tree species were tested for *P. ramorum* by (1) isolation onto *Phytophthora* selective media and (2) real time PCR. Live *P. ramorum* (EU1) cultures were obtained from all species except *Abies grandis* where only rtPCR confirmation of *P. ramorum* was obtained.

These observations suggest that even when the opportunity for successive years of re-infection is eliminated by removal of the spore generating host, *P ramorum* can remain viable and continue to cause expanding stem lesions in some terminal hosts for at least 5 years. This has biosecurity implications for timber processing if the pathogen can remain viable in infected tissue over several years. It also raises the question of why larch trees succumb so rapidly to the disease, often in just 2-3 years, when other susceptible conifer terminal hosts remain alive for much longer despite suffering active and potentially lethal stem infections.

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Resequencing of the *Phytophthora ramorum* Genome to Characterize Genetic Variation and Population Dynamics of the Invasive Pathogen[1]

Jennifer Yuzon,[2] David Rizzo,[2] Mathu Malar C,[2] Sucheta Tripathy,[3] and Takao Kasuga[4]

Abstract

Phytophthora ramorum has spread and diversified throughout California's northwestern coast since its introduction in the 1990s. Tracking the spread of *P. ramorum* and the functional response of the pathogen to the environment is of particular interest to managing the epidemic. Using genetic tools such as microsatellite markers, researchers have learned much of the pathogen's epidemiology by identifying migrational pathways and new introductions. However, higher resolution markers may reveal previously undetected substructure.

Work at the genetic and genomic level is underway to identify markers in the form of single nucleotide polymorphisms (SNPs), not only for a higher resolution of population structure, but to identify genetic responses of the pathogen to its adopted environment. Because SNPs are more common throughout the genome and have a lower likelihood of violating the infinite sites assumption, a genome-wide SNP dataset will help reconstruct the evolutionary past of *P. ramorum*; identify fitness and selection for genes; and possibly associate population subdivision based on geography, climate, or other influences on population dynamics. Structural variants and chromosomal abnormalities are also of interest because such genetic variation can add another layer of evolutionary potential for the rapidly expanding population.

Characterizing genetic variation has been challenging given the current reference genome. As much as 30% of the sequence is missing in the first draft of the *P. ramorum* genome published in 2006. Copy number variation (CNV) analysis and flow cytometry (FCM) revealed that the genome of the reference strain Pr102 was aberrated and unstable. Using such a reference genome can lead to biases for characterizing important genetic variation in extant lineages.

We have chosen the NORS-DUC standard isolate CDFA1418886 and started to revise the reference genome of *P. ramorum*. CDFA1418886 was cultured from the foliar host camellia and is more likely to depict the type of genetic variation seen in the transmissive population of *P. ramorum* in California. Phenotypically, CDFA1418886 is stable and more aggressive compared to Pr102. Genome analyses and FCM confirm that the genome lacks aberration and is more stable.

Using PacBio sequencing and an in-house pipeline for assembly, the genome is larger (~75-80MB) than initially reported (65MB) and reflects the genome size seen in FCM. Further refinement of the reference utilizes the conserved synteny between *P. ramorum* and closely related species.

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Work on identifying genetic variants by resequencing nursery and forest isolates from foliar hosts in California is underway. The software BIC-seq is used to call structural variants such as CNV and LOH. SNP caller software are also being tested.

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