United States Department of Agriculture
Forest Service

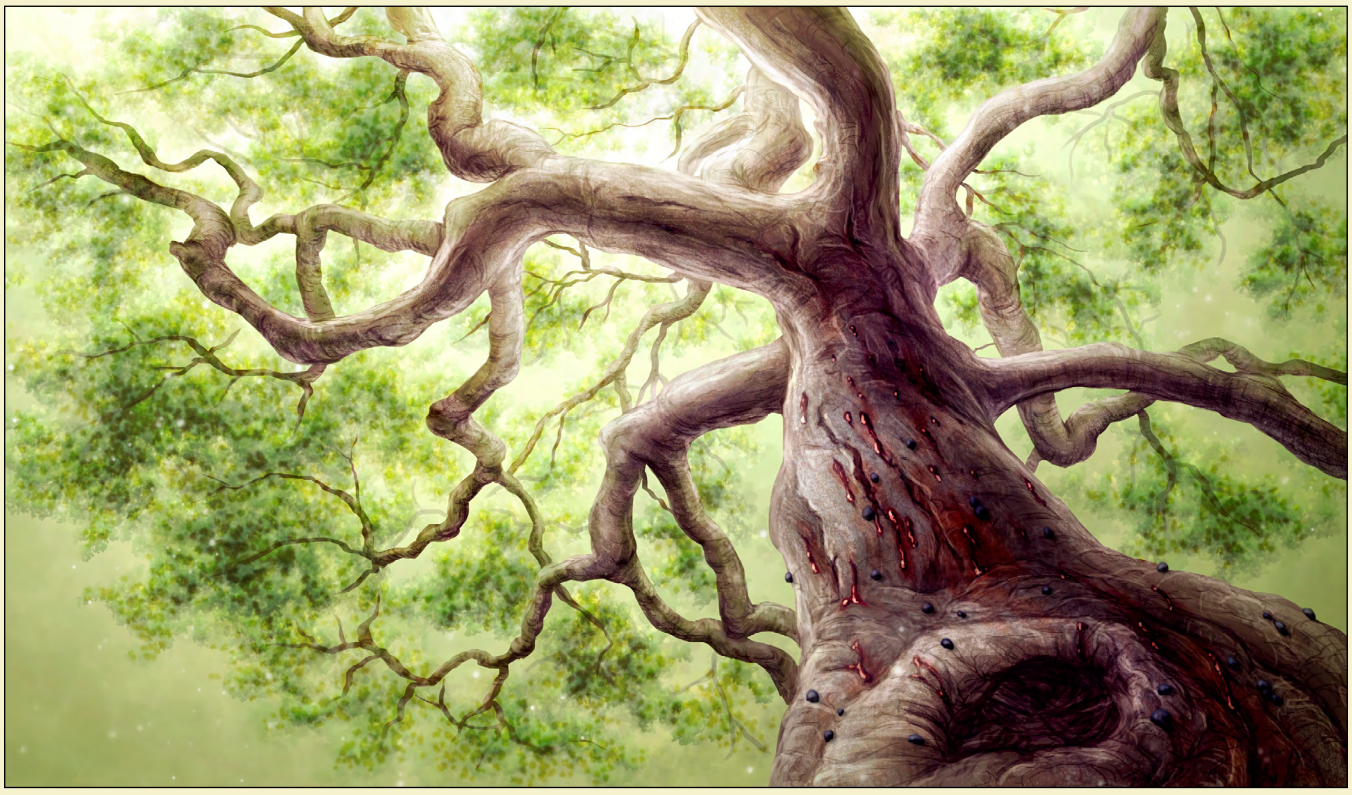
Pacific Southwest
Research Station

General Technical Report
PSW-GTR-243

June 2013

Proceedings of the Sudden Oak Death Fifth Science Symposium

June 19 to 22, 2012, Petaluma, California



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U.S. Department of Agriculture, Forest Service
Pacific Southwest Research Station
Albany, California
General Technical Report PSW-GTR-243
June 2013

Cover art by Jillian Walters, Monterey, California; http://jillianwalters.com/.

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Abstract

Frankel, S.J.; Kliejunas, J.T.; Palmieri, K.M.; Alexander, J.M. tech. coords. 2013. Proceedings of the Sudden Oak Death Fifth Science Symposium. Gen. Tech. Rep. PSW-GTR-243. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. 169 p.

The Proceedings of the Sudden Oak Death Fifth Science Symposium provides an update on research to address sudden oak death, caused by the exotic, quarantine pathogen, *Phytophthora ramorum*. Over 60 submissions present national and international investigations covering pathogen biology, biosecurity, genetics, monitoring, fire ecology, and diagnostics. Several papers on disease status and progress toward nursery and wildland management are also included.

Keywords: Sudden oak death, *Phytophthora ramorum*, invasive species, tanoak, *Notholithocarpus densiflorus*, coast live oak, *Quercus agrifolia*, Japanese larch, *Larix kaempferi*.

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Plant Imports, Phytophthoras, and Forest Degradation[1]

Clive Brasier[2,3]

Abstract

Numerous 'exotic' tree pathogens are arriving in Europe, North America, and elsewhere due to flaws in current international plant health sanitary and phytosanitary (SPS) protocols. These include lack of protection against the many organisms unknown to science, an emphasis on promoting trade rather than promoting environmental biosecurity, a steadily increasing globalization of the trade in rooted plants, and the failure of regulatory authorities to take meaningful and effective action (Brasier 2005, 2008; Liebhold et al. 2012,Webber 2010). The United Kingdom, for example, has experienced multiple major tree disease events involving introduced pathogens in commercial forests, woodlands, and urban trees over the past decade, from alders and horse chestnuts to pines and larches (Brasier 2012). The situation in the United Kingdom is effectively a full blown, though largely un-trumpeted, forest and amenity tree biosecurity emergency.

Phytophthora species are particularly well suited to spread on imported plants, being frequently soil inhabiting, favored by irrigation and other factors in intensive nurseries, and occurring as latent sporulating infections in symptomless host material (e.g., Denman et al. 2009, Vercauteren et al. 2013). About half of the current disease outbreaks in the United Kingdom are caused by introduced *Phytophthora* spp., among them two different evolutionary lineages of *P. ramorum* (EU1 and EU2). The possible scale of the *Phytophthora* threat is further indicated by an estimate that there may be 100 to 500 *Phytophthora* spp. unknown to science in underexplored ecosystems – the "invasives in waiting" (Brasier 2009). A significant proportion of these unknowns could be a threat to forest health in the future if they are introduced beyond their native range.

Much of the blame for the forest biosecurity crisis is attributed to high-volume plant imports. However, specialist plant collecting nurseries, amateur and professional plant collectors, and person-to-person transfer of imported plants are also part of the problem; although it should be emphasized that many plant collecting professionals, such as those attached to botanic gardens, often operate to the highest quarantine standards. It is now well documented that chestnut blight was introduced into Britain in 2011 on highly specialized imports of *Castanea sativa* Mill. Small, highly specialized plant imports are suspected to have been involved in the recent introduction of *P. tropicalis*, *P. kernoviae*, *P. niederhauseri* and the new EU2 lineage of *P. ramorum* (Van Poucke et al. 2012) into the United Kingdom. However, conclusive evidence is often difficult to obtain. Sometimes this is because affected plant material or relevant documentation has been destroyed. Sometimes it results from a requirement for official confidentiality about the relevant parcels of imports and the locations of infested propagation sites. The latter practice is highly questionable as it is probably one of the major blocks to achieving adequate plant biosecurity, obscuring reality from the public and the press. The need for better education of nurserymen, horticultural journalists, and the wider public is another (Brasier 2008).

The risk of bringing in unknown *Phytophthora* spp. on plants from underexplored forest ecosystems is illustrated by recent dedicated surveys in parts of Asia, such as Nepal and Taiwan, where the apparently endemic species *P. himalsilva* sp. nov. and *P. lateralis* have been discovered in remote forest locations (Brasier et al. 2010, Vettraino et al. 2011). Similar surveys indicate that Nepal may also be inadvertently introducing exotic *Phytophthora* spp. into its Himalayan foothill areas. Thus, in recent soil samples from a visually healthy forest area in the remote Bajura District of western Nepal, only two, probably endemic, forest *Phytophthora* spp. were found. This was in marked contrast to similar samples from a degraded forest area near Kathmandu, in the vicinity of a nursery specializing in introduced *Castanea* spp. These samples yielded nine *Phytophthora* species, many of them species already prevalent in forests elsewhere in the world and therefore probably recently introduced exotics (A. Vannini, A.M. Vettraino and C.M. Brasier, unpublished data). Until the present,

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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such studies have been fragmentary and carried out largely by committed volunteers. Many more such studies are needed, as they are critical to enhancing our knowledge base in the fight against the importation of exotic pathogens into forest ecosystems. Is it time for regulators, funding agencies, and plant collectors to get involved?

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North American Disease Status Updates

Detection and Eradication of *Phytophthora ramorum* From Oregon Forests, 2001-2011[1]

Alan Kanaskie,[2] Everett Hansen,[3] Ellen Michaels Goheen,[4] Nancy Osterbauer,[6] Michael McWilliams,[2] Jon Laine,[2] Michael Thompson,[2] Stacy Savona,[2] Harvey Timeus,[2] Bill Woosley,[2] Randall Wiese,[2] Wendy Sutton,[3] Paul Reeser,[3] Joe Hulbert,[3] Rick Shultz,[5] and Dan Hilburn[6]

Abstract

Sudden oak death (SOD), caused by *Phytophthora ramorum,* is lethal to tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H. Oh), and threatens this species throughout its range in Oregon. The disease was first discovered in coastal southwest Oregon forests in July 2001. Since then an interagency team has been attempting to eradicate the pathogen through a program of early detection surveys followed by destruction of infected and nearby host plants. Eradication treatments eliminated disease from many infested sites, but the disease continued to spread slowly, predominantly northward, in the direction of winds that prevail during storms and wet weather. During the 10-year period, the disease spread from the initial infestations southward 1.9 km (1.2 mi), and northward and eastward 28 km and 7.6 km (17.3 mi and 4.7 mi), respectively. The area under quarantine has expanded five times, from 22 km^2 (9 mi^2) in 2001 to 505 km^2 (202 mi^2) in early 2012. Continued spread of SOD is attributed to the slow development of symptoms in infected trees which hinders early detection, and to delays in completing eradication treatments due to inconsistent funding. A sharp increase in disease in 2010 and 2011 necessitated major changes to the SOD management program and quarantine regulations.

Keywords: *Phytophthora ramorum*, sudden oak death, eradication, Oregon, tanoak

Introduction

Sudden oak death (SOD) is caused by *Phytophthora ramorum,* a pathogen of unknown origin and world-wide importance. It has been causing widespread mortality of coast live oak (*Quercus agrifolia* Née), tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H. Oh), and California black oak (*Quercus kelloggii* Newb.) in California for nearly 2 decades, where it now occurs in 14 counties (Rizzo and Garbelotto 2003, Rizzo et al. 2002). In Europe, where the pathogen formerly occurred only in the nursery trade, it has spread to trees and forests (Brasier and Webber 2010). The pathogen has potential to spread throughout coastal forests of the United States west coast (Meentemeyer et al. 2004, Vaclavik et al. 2010) and to cause considerable ecological and economic damage to the forestry and nursery industries (Hall 2009). Risk models show that many forests of the world, including the hardwood forests of the eastern United States, are highly susceptible to this pathogen. Accordingly, the pathogen is highly regulated through international and domestic quarantines.

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Sudden oak death was first discovered in Oregon forests in July 2001 near the coastal city of Brookings, 8 km (5 mi) north of the California border. Archival aerial photographs revealed tanoak mortality in at least one of the infested sites, suggesting that disease probably was present there since 1998 or 1999. At the time of discovery in Oregon, we knew of five infested sites encompassing a total of 14.6 ha (36 ac) distributed over an oblong area 4 km (2.5 mi) (north-south) by 1.9 km (1.2 mi) (east-west). Soon after the initial detection, we convened an emergency meeting of personnel from the Oregon Department of Agriculture (ODA), Oregon Department of Forestry (ODF), Oregon State University (OSU), and the U.S. Department of Agriculture Forest Service (USDA FS). Because of the apparently small number of infestations and the unknown potential for damage, we decided to attempt eradication of the pathogen by cutting and burning all infected and symptomatic host plants in the infested sites. Also at that time, the ODA established an emergency quarantine area of 22.5 km^2 (9 mi^2) from which movement of all host material was prohibited (Goheen et al. 2002).

Ten years later, it is clear that we failed in our initial goal of complete eradication of the pathogen from Oregon forests. The program now continues with a revised goal of containment and slowing spread, using early detection and eradication as the primary tools for reducing inoculum available for disease intensification and spread.

Ecology of *P. ramorum* in Oregon Forests

Phytophthora ramorum produces aerial propagules under wet conditions and mild temperatures. In the wet, mild climate of Curry County, spore production has been documented year-round and disease can spread anytime suitable weather conditions occur. In Oregon, the primary host is tanoak, which is killed by the pathogen and acts as a source of inoculum throughout the year (Hansen et al. 2008). The disease spreads locally by rain splash and over long distances (several km) via wind and wind-driven rain from the canopy of infected tanoak trees (Reeser et al. 2010). Many other forest plant species are also susceptible to the pathogen when growing close to tanoak. These include Pacific rhododendron (*Rhododendron macrophyllum* D. Don ex G. Don), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), evergreen huckleberry (*Vaccinium ovatum* Pursh), red huckleberry (*Vaccinium parvifolium* Sm.), Oregon myrtle (*Umbellularia californica* (Hook. & Arn.) Nutt.), cascara (*Rhamnus purshiana* DC.), and poison oak (*Rhus diversiloba* Torr. & A. Gray) (Hansen et al. 2005). They do not appear to be important for disease spread in Oregon forests, at least under the climatic conditions prevailing during the past 10 years. Humans also can spread disease by transporting infected plants or infested materials, but this has not been documented in Oregon forests.

The time between initial infection of tanoak and the development of disease symptoms is not clearly understood in natural forest conditions. Infections on leaves and fine twigs can become apparent within weeks of infection as leaf blotches and small lesions, but these are difficult or impossible to detect in standing tanoak trees until infection is abundant and causes discoloration of foliage en masse. The time between initial infection in the crown of tanoaks and the development of trunk cankers and tree death appears to range from several months to several years (McPherson et al. 2005, McPherson et al. 2010). This latent period, when the pathogen is present but not readily detectable, is extremely important to the early detection and eradication program because spore production can occur throughout this period, potentially compromising the effectiveness of eradication treatments.

Early Detection Surveys

The detection program consists of several types of survey, each with their own strengths and limitations. As a group, these surveys have proven highly effective. Even though inconsistent funding has impeded the treatment program, we have maintained a consistent detection survey effort since 2001.

Aerial Survey Followed by Ground Checks

Aerial surveys provide the most extensive, but least "early" detection. Four surveys are conducted annually (February, May, July, and October) that extend from the California border north to the Rogue River, an area of approximately 1250 km^2 (500 mi^2). In the first stage of the survey, observers in a fixed-wing aircraft record the approximate location of recently killed tanoak trees (red-brown foliage) on maps. These records are then used to guide a helicopter to the dead trees. While hovering over the dead trees, the number and condition of the trees are noted and the geographic coordinates determined with hand-held Global Positioning System (GPS) units. Ground crews then use maps, GPS units, and compasses to find the dead trees. All dead-tree locations are visited and trees and plants in the vicinity are checked for symptoms of SOD or other disease. If symptoms are present, two samples of symptomatic plant tissue are collected. One is plated in the field onto *Phytophthora*-selective agar and the other sample is taken to the laboratory for plating and polymerase chain reaction analysis (PCR).

Because aerial surveys are based on the presence of dead trees, they do not offer true early detection. Infested sites detected by this method will have had *P. ramorum* present for months or years prior to the detection. However, because the surveys are flown at frequent intervals and detect recently killed trees, they typically capture new infestations in relatively early stages of development.

Ground Surveys

Ground surveys are very labor intensive, but allow detection of an infestation before dead trees are present or visible to aerial observers. They are conducted by two- or three-person crews walking transects spaced 30 to 50 m (100 to 150 ft) apart while looking for symptoms such as bleeding cankers, stem lesions, wilting shoots, leaf spots, and branch dieback on understory plants and live trees. Samples are collected and treated as described for ground checks associated with aerial surveys.

Ground surveys supplement the aerial surveys and provide data necessary to certify areas as "disease-free" to fulfill quarantine requirements for transporting host material. They are undertaken in areas where landowners request surveys or where presence of the disease is likely based on risk maps or proximity to known infestations.

Stream Baiting

Stream baiting with native rhododendron and tanoak leaves is an extensive survey that offers the possibility of detecting *P. ramorum* before tree mortality is evident (Sutton et al. 2009). Stream baiting is carried out in areas considered at risk of new infestation within and beyond the quarantine area. Streams draining known infested sites also are sampled as positive controls. Year-round sampling of approximately 60 bait stations at 2-week intervals is interrupted only by summer drought or winter floods. The area of drainages sampled ranges in size from 8 to 3634 ha (20 to 8,980 ac) and totals 32 192 ha (80,000 ac).

In several cases, stream baiting indicated an infestation in a drainage area before we detected it by any other means. However, there have been a few cases where an infestation occurred in a drainage, but the stream baits were negative for *P. ramorum* (false negative). Conversely, there have been a few instances (very rare) where we recovered *P. ramorum* from stream baits, but have not found upstream infected plants. We do not fully understand the ecology of *P. ramorum* in streams.

Eradication Treatments

Mandatory eradication began in the autumn of 2001 under the statutory authority of the ODA. Funding initially was provided by the USDA FS and in subsequent years by ODF, U.S. Department of the Interior Bureau of Land Management (USDI BLM), and USDA Animal and Plant Health Inspection Service (USDA APHIS). Although there was no direct cost to landowners, no compensation was made for loss of timber or other values. All eradication activities on federal lands

managed by the USDI BLM or USDA FS have been funded by the respective agencies and have been uninterrupted to date.

After initial detection of *P. ramorum*, each infested site is surveyed for symptomatic plants and a treatment area delimited. In 2001 and 2002, the treatment area boundary was 15 to 30 m (50 to 100 ft) from infected or symptomatic plants. In subsequent years it was increased to 100 m (300 ft), reflecting monitoring data showing that smaller treatment areas often were not large enough to capture the extent of the infestation.

On private and USDA FS land, eradication treatments consist of felling and burning all host plants within the treatment area as soon as possible after detection. Cutting, piling, and burning are accomplished by hand crews, heavy and light equipment, broadcast burning, and any combination thereof. On USDI BLM land, host plants are cut, piled, partially covered with plastic, allowed to cure, and burned 6 to 14 months later. In early 2011, USDI BLM modified this approach by cutting and burning the actual infected trees immediately and making piles of everything else in the treatment area for burning later.

After the first 2 years of treatments, tanoak stumps had sprouted prolifically and *P. ramorum* was occasionally isolated from the new shoots. In 2004 and 2005, all sprouts from previously burned sites were sprayed with herbicide to kill sprouts. Since 2004, all tanoaks in treated areas (other than those on USDI BLM land where herbicide use was restricted prior to 2011) have been injected with herbicide (imazapyr or glyphosate) prior to felling to prevent sprouting. Follow-up treatments often are necessary to destroy residual host material and stump sprouts that may harbor the pathogen. Upon completion of burning, most sites are planted with non-host or conifer seedlings.

Infestations detected in February to May often can be treated immediately if fire precaution levels allow burning and if funds are available. We make this a priority because late winter and early spring are important times for disease spread and intensification. For infestations found in summer and fall, we often start cutting immediately and finish burning when fall rains begin. Our goal has been to complete treatments by the end of December to minimize inoculum availability during winter and spring. It is a good plan, but operationally and administratively it has been difficult to achieve, especially in recent years when funding was inconsistent and the amount of disease had increased.

Eradication treatments on private lands were delayed several times because of lack of funds. Treatments were suspended from January to May, 2008, from April to September, 2009, and from November 2009 to April 2010. During these periods we observed disease intensification and spread in several areas. By the time new funds became available in 2010, we had accumulated a large backlog of untreated or partially treated sites. Priority was given to treating outlying sites and sites considered most important in terms of spread outside the quarantine area, while allowing sites near the center and western part of the quarantine area to remain untreated or partially treated for many months. As funds again became limiting in late 2010 and 2011, we gave priority to treating sites nearest the quarantine boundary, once again allowing sites near the center of the quarantine area to remain untreated or partially treated for extended periods of time.

Since 2001, eradication treatments have been completed on approximately 1215 ha (3,000 ac) of land, at a cumulative cost of $7.5 million. There has been no compensation to landowners for the value of timber or other resources lost as a result of the eradication treatments. For the period 2001 to 2009, the area treated for eradication was distributed among landowner groups as follows: private industrial (72 percent); non-industrial private forests (18 percent); rural-residential (6 percent); USDI BLM (3 percent); USDA FS (<1 percent); and state of Oregon (<1 percent).

Preventive Host Removal

Since 2001, 565 ha (1,400 ac) of tanoak forest have been felled or killed with herbicide in advance of the disease in areas of probable disease spread, mostly in the northern part of the quarantine area. These host removal activities were voluntary landowner activities supported in part by state or federal funding.

Eradication Post-Treatment Monitoring

Eradication of *P. ramorum* from individual infested forest sites is difficult, but not impossible. The disease usually does not persist on infested sites following cutting and burning, but the pathogen frequently can be recovered from soil several years after treatment. From 2008 to 2010 we surveyed treated sites to determine the presence of *P. ramorum* in soil or vegetation. Soils samples and vegetation were collected from sample plots established around stumps of known infected trees on sites treated between 2001 and 2008. We established 145 plots in 2008 and 2009 and 143 plots in 2010 (Goheen et al. 2009, Goheen et al. 2010).

In the sample period from 2008 and 2009, *P. ramorum* was not recovered from soil or vegetation on 51 percent of the plots sampled. *Phytophthora ramorum* was present in soil only on 32 percent of plots, in soil and vegetation on 12.5 percent of plots, and in vegetation only on 4.5 percent of plots. In the 2010 sampling, *P. ramorum* was not recovered from soil or vegetation on 63 percent of plots sampled. *P. ramorum* was present in soil only on 25 percent of plots, in soil and vegetation on 7 percent of plots, and vegetation only on 5 percent of plots.

Almost all infected vegetation was tanoak stump sprouts. On plots where *P. ramorum* was baited from soil, recovery was generally low, usually only one of 20 soil samples. The pathogen was recovered from soil up to 8 years post treatment. Further analysis of these data and additional data collected in 2012 is underway (Goheen et al., Monitoring the effectiveness of *Phytophthora ramorum* eradication treatments in Oregon tanoak forests, this proceedings).

Disease Spread, 2001 to June 2012

Continued spread of SOD is attributed to the slow development of symptoms in infected trees which hinders early detection and to delays in completing eradication treatments which allow disease spread from known infestations. From 2001 to 2004, the number of new infested sites discovered in surveys remained steady or decreased, suggesting modest success at containment and eradication. In 2005 and 2006, the number of new infested sites and the distance between them began increasing, possibly the result of 2 consecutive years of unusually wet spring weather which favored spread of the pathogen. Several new sites found during this period were more than 3 km (2 mi) from previously known infected trees and outside of the existing quarantine boundary. From 2007 to 2009 the trend in occurrence of new infested sites appeared to stabilize at approximately 60 new disease patches per year, with no new sites outside of the existing quarantine boundary (fig. 1).

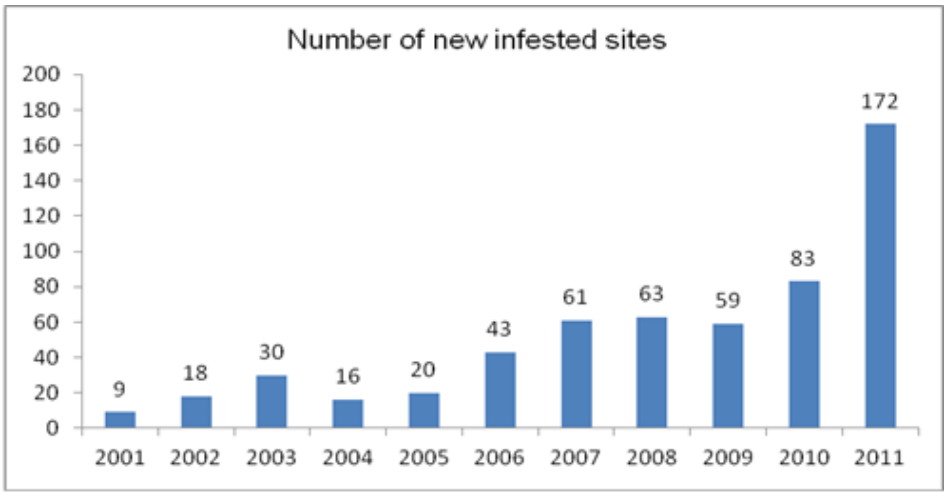

Figure 1—Number of new sites infested with *Phytophthora ramorum* discovered annually between 2001 and 2011 in Curry County, Oregon forests.

By the end of 2010, the number of new infested sites had increased to 83. All were well within the existing quarantine area, and most were small with few infected trees, suggesting relatively early detection. Distribution of new sites was uneven with noticeable intensification at Cape Ferrelo in the Taylor-Duley creek drainages (west side of quarantine area) where treatment delays had occurred in prior years.

In 2011 we detected 172 new infested sites, nearly triple the 3-year average. The majority of new sites were in the core of the quarantine area, mostly on private land and mainly in the Cape Ferrelo area. Many new sites were very close to previous infestations, probably a result of delays in completing treatments promptly. One of the sites (Cape Sebastian) detected in September 2011 was 10.5 km (6.5 mi) north of the quarantine boundary and 19.3 km (12 mi) from the nearest known infested site. At least 25 infected trees were identified at the site, suggesting that the pathogen had been there for at least a year. We do not know if this infestation was the result of natural or human-assisted spread.

In May, 2012, a new infestation was confirmed on USDA FS land along Wheeler Creek, just outside of the southwest corner of the quarantine area. The area is remote and rugged, with very little human activity other than occasional hikers. Treatment of the 19 ha (48 ac) unit began in June immediately following delimitation surveys. An emergency quarantine area of 68 km^2 (27 mi^2) was established to include the Wheeler Creek infestation.

Apparent long-distance spread has been observed several times with distances of 3 to 5 km (2 to 3 mi) (in one case 20 km) between infested sites with little evidence of infestations between them. The likelihood of long-distance spread increases with the amount of source inoculum and the length of time it is present on the landscape. As untreated infestations intensify and expand, we expect an increasing number of long-distance spread events.

New infestations often occur very close to eradication sites within a year of treatment. In most cases we believe this is due to latency of the pathogen rather than spread during the treatment process or failure to detect symptomatic trees at the time of delimitation. This problem can be solved somewhat by large treatment area buffers to capture pre-symptomatic or cryptic infections. In at least three instances where we found the disease in early stages and used large treatment areas of 10 to 16 ha (25 to 40 ac). the disease has not appeared in the adjacent forest 4 years post treatment. Treatment area buffers of 200 m (600 ft) or more from infected or symptomatic plants probably are necessary to capture most nearby (but not readily detectible) infected plants.

Disease spread during the 10-year period has been predominantly northward, following the prevailing wind direction during storms and wet weather. The disease has spread from the initial infestations southward 1.9 km (1.2 mi), and northward and eastward 28 km and 7.6 km (17.3 mi and 4.7 mi), respectively. The area under quarantine has expanded five times: from 22 km^2 (9 mi^2) in 2001 to 505 km^2 (202 mi^2) in early 2012. The current quarantine area and distribution of the disease are shown in fig. 2.

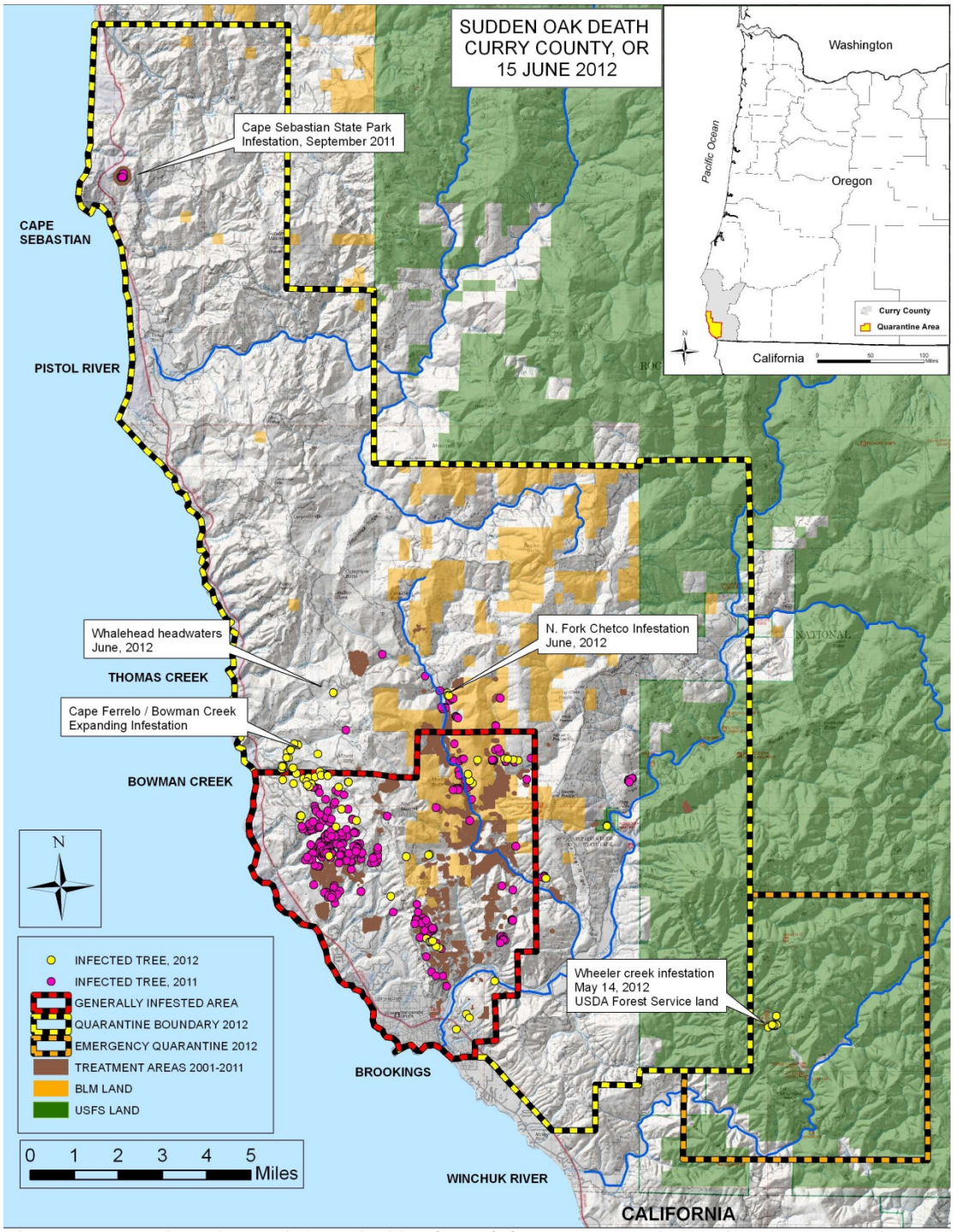

Figure 2—Location of trees infected with *Phytophthora ramorum* in southwest Oregon that were discovered in 2011 through June 2012 (enlarged for visibility). Brown polygons indicate eradication treatment areas.

Costs and Funding

Program expenditures from 2001 to 2011 totaled $11.5 million for detection surveys, eradication treatments, and administration (does not include university research or the *P. ramorum* nursery program). Approximately $7.5 million of this went toward eradication treatments. Current annual cost

of detection surveys and administration is $400,000 (Brookings staff, Salem staff, aerial surveys, lab support). Eradication treatments average approximately $6,200 per ha ($2,500 per ac). The major sources of funds were: USDA FS, $7 million (59 percent); USDI BLM, $2.2 million (18 percent); USDA APHIS, $470,000 (4 percent); state of Oregon, $2.2 million (18 percent); and private industry, $80,000 (1 percent).

Despite consistent high levels of support from the USDA FS, several aspects of funding have hindered the eradication effort. Many times funds that were expected early in a federal fiscal year were not available until the federal budget was officially passed, often as late as spring or summer of the following year. Unfortunately, the waiting period was during winter and spring when most disease spread occurs. At other times, federal funds were available, but the state could not secure the required non-federal matching funds, so we could not accept the funds. Nearly $1 million in federal funds were forfeited for this reason. In addition, when the economy declined in 2008, the state chose to offset budget reductions by returning $265,000 of state general funds specifically allocated for SOD treatments. The net effect of these funding problems was that we fell farther and farther behind in our eradication treatments, allowing sites that should have been treated promptly to carry over into the following year, creating a backlog of sites requiring treatment. Meanwhile, new, often higher priority sites (in terms of disease spread) continued to appear.

In an attempt to recover lost ground and begin host removal in advance of the disease front, we applied for $4.4 million through the American Recovery and Reinvestment Act, the so called "stimulus program." We were awarded $2.7 million (no requirement for matching funds), which finally became available in April 2010, allowing us to add staff in Brookings, resume work on the backlog of untreated sites, and complete treatment of all high-priority sites identified in 2010. But it may have been too late. The previous delays had allowed the disease to intensify and spread, and by mid-2011 it was clear that the area requiring treatment on private land would exceed available or expected funds, and a major program change would be necessary.

Finding non-federal funds to match available federal dollars remains a challenge. In 2011 we were able to obtain matching funds from private landowners through a 50-50 cost share program for treatments and in-kind services for work related to SOD management. Even with ample matching funds, the combined state and federal fund sources were not sufficient to continue treating all sites. At the 2010 levels of disease and staffing, we estimated an annual program cost of $2 million to $2.5 million dollars to continue the early detection surveys and treat infested sites with 100 m (300 ft) treatment buffers. The expected budget, however, was approximately half of that.

Changes to the Sudden Oak Death Program

In early 2011, the Oregon SOD "task force" (ODA, ODF, USDA FS, and OSU) and stakeholders met and considered various options for a sustainable SOD management program. Options included stopping the program altogether, establishing a broad host-free zone north of the current infestation, extensive aerial application of fungicides, and numerous variants of the existing eradication program.

With funding as a major constraint, we settled on a program with the goal of slowing spread of the disease. Pest spread models suggest that slowing spread is best accomplished by early detection and rapid suppression of new infestations that occur beyond the leading edges of the main infestation. This approach is analogous to treating spot fires when controlling wild fire. Additional benefit can be gained by reducing overall inoculum levels elsewhere within the quarantine area by destroying infected host plants. Reducing inoculum lowers the chance that disease will intensify on site or be spread long distance naturally or by human activities.

The base function of the program and highest priority for funding is the early detection of infected trees through a variety of survey methods. Eradication treatments will be scaled to funding levels. The highest priority sites (in terms of potential for disease spread) will be treated first with treatment costs paid by the state and federal agencies. Lower priority sites will remain untreated or be treated voluntarily by landowners. Federal agencies will continue eradication on all infested sites on their

land. Treatment priorities are assessed bi-monthly by agency staff conference (ODF, ODA, and USDA FS).

The new program required several changes to the Oregon quarantine regulations which became effective in March 2012. The key provisions of the quarantine rule are:

1. Establishes a "generally-infested area" within the quarantine boundary where *P. ramorum* has been commonly found or where the disease has persisted or intensified and complete eradication of the pathogen is impossible or impractical (fig. 2). Parts of the generally infested area currently are uninfested, but these likely will become infested over time if host plants are present. The size and shape of the generally infested area will be updated periodically by the ODA and ODF depending on disease distribution and funding available for treatments, and will be available as a map or shape file on the ODA/ODF website. Within the generally infested area, eradication treatments are no longer required by the state.

2. Defines two types of infested sites based on their importance for spread of disease.

A. Type 1 sites are infested sites considered to be of highest risk for spread of *P. ramorum* into previously uninfested areas. They typically are located outside of the generally infested area. The highest priority sites are those closest to or beyond the existing quarantine boundary. Eradication treatments are required: all host plants within 15 to 100 m (50 to 300 ft) of infected or symptomatic plants must be cut and burned as soon as possible after the treatment areas have been delimited. Cost of treatment will be borne by the state if funds are available.

B. Type 2 sites are infested sites considered to be of less risk for spread of *P. ramorum* into previously uninfested areas. Type 2 sites typically are located inside of the generally infested area. Eradication treatments are not required, but disease suppression through best management practices is encouraged. A 50-50 cost-share program may be available through ODF to help defray costs of implementing best management practices to reduce disease spread. Host trees within a Type 2 treatment area may be used as firewood within the treatment area.

3. Allows increased utilization of tanoak within the quarantine area.

A. Inside the generally infested area, tanoak maybe used as non-commercial firewood, but it cannot leave the generally infested area.

B. Outside of the generally infested area, tanoak cannot leave an infested site or eradication treatment area, but it can be transported out of the quarantine area if from a "disease-free area," which is defined as an area located more than 402 m (1/4 mile) from the generally infested area or any other infested site, and which has been officially surveyed within the past 6 months and found free of *P. ramorum*.

Changes to the quarantine regulations reflect the financial reality of managing an expanding new disease. The initial goal of complete eradication in Curry County is unachievable. Our goal now is to slow spread by 1) early detection and rapid eradication of new infestations that are epidemiologically important; 2) reducing inoculum levels wherever practical through cost-share projects and using best management practices; and 3) improved education and outreach to prevent spread by humans. The current planned annual budget for the program is approximately $1 million to $1.2 million.

Conclusions

Spread at the landscape level continues because latency of the pathogen and cryptic infections hinder early detection. Delays in completing eradication treatments allow disease to intensify and spread between the time of detection and completion of treatments. In the wet, mild climate of Curry County, disease can spread anytime suitable weather conditions occur. Delays anywhere in the detection-treatment process are very costly.

Phytophthora ramorum eventually may spread throughout range of tanoak and possibly farther on other host species such as rhododendron and evergreen huckleberry. Pest risk models predict that without control, it could eventually spread to 19 western Oregon counties. As the disease spreads, the quarantine area and associated regulations will expand with it. These regulations likely will increase

production and shipping costs for the nursery and forest industries, especially when moving host plant material out of the quarantine area or out of state. Markets may be lost as importers of Oregon products enact their own quarantines or decide not to purchase Oregon products because of perceived risk. This already has happened to log exporters and lily bulb growers. The economic justification for slowing spread of the disease is based on the value of preventing or delaying these costs (Hall 2009).

Despite continued spread and intensification of disease, the program has by no means been a failure. In the 10 years since first detected, SOD still is confined to a relatively small quarantine area near Brookings. Disease spread and mortality are less than they would be without treatment. Although *P. ramorum* will not be eradicated from Oregon forests, an ongoing well-funded disease management program will slow its progress, prevent or delay environmental and economic damage, and reduce the probability of spread to other forests.

Acknowledgments

Funding for detection, monitoring, and eradication was provided by USDA FS Pacific Northwest Region, Forest Health Protection; USDA FS Pacific Southwest Region, Forest Health Protection; USDA FS Pacific Southwest Research Station; USDI BLM; USDA APHIS; OSU; Oregon Parks and Recreation Department, ODA, and ODF. Finally, we extend appreciation to landowners in Curry County, especially the South Coast Lumber Company, for their cooperation and tolerance.

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An Overview of *Phytophthora ramorum* in Washington State[1]

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

Phytophthora ramorum, the exotic water mold that causes sudden oak death and ramorum shoot blight, was first detected in Washington State on ornamental nursery stock in 2003. Since then, three lineages (NA1, NA2, and EU1) have been detected in a total of 49 nurseries in western Washington (fig. 1).

The number of positive nurseries has decreased since a high of 25 in 2004 (fig. 2). During the past eight years, most positives are repeat nurseries.

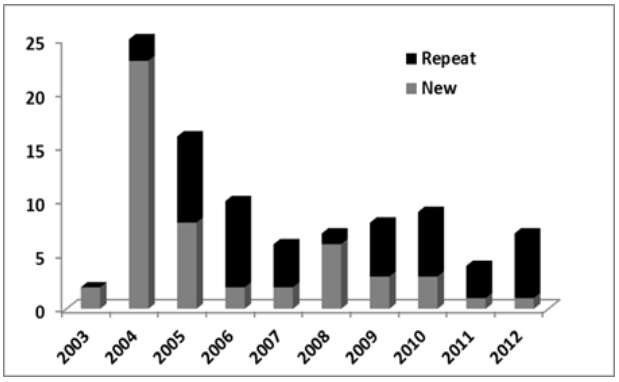

Figure 2—Yearly number of new and repeat positive nurseries in western Washington, 2003 to May, 2012.

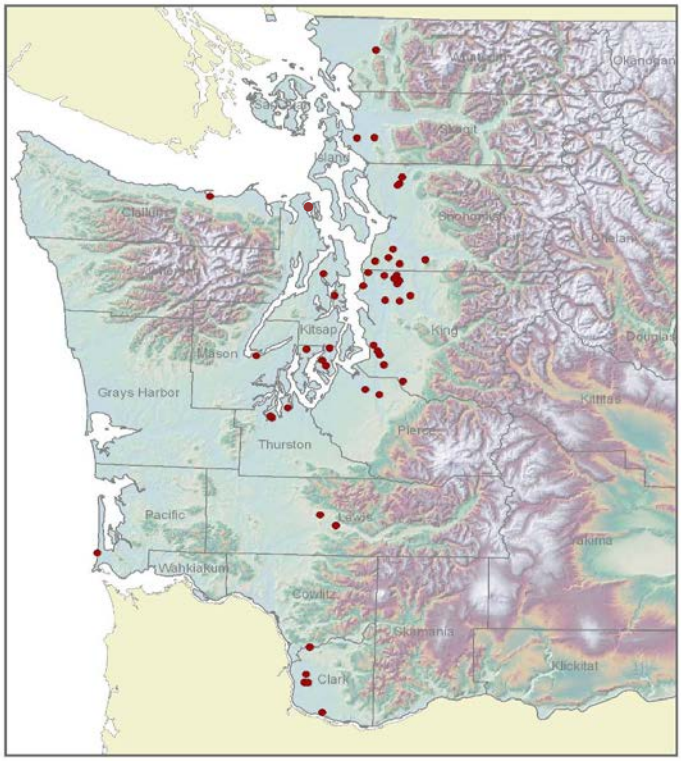

Figure 1—*Phytophthora ramorum*-positive nursery sites in western Washington, 2003 to May, 2012.

In 2006, stream baiting revealed that *P. ramorum* had spread from a nursery in Pierce County into a nearby stream. Subsequent yearly stream baiting has resulted in the detection of *P. ramorum* in a total of 11 drainage ditches and/or streams in five western Washington counties (fig. 3). Genotype analysis indicates that all three lineages of this pathogen have spread into waterways and that contamination of waterways has typically resulted from spread of inoculum from nearby positive nurseries. Stream baiting has also shown that once a waterway becomes infested, it remains infested even after successful mitigation steps have eliminated the pathogen from infested nurseries.

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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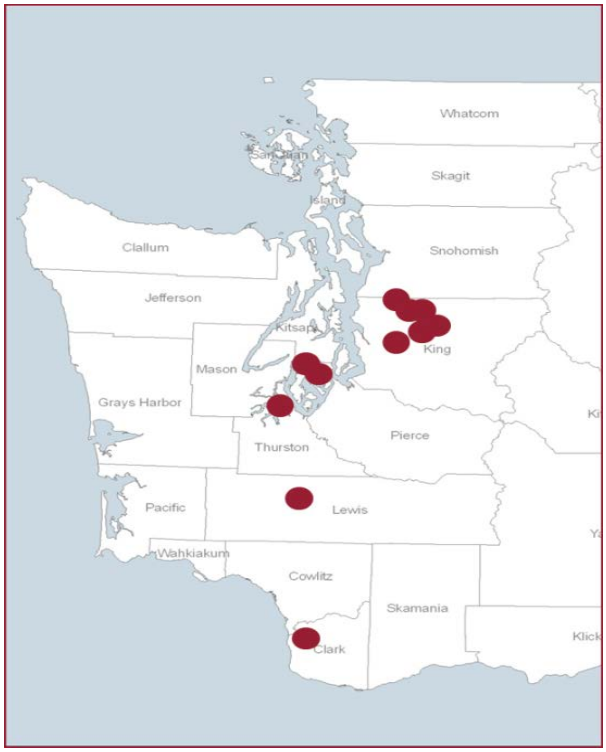

Figure 3—Locations of drainage ditches and/or streams where *Phytophthora ramorum* has been baited since 2006.

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

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

Acknowledgments

The authors wish to thank WSDA for information relating to positive nurseries and waterways, isolates, DNA, and plant samples; Gil Dermott and Kathy Riley, WSU, for assistance in compiling data and preparation of maps; and the WSDA Nursery Research Program for providing funding to genotype isolates of *P. ramorum*. The assistance of Dan Omdal and Amy Ramsey-Kroll, Washington State Department of Natural Resources, and Steve Oak, USDA Forest Service, Southern Region is also gratefully acknowledged.

Phytophthora ramorum Regulatory Program: Present, Past, and Future Direction[1]

Prakash Hebbar,[2] Scott Pfister,[2] Stacy Scott,[3] Anthony Man-Son-Hing,[4] and Russ Bulluck[5]

Abstract

Since the publication of the *Phytophthora ramorum* interim rule in 2007, the Animal and Plant Health Inspection Service, Plant Protection and Quarantine (APHIS PPQ) has explored several avenues to obtain consensus on the objectives of the regulatory program and meet its goals. The principal objective of the program is to protect native biodiversity and wildland environments from sudden oak death (SOD). For the past several years, input from stakeholders, better scientific knowledge, and novel detection methods have helped APHIS, in collaboration with their partners, to develop and implement several scientifically-based protocols and remediation strategies that have reduced the risk of the pathogen being moved through shipments of infested nursery stock. Despite the progress made, *P. ramorum* continues to be moved around and has subsequently established itself in a number of retail and wholesale nurseries in several non-regulated states and, through these nurseries, into streams and waterways. Based on 10 years (2001 through 2010) of regulatory data, enhanced understanding of the science, and current realities, the general consensus is that a more targeted and focused regulatory framework is needed to reduce the potential for pathogen movement in nursery stock in order to protect valuable forest resources and the nursery industry. In addition to survey and detection within nurseries, it is imperative that best management practices (BMPs) be implemented to avoid the introduction and/or re-occurrence of *P. ramorum* within the nursery production system. The agency (APHIS) is currently investing in several pilot programs to test and improve implementation of BMPs in nurseries. This presentation describes several aspects of a revised regulatory framework being currently discussed and how it can be related to the implementation of BMPs and critical control point (CCP) assessment for regulated nurseries.

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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International Updates

The New *Phytophthora ramorum* Dynamic in Europe: Spread to Larch[1]

Anna Harris[2] and Joan Webber[2]

Abstract

Phytophthora ramorum has been reported from most European Union member states, mainly affecting ornamental plants in nurseries. The most epidemiologically important hosts are those that support abundant sporulation, and, until recently, in Europe this applied primarily to rhododendron and vaccinium. However, following the first findings of *P. ramorum* on Japanese larch (*Larix kaempferi* (Lam.) Carrière) in southwest Britain in 2009, it soon became clear that infected foliage of Japanese larch could produce abundant numbers of sporangia, as demonstrated in the laboratory (see Webber, J.F.; Mullett, M.; Brasier, C.M. 2010. Dieback and mortality of plantation Japanese larch (*Larix kaempferi*) associated with infection by *Phytophthora ramorum*. New Disease Reports. 22: 19.) and on naturally infected needles, leading to bark infections on larch and other nearby susceptible tree species.

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

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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EU2, a Fourth Evolutionary Lineage of *Phytophthora ramorum*[1]

Kris Van Poucke,[2] Selma Franceschini,[3] Joan Webber,[3] Kurt Heungens,[2] and Clive Brasier[3]

Phytophthora ramorum is an aggressive Oomycete pathogen introduced into western North America and western Europe in the late twentieth century by the ornamental plant trade (Goss et al. 2011, Grünwald et al. 2012, Mascheretti et al. 2008, Prospero et al. 2007). The pathogen attacks a wide range of trees and shrubs, causing foliage blights and bleeding stem lesions both in nurseries and in the field (Rizzo et al. 2002, Werres et al. 2001). In North America, *P. ramorum* is known for causing sudden oak death, the dieback and mortality of millions of coast live oak (*Quercus agrifolia* Née) and tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H. Oh) trees along 1 500 km of near-coastal native forest in California and southwestern Oregon (Grünwald et al. 2008, Rizzo et al. 2002). The pathogen has also spread rapidly and widely across Europe within the nursery trade. From 2003 onwards, it was found attacking rhododendron and some broadleaf trees in the United Kingdom (Brasier et al. 2004) and subsequently native *Vaccinium* heathlands (P. Beales, Central Science Laboratory, personal communication). Since 2009, *P. ramorum* has caused sudden larch death, heavy dieback and mortality of plantation Japanese larch (*Larix kaempferi* (Lam.) Carrière) trees in western Britain and Northern Ireland, resulting in the felling of millions of trees (Brasier and Webber 2010, Webber et al. 2010).

Ivors et al. (2006) demonstrated three distinct genetic lineages in *P. ramorum.* These have since been informally designated NA1, NA2, and EU1 after their initial outbreak locations (Grünwald et al. 2009). NA1 and NA2 are confined to western North America, NA1 being predominant in the forests and found in most nurseries and NA2 so far confined to nurseries and adjacent waterways. Until recently, EU1 was the only lineage found in Europe. EU1 has also been found at a small number of nurseries in the Pacific Northwest. The recent appearance of the lineages is believed to involve independent introduction of NA1 and NA2 into North America and EU1 into Europe; with the appearance of EU1 in North America resulting from a secondary introduction from Europe (Goss et al. 2009a, Goss et al. 2011, Grünwald et al. 2012).

All three lineages are near clonal at their presumed centers of introduction, consistent with introduction bottlenecks (Goss et al. 2009b, Grünwald et al. 2008, Ivors et al. 2006, Vercauteren et al. 2010). Significant differences exist among them for important fitness characteristics, such as growth rate, colony stability, and aggressiveness (Brasier et al. 2006a, 2006b; Elliott et al. 2011). *Phytophthora ramorum* is heterothallic, and to date all NA1 and NA2 lineage isolates have been of A2 sexual compatibility type and EU1 isolates largely of A1 type (Brasier and Kirk 2004, Werres and Kaminski 2005). In Belgium, rare A2s of EU1 lineage have been observed, but these are probably products of somatic recombination from an A1 isolate (Vercauteren et al. 2011b). Gametangial formation between A1s and A2s is unusually sparse and gametangial meiosis often abnormal (Brasier and Kirk 2004, Boutet et al. 2010, Vercauteren et al. 2011a), and a coalescence analysis indicates the lineages may

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California. The original paper is available through the Fungal Biology website: http://dx.doi.org/10.1016/j.funbio.2012.09.003.

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have diverged 150,000 to 500,000 years ago (Goss et al. 2009a). The lineages therefore appear to be partially reproductively isolated, adaptively different populations within *P. ramorum*.

In 2011, following the spread of *P. ramorum* onto larch in the United Kingdom, preliminary screening of larch isolates with SSR markers and *Cox* II sequencing led to evidence of a novel genotype on larch in Northern Ireland and western Scotland, potentially distinct from the three known lineages. The seven isolates were assigned to a new lineage: EU2. They came mostly from *Larix* but also from *Quercus*, *Rhododendron*, *and Vaccinium* (Van Poucke et al. 2012).

In a detailed SSR analysis with 18 primer pairs, all seven novel isolates had an identical SSR profile distinct from that of the EU1, NA1, and NA2 lineages. The differences were similar to those previously observed between the other lineages. No intra-EU2 lineage genotypic diversity was detected. Multilocus sequencing was carried out to determine the phylogenetic position of the EU2 lineage. Single sequences were obtained for all 11 loci except the heterozygous nuclear locus *Avh120*. For the five mitochondrial loci, the new EU2 lineage clustered with the three other *P. ramorum* lineages and separately from *P. hibernalis*. Within *P. ramorum*, the EU2 lineage was in most cases closest to the NA2 lineage and ancestral to it, though with weak support (<70 percent bootstrap). With the six nuclear loci, segregating sites were observed in all three known lineages as reported by Goss et al. (2009a). As only a single heterozygous site was observed in the ITS of the rDNA, this locus was not taken further into account. With the EU2 lineage, heterozygosity was observed in only one (*Avh120*) of the five remaining nuclear loci. The other three lineages were heterozygous at three (NA1) to five (EU1) loci. In phylogenetic trees, the unique EU2 lineage typically clustered with the three other *P. ramorum* lineages and separate from *P. lateralis* and *P. hibernalis*. Based on the β-*tub* and the *Cox* I loci, two PCR-RFLP tests were developed that effectively discriminate between all four lineages (Van Poucke et al. 2012). Both methods involve a restriction enzyme that cuts at least once in all lineages, so that the activity of the enzyme can be verified in all *P. ramorum* samples.

In sexual compatibility tests on carrot agarose medium between the EU2 isolates (of unknown mating type) and EU1, NA1, and NA2 tester isolates, gametangia were only produced in pairings with isolates of the NA1 or NA2 lineages and not with those of the EU1 lineage, establishing the EU2 isolates as A1 type. As previously observed with *P. ramorum* (Brasier and Kirk 2004), not all pairings (even between known A1s and A2s) were fertile, and gametangia were generally rare or very rare even in the fertile mixtures. The size and morphology of the gametangia produced in inter-lineage pairings involving EU2 isolates was similar to that in the control pairings and to that previously published for intra-specific pairings of *P. ramorum* (Brasier and Kirk 2004, Werres and Kaminski 2005).

The EU1 lineage has been present in Europe since at least 1993 and is now very widespread, occurring in most of western and central Europe (Anonymous 2011, Grünwald et al. 2012, Webber 2008, Werres et al. 2001). In contrast, the first recorded disease outbreak caused by EU2 is only in 2007 in Northern Ireland, and its known distribution is still restricted to Northern Ireland and to an area in southwest Scotland about 100 km away. The SSR profiles of the seven EU2 isolates examined were entirely uniform. The recent detection, limited geographical distribution, and genetic uniformity of EU2 suggest that it is a much more recent introduction than EU1. *Phytophthora ramorum* is particularly well suited to long distance spread via infested plant material, as has been demonstrated by its rapid spread on susceptible nursery stock, especially on *Rhododendron*, *Viburnum* and *Pieris* (e.g., Frankel 2008, Grünwald et al. 2012). Introduction via plant movement or plant trade is therefore the most likely mode of arrival of EU2 in the United Kingdom. Spread within the United Kingdom is also most likely to be associated with movement of infected plants, although other pathways such as movement of 'sporangial clouds' from heavy sporulation of EU2 on infected larch plantations, or movement between sites of spores attached to boots or machinery, cannot be ruled out. Brasier and Webber (2010) suggested that the epidemic on larch could simply reflect the intrinsic properties of *P. ramorum* or could be a result of its adaptation to larch. The fact that two lineages of *P. ramorum*, EU1 and EU2, are now involved suggests the epidemic is more likely to be an intrinsic property of the pathogen.

As with the other three lineages, the geographic origin of EU2 remains unknown. It has been suggested that the lineages might have separate geographic origins and could be at least equivalent to taxonomic subspecies (Brasier et al. 2006b). The coalescence analysis of Goss et al. (2009a) indicates that EU1, NA1, and NA2 have been evolutionarily divergent for a minimum of 100,000 years. Whether this is a result of their arising in different geographic locations or their becoming reproductively isolated within a single center of origin, has yet to be determined. A proper understanding of the evolution and behaviour of *P. ramorum* and its lineages may only come from studying its behaviour at its geographic source. Indeed the arrival of EU2 highlights an urgent need to identify the geographic origins of *P. ramorum* in order to understand the organism's natural ecology, the processes that have produced the lineages, and whether further lineages exist. Presently, studying the organism in the context of introduction and invasion, we may only be looking at half the picture.

All the isolates of the EU2 lineage examined here were of the A1 compatibility type, hence EU2 may be solely of A1 type, at least in its current introduced, as opposed to its endemic form. Since the other lineage present in Europe (EU1) is also of A1 type, the arrival of EU2 should not increase the theoretical risk for sexual recombination of *P. ramorum*, although inter-lineage somatic recombination is a possibility. Currently, findings of *P. ramorum* in the United Kingdom are subject to emergency European Union and United Kingdom plant health phytosanitary measures. Indeed, present evidence suggests EU2 may have arrived in the context of these ongoing emergency measures. The true significance of the arrival of EU2 for the future health of United Kingdom forests and plant heritage should become clearer when more information is available on its distribution across the British Isles and when the comparative behaviour of the EU2 and EU1 lineages is better understood, in particular their comparative aggressiveness and host range. For a full account of these observations see Van Poucke et al. (2012).

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The Epidemiology of *Phytophthora ramorum* and *P. kernoviae* at Two Historic Gardens in Scotland[1]

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Abstract

This study looked at the factors that facilitated the spread of *Phytophthora ramorum* and *P. kernoviae* at two locations in the west of Scotland. Spore traps, river baiting, bait plants, and soil sampling were used to both confirm the presence of, and measure the amount of, inoculum in the environment in order to quantify the relationship between inoculum levels and disease development. *Phytophthora ramorum* was detected in spore traps at high levels under a sporulating host, but also at sites where hosts were not present, leading to the conclusion that inocula in low-level spore traps were the result of soil splash. *Rhododendron* and *Vaccinium* bait plants were also infected with *P. ramorum* via soil splash at sites where there was no sporulating host present. *Phytophthora kernoviae* was only detected in spore traps where there was a sporulating host overhead. Water baiting confirmed the presence of *P. ramorum* in two streams in one of the gardens, but *P. kernoviae* was not detected using this method at the other garden despite the large-scale *P. kernoviae* infection there. Inoculum continued to be detected in soil in areas where infected hosts had been removed 2 years ago, confirming that both of these pathogens can survive in soil for a considerable period. Evidence of the movement of infested mulch during horticultural activity was found. These findings have clear implications for the control of disease spread within the garden setting.

Keywords: *Phytophthora ramorum*, *Phytophthora kernoviae*, epidemiology, spread

Introduction

In Europe, *Phytophthora ramorum* was initially discovered within the nursery industry and was found infecting container grown *Rhododendron* and *Viburnum* plants (Werres et al. 2001). The extent of the host range and the damage that this pathogen could cause in parks and gardens was not immediately apparent; it was not until outbreaks occurred in forests in California that the true potential scale of damage was observed (Rizzo et al. 2002). During surveys undertaken to find *P. ramorum* in 2003, *P. kernoviae* was discovered infecting trees and shrubs in woods in Cornwall (Brasier et al. 2005). It soon became clear that *P. kernoviae* could cause as much damage as *P. ramorum* in gardens, particularly if the garden contained a large proportion of *Rhododendron* species (Webber 2008).

The trees and shrubs that have traditionally been grown in United Kingdom historic gardens have transpired to be particularly susceptible to both pathogens, particularly *P. ramorum*. Initially, ornamental *Rhododendron* species, *Viburnum*, and the invasive *R. ponticum* were found to be infected. As *P. ramorum* garden infections developed, many more commonly used horticultural species became infected such as *Magnolia*, *Pieris*, *Osmanthus*, *Fagus*, and *Camellia*. The garden hosts of *P. kernoviae* remained more restricted; *Rhododendron* is still an important host, but infections have also occurred on *Magnolia*, *Pieris*, *Fagus*, and *Vaccinium*. The proliferation of hosts

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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and the ideal environmental conditions in the west of England, Wales, and Scotland have provided these *Phytophthora* species with particularly conducive conditions for establishment and spread.

Methods

Field Sites

The first study location was a botanic garden in Argyll and Bute, where both *P. ramorum* and *P. kernoviae* were present. The second was a castle garden on the island of Arran, which was severely infested by *P. kernoviae*. A woodland *P. kernoviae* outbreak on *Vaccinium myrtillus* L. near the castle garden was also studied. Spore traps, river baiting, bait plants, and soil sampling were used to measure the amount of inoculum in the environment at the gardens. Four investigation sites within the botanic garden were chosen for spore trap locations and six at the castle garden. The sites at the botanic garden were: site 1, cleared infection site where both *P. ramorum* and *P. kernoviae* had infected a number of *Rhododendron* 'Elizabeth Hobbie' plants; site 2, under an infected *Magnolia kobus* DC.; site 3, cleared infection on *Kalmia latifolia* L.; and site 4, infection on two *Osmanthus* plants now cleared.

The investigation sites at the castle garden were: site 1, within an area of infected and partially cleared *Rhododendron ponticum* L. near the garden entrance; sites 2, 3, and 4, in the main infection area at the bottom of the garden, which was extensively infested with *P. kernoviae* and once contained a large number of *Rhododendron* species and cultivars which were partially cleared; site 5, under a grove of mature *Pieris japonica* (Thunb.) D. Don ex G. Don trees; and site 6, at the stump of a felled *Drimys* tree that was infected and cleared from the bottom of the garden.

Spore Traps

Two types of spore traps were designed to catch rain and water splashes, potentially containing sporangia and zoospores. The low-level traps were located at ground level and constructed by digging a 2-L bottle into the ground and placing a funnel on top. Wire mesh on the top of the funnel prevented blockage with fallen leaves and other debris. The high-level water traps were approximately 1 m above ground level. They consisted of a 2-L bottle on the ground, a funnel, and a length of hose connecting the two. The low-level traps are designed to record mainly rain splash dispersal from the surrounding soil, while the high-level traps record dispersal from rain and leaf splash from the nearby plants. The 2-L bottles were taken to the quarantine unit at Science and Advice for Scottish Agriculture (SASA) every 2 weeks. The water they contained was filtered through a 3µl membrane filter and the filters were processed to extract the DNA they contained using the Macherey-Nagel Nucleospin® Plant Kit. Any extracted DNA was then quantified using Real-Time PCR.

Bait Plants

To test the viability of the inoculum in the environment, and to test for a link between inoculum levels and infection, one potted *R. ponticum* bait plant and one *V. myrtillus* bait plant was placed monthly at each investigation site in both gardens for a 4-week period in order to see whether they became infected. The bait plants were taken to the quarantine unit at SASA after the 4-week period in the gardens, were kept for 3 months post-exposure, and were checked daily for symptoms. Infected leaves were plated onto V8 agar plus antibiotics medium and the resulting culture was identified under the microscope or by using PCR testing if required. Water baiting also took place in a number of streams around the gardens. The water baits were made by placing six cut *Rhododendron* leaf pieces in a muslin bag and attaching a length of string to be used to anchor the bait once it was placed in a stream. Recovered baits were surface sterilized and plated onto V8 agar plus antibiotics medium, and the resulting culture was identified using microscopy or PCR.

Soil Samples

To establish inoculum levels in the soil, soil samples were collected monthly from the investigation sites, but also extensively around selected sites within the gardens at 3 monthly intervals. Random soil samples were also taken around each garden at various times over the study period. Soil samples were dried, mixed, and then added to a mixing bowl from a large planetary ball mill with 12 steel ball bearings and 120 ml of cetyltrimethyl ammonium bromide (CTAB) buffer. They were milled at 300 rpm for 5 minutes. A robotic workstation for DNA extraction based on magnetic-particle purification (e.g., Qiagen Biosprint 15) and the Wizard Magnetic DNA purification system for food (Promega) was used for DNA extraction from the soil.

Water Baits

Four *Rhododendron* leaves were cut into pieces and placed in a square of muslin which was then tied with twine. The twine was about 1 m long, which allowed it to be anchored in the field. These baits were then placed in watercourses around each garden for 24 hours. As with the bait plants, the use of *V. myrtillus* and *Pieris* as water baits was tried in order to establish levels of *P. kernoviae* and *P. ramorum* in water courses at both gardens. Once the water baits had been recovered, the leaves in them were surface sterilized and plated onto V8 agar plus antibiotics. The plates were checked under a compound microscope after 5 days for the presence of mycelium and sporulation structures. If the presence of either of these *Phytophthora* species was suspected, the Nucleospin® Plant Kit protocol for DNA isolation from plants was used to extract any DNA and PCR was used for confirmation.

After initial investigation of the watercourses at the botanic garden, a stream which ran from where it entered the garden to a small pond and then down to site 1 was chosen to be baited for *P. ramorum* on a monthly basis. There were five baiting locations along this stream. Another regularly tested watercourse was near the car park and horticultural sheds; this stream originated from a different source.

Molecular Detection

Once the samples were processed using the extraction processes described above, Taqman Real-Time PCR, using a thermo-cycler, was used to quantify the levels of a target sequence, therefore quantifying the presence of these pathogens. This PCR method uses fluorophore-labelled DNA probes to measure the amount of amplified product in a sample in real time, giving results in cycle threshold (Ct) values. The Ct value is the number of cycles needed to get a fluorescent signal that is significantly higher than background levels. The lower the number of cycles, the more DNA was present in the sample. In order for the Ct value to be converted into a more useful measurement (e.g., the amount of DNA per sample in picograms), four standards of known concentrations were added to each PCR run along with the samples. Reaction mixtures of 25 µl were used for the Real-Time PCR containing:

Component	**Amount (µl)**
Double distilled H_20	8.0
Taqman Master Mix[7]	12.5
Forward Primer (5 molar concentration)	1.5
Reverse Primer (5 molar concentration)	1.5
Taqman Probe (5 molar concentration)	0.5
DNA (Sample)	1.0

[7] Taqman Universal Master Mix, No AmpErase UNG (Applied Biosystems, part No. 4324018) containing DNA polymerase and dNTPs.

The primers and probes sequences are as follows:

P. ramorum:

Pram 114-FC	5’ - TCA TGG CGA GCG CTT GA - 3’
Pram 1527-190-R	5’ - AGT ATA TTC AGT ATT TAG GAA TGG GTT TAA AAA GT - 3’
Pram 1527-134-T	5’- [FAM] - TTC GGG TCT GAG CTA GTA G - [TAMRA] - 3’

P. kernoviae:

Pkern 615F	5’ - CCG AAC AAT CTG CTT ATT GTG TCT - 3’
Pkern 722R	5’ - GTT CAA AAG CCA AGC TAC ACA CTA - 3’
Pkern 606T	5’- [TET] - TGC TTT GGC GTT TGC GAA GTT GGT - [TAMRA] -3’

TET and FAM were the dyes used and TAMRA was the quencher.

The thermal cycler program used was an initial 10-minute denaturing stage of 94 °C, then:
15 seconds at 94 °C
60 seconds at 60 °C
} X 40 cycles

Results

Spore Traps and Bait Plants

Phytophthora ramorum was detected in spore traps at the botanic garden 33 times over the 2 years (fig.1). Of these, 11 findings were at site 2 where the infected *M. kobus* had been left in *situ*. Four of these findings were in the high-level traps and seven in the low-level traps. When inoculum was recorded in the high-level traps, it was also recorded in the low-level traps in three of the four instances (April 10, December 10, November 11). Also, proportionally more inoculum was collected in the low-level trap (average 1.75 pg/µl) compared to the high-level trap (average 0.5 pg/µl). In fact, the high-level trap on average contained the least amount of inoculum found at any site throughout the study. At the other three botanic garden investigation sites, the infected hosts had been removed prior to the start of the study. Inoculum was recorded in these low-level traps on nine occasions at site 4, seven at site 3, and six at site 1. These data show that *P. ramorum* is readily splashed from soil into the low-level traps. *Phytophthora kernoviae* was only recorded on four occasions in spore traps at this garden; these four incidences were at very low levels (between 1.12 pg/µl and 2.13 pg/µl) and occurred at four different spore trap locations.

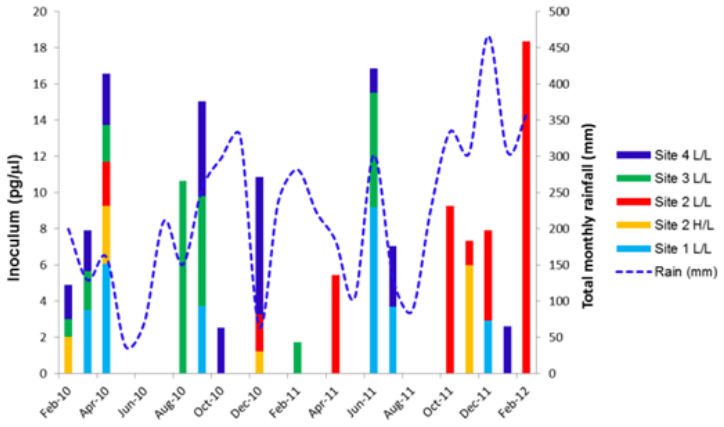

Figure 1—The incidence of *Phytophthora ramorum* inoculum (picograms/µl) in spore traps at the botanic garden.

Bait plants were found to be infected with *P. ramorum* on 16 occasions at site 2 under the *M. kobus* over the 2 years (fig. 2). This is not surprising because of the presence of an overhead sporulating host at this site. Site 4 bait plants, however, became infected almost as often (14 times) despite the removal of the infected host before the start of the study. This is also the case with the four infections at site 3. The bait plant infections at sites 3 and 4 have most likely resulted from inoculum splash from the surrounding infested soil, although the presence of infected plants without symptoms in these areas cannot be ruled out. The infection of bait plants indicates that the inoculum recorded under the sporulating *M. kobus* in April 11, October 11, December 11, and February 12 was viable because these were the months that the bait plants became infected (fig. 2). There were far more bait plant infections than inoculum detections in the trap, with a total of 10 instances of infection with no spore trap inoculum. The low level trap at site 3 only recorded inoculum with a bait plant infection once on April 10. There were also only three more bait plant infections at site 3 which all occurred when inoculum was not recorded in the spore trap.

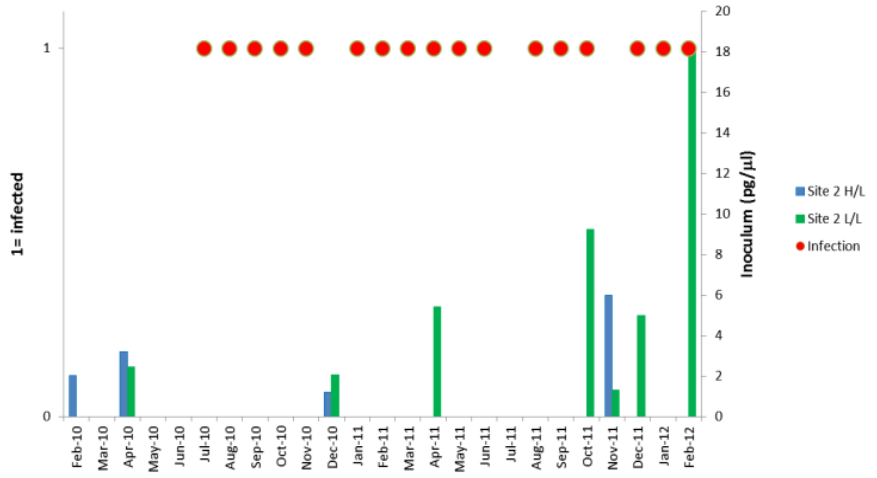

Figure 2—Spore trap inoculum (*Phytophthora ramorum*) at the botanic garden site 2 and bait plant infection.

At the castle garden, *P. kernoviae* was recorded on 12 occasions in the spore traps at site 5; nine of these were in the low-level trap and three in the high. The only other findings were in March 2010 at site 3 and site 4 (fig. 3). The high levels of inoculum recorded in the site 5 low-level traps in December 2010 (30.4 pg/µl) and January 2011 (19.7 pg/µl) were preceded by a month of high rainfall in November 2010 (total of 296 mm). The high-level traps did not record inoculum during this period, so these findings could have been due to water splashing off the soil and leaf litter around the low-level trap. Given the lack of *P. kernoviae* recorded at the botanic garden and most of findings at the castle garden occurring under the infected *Pieris*, it appears that high inoculum levels are required to be present in the environment before the spore traps pick up the presence of *P. kernoviae*. Another factor could be that there was less *P. kernoviae* soil contamination to splash up into the traps at the castle garden than there was *P. ramorum* soil contamination at the botanic garden.

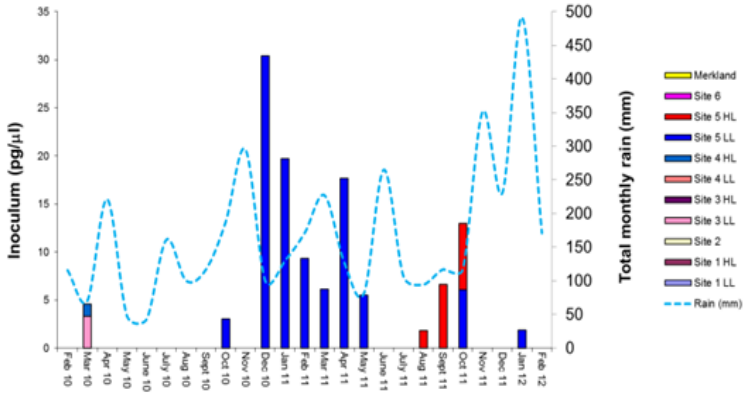

Figure 3—Incidence of *Phytophthora kernoviae* inoculum in spore traps at the castle garden with monthly rainfall.

The *Rhododendron* bait plants were largely ineffective at the castle garden. That was surprising because of the high prevalence of *P. kernoviae* infection of planted *Rhododendron* and *R. ponticum* at this garden. This could be because the bait plants were only exposed for one month whereas the *Rhododendron* plants in the garden may have been exposed for longer before succumbing to infection. It was not until a particularly susceptible batch of *Vaccinium myrtillus* bait plants were placed in the garden in January 2011 that infection started to occur. Once the *Vaccinium* bait plants were placed at site 5 in January 2011 infection was recorded in 10 of the subsequent 12 months (fig. 4). The bait plants were often very heavily infected upon their recovery from site 5b. Infection was also recorded on five occasions at site 4 which is in the heart of the original infection although the symptoms on the bait plants here were more subtle. Of the 11 instances of bait plant infection at site 5, inoculum was recorded in a spore trap (high and/or low) on six occasions. The lowest amount of *P. kernoviae* inoculum recorded whilst a bait plant was infected was 1.836 pg/µl in August 11 in the site 5 low level trap.

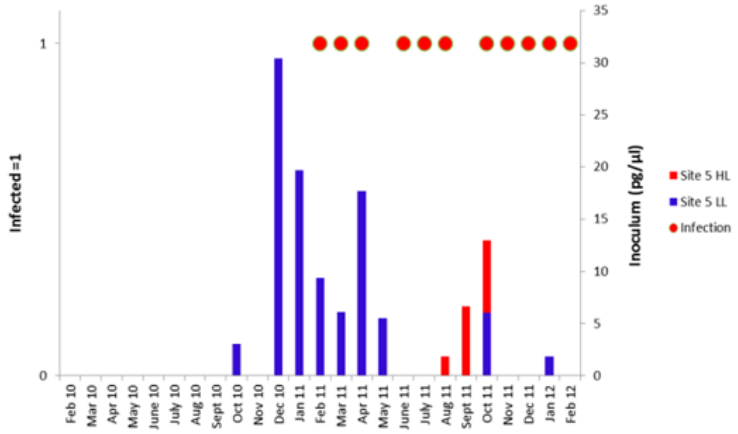

Figure 4— *Phytophthora kernoviae* inoculum in spore traps at site 5 at the castle garden and associated bait plant infection.

Water Baits

Water baiting to establish the presence of these pathogens was only successful at detecting *P. ramorum* at the botanic garden; *P. kernoviae* was not detected in watercourses at either garden. *Phytophthora ramorum* was successfully isolated from the stream that enters the botanic garden then runs down to site 1 as follows: four times where the stream enters the garden, seven times 50 m downstream, once in the small pond, seven times above site 1, and 11 times at site 1. At the other baiting site near the horticultural sheds, *P. ramorum* was isolated 11 times. This stream originates from outside the garden at a former commercial forestry plantation above the garden. The only months where *P. ramorum* was not detected was when there was either not enough rain to fill the streams or the streams were frozen. If there was enough water in the streams for baiting, *P. ramorum* was usually detected.

Soil Infestation

The soil inoculum levels of both *P. ramorum* and *P. kernoviae* at these gardens did not deplete over the 2 year study, despite the removal of the sporulating hosts at most of the sites (fig. 5 and 6). There was some evidence of seasonal variation in *P. ramorum* soil inoculum levels at the botanic garden under the infected *M. kobus* at site 2, with findings of 11,205 pg/ml in August 2010 and 15,805 pg/ml in August 2011, but these high peaks coincide with low levels of rain, not high levels. This inoculum does not appear to 10 m away at site 3, as the average levels there remained relatively low (average 112 pg/ml) (fig. 5).

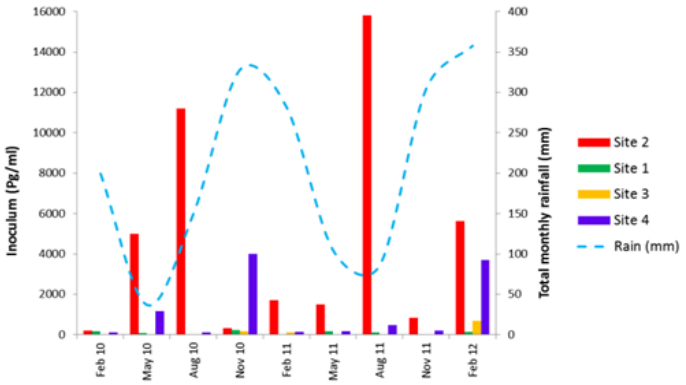

Figure 5—*Phytophthora ramorum* inoculum in soil at the botanic garden (picograms/ml) and total monthly rainfall.

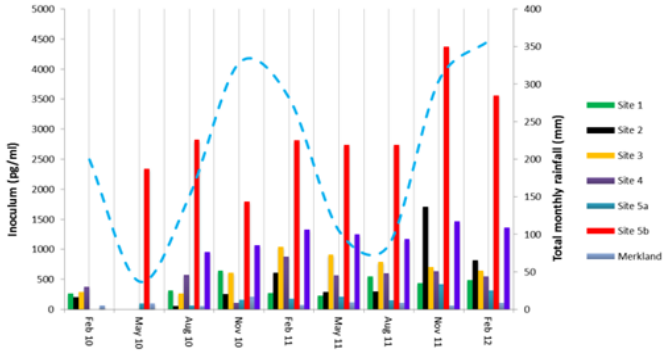

Figure 6—*Phytophthora kernoviae* inoculum in soil at the castle garden (picograms/ml) and total monthly rainfall.

There were peaks of 4,010.5 pg/ml at site 4 in November 2010 and 3,706 pg/ml in February 2012 which coincided with particularly wet months at the garden. This site is in a depression in the ground where the soil was often found to be saturated. It may be expected that, of all of sites where the host had been removed at the botanic garden, site 3 would contain the highest levels of soil inoculum (average 112 pg/ml) because it is only 10 m from sporulating *M. kobus* at site 2, but much higher levels were actually recorded at site 4 (average 1,129 pg/ml), the furthest site from this source of inoculum. The overall level of *P. kernoviae* inoculum in the soil at the botanic garden was low. It was only consistently found throughout the study period in the soil at site 1, with an average of 136.7 pg/ml. The inoculum level also depleted from 559 pg/ml in May 2010 to 74 pg/ml in February 2012. This site is where *P. kernoviae*-infected *Rhododendron* plants had been removed in 2009.

At the castle garden (fig. 6), inoculum levels at site 5b were particularly high with an initial finding of 2,347.5 pg/ml in May 2010, a maximum of 4,380 pg/ml in November 2011, and an average over the 2 years of 2,579.5 pg/ml. These findings combined with infected leaf litter on the ground indicated that one or more of the *Pieris* at this site were infected. Site 6 was added in August 2010 to measure inoculum around the stump of a large infected *Drimys* that had been removed before the study started. This was in the heart of the worst affected area and the soil inoculum levels there remained high, with an average of 958 pg/ml at site 6, 480.7 pg/ml at site 4, and 586.5 pg/ml at site 3.

The soil at the *V. myrtillus*-infected woodland site showed low levels of infestation over the 2 years with an average of 102.4 pg/ml. In addition, only small patches of soil were found to contain inoculum as opposed to parts of the castle garden where whole areas were infested, around site 4, for example.

Random soil sampling around the gardens detected very high levels of *P. ramorum* inoculum (8,540 pg/ml) near to a pond in the botanic garden, despite there being no confirmed cases of plant infections in this area. This sampling event was followed up with six more samples which were all positive; the highest level was found to be 4,670 pg/ml. The garden managers were able to confirm that the area had been redesigned the previous year and that mulch had been added to the soil. The mulch heaps in the garden were subsequently tested and *P. ramorum* was found to be present at an average level of 238 pg/ml. These findings were followed up by testing the wood chip piles which would have been used for mulching the beds. *P. ramorum* inoculum was detected in the wood chips.

On a number of occasions, the downhill sides of wooden slot drains were tested and found to contain high levels of *P. ramorum* inoculum, particularly the slot drain below the first confirmed case in the garden. This particular drain was first tested in November 2010 and a high-inoculum level of 3,975 pg/ml was found. Testing then continued over the next year, revealing that the area was heavily infested, with average inoculum levels ranging from 3,488 pg/ml to 6,305.5 pg/ml. Furthermore, the highest inoculum level found during this whole study (19,760 pg/ml) was found at this site in February 2012. This is higher than any of the samples taken from under the infected *M. kobus* (maximum 11,205 pg/ml). These drainage channels across the paths increase the amount of water on the downhill side of paths which in turn appears to concentrate the inoculum.

Random soil sampling at the castle garden detected *P. kernoviae* throughout the lower part of the garden, with 169 of the 211 samples collected containing inoculum. Most of the infections within this area were on planted rhododendron species and cultivars, or *R. ponticum*. Infested soil was also found in moderately high concentrations (510 pg/ml) in higher areas of the garden that contained no previously infected plants. It is assumed that *P. kernoviae* was introduced into these areas by horticultural activity. At the *Vaccinium*-infected woodland, random sampling uncovered no new infested areas of soil and the original patches of known infestation remained limited.

Discussion

Spore traps have been used widely for detecting the presence of plant pathogens in the past, and *P. ramorum* is no exception (Davidson et al. 2005, Hansen 2008, Turner et al. 2008).The overall findings of inoculum in spore traps at these gardens were relatively low, with only 13 percent of traps

recording the presence of inoculum. This could be due to the effects of the legislation that requires the removal of infected plants as soon as they show symptoms; the spore traps reliably detected inoculum under infected hosts. *Phytophthora kernoviae* was not detected in the spore traps at the castle garden despite the large extent of the initial infestation, which may suggest that *P. kernoviae* does not persist in the environment for as long as *P. ramorum*.

The low-level traps at the botanic garden where there were no sporulating hosts present, but where *P. ramorum* inoculum was still recovered, show that inoculum was most likely splashed from soil during rain events and into spore traps at these sites. This argument is strengthened by the frequent infections of bait plants at sites where the sporulating host had been removed. However, it must be noted that *P. ramorum* could be sporulating from an asymptomatic infected host nearby. Infection of conifer seedlings and *Rhododendron* plants via soil splash have also been found to occur under infected California bay laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.) in the United States (Chastagner et al. 2008).

This study showed that both *P. ramorum* and *P. kernoviae* persist in soil in the west of Scotland for at least 2 years after their host is removed. This concurs with work carried out in other parts of the United Kingdom and further afield that have found that these pathogens persist in soil for at least 2 years (Turner et al. 2005, Shiskoff 2007, Widmer 2011; Alexandra Schlenzig, SASA, unpublished). An important consideration of inoculum survival in soil at the castle garden is that although the above ground parts of the main host, *R. ponticum*, had been largely removed, there was some evidence of regrowth and reinfection at some sites around the garden. This, combined with the possible asymptomatic infection of some of the other *Rhododendron* species present in the most infested parts of the garden, could effectively 'top up' the inoculum in the soil.

This study has also shown that infested soil is inadvertently moved around a garden by horticultural activity in infested mulch. This highlights the importance of correct composting techniques. In large scale compost heaps, Noble et al. (2011) found that *P. ramorum* had not survived after 5 days at a mean temperature of 41.9 ^{o}C (32.8 ^{o}C for *P. kernoviae*) or for 10 days at 31.8 ^{o}C. If these composting procedures cannot be followed, horticultural practices should be modified in gardens that become infested by these pathogens so that infested material is burned and no compost or leaves are gathered to be used again.

These findings highlight the need for careful disease management to prevent the spread of these pathogens around a garden, the prevention of the establishment and spread of hitherto unknown pathogens that may be introduced in the future, and the potential risk of spread from gardens to surrounding plantations and forests.

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Genetic Diversity of *Phytophthora ramorum* in Nursery Trade and Managed Environment in Scotland[1]

Alexandra Schlenzig[2] and David Cooke[3]

Abstract

Scottish *Phytophthora ramorum* isolates (228) collected between 2002 and 2011 from almost every outbreak site in Scotland were genotyped using seven microsatellite markers as described by Vercauteren et al. (2010). Thirty multilocus genotypes were identified within the Scottish population, with 51 percent of the isolates belonging to the main European genotype EU1MG1 and 13 unique detected genotypes. Ten of those genotypes were site specific, often represented by single isolates. Three *P. ramorum* isolates, all from the same location, belonged to the new EU2 European lineage.

The number of genotypes found in the managed environment was higher than the number found in the horticultural trade (25 vs. 11), probably due to the fact that outbreaks in nurseries are usually detected earlier and are quickly eradicated. Evidence of locally-evolved genotypes was found in some outbreak sites.

Keywords: *Phytophthora ramorum*, genetic diversity, nursery, managed environment

Introduction

Although *Phytophthora ramorum* was first discovered in 1993 on *Rhododendron* and *Viburnum* spp. in nurseries in Germany and The Netherlands (Werres et al. 2001), it only raised the attention of a wider range of scientists when it became apparent that it was responsible for sudden oak death on oaks (*Quercus* spp.) and tanoaks (*Notholithocarpus densiflorus* Hook. & Arn.) Manos, Cannon & S.H. Oh) (Rizzo et al. 2002) in North America. In contrast, in Europe the pathogen caused only limited ecological damage, despite being present in most European countries and having a remarkably wide host range. Findings outside the nursery trade were limited, the main host being *Rhododendron ponticum* L. Even in the United Kingdom, as the worst affected European country, only 28 trees were infected with the disease by March 2008 (Tracy 2009). However, since 2009 it has been found on Japanese larch (*Larix kaempferi* (Lam.) Carrière) and, to lesser extent, also on other *Larix* spp. in the United Kingdom. By 2010, an estimated 1900 ha of larch plantations were showing symptoms of the disease, triggering the invention of the term sudden larch death (Brasier and Webber 2010).

Phytophthora ramorum has so far only been found in North America and Europe. Early AFLP (amplified fragment length polymorphism) and SSR (simple sequence repeats) studies (Ivors et al. 2004, 2006) showed different populations on both continents, but only limited genetic variation within these populations. More recently, additional new SSR markers revealed more variation within the United States population of *P. ramorum* (Mascheretti et al. 2008; Prospero et al. 2004, 2007) and within the European population (Vercauteren et al. 2010). The latter were able to distinguish 30 multilocus genotypes within the Belgian population using four previously described and three newly identified markers.

As of early 2012, three distinct genetic lineages had been reported: the EU1 lineage present in Europe and a small number of nurseries along the United States west coast, and the NA1 and NA2

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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lineages currently restricted to North America (Goss et al. 2009, Grünwald et al. 2009, Ivors et al. 2006, Martin 2008). The dominant NA1 lineage is found in forestry and nursery environments; whereas, NA2 has been recovered only from nurseries and from a few waterways. Recently, a new *P. ramorum* lineage, designated EU2, has been reported (Van Poucke et al. 2012), with the known geographical origin of those isolates currently limited to Northern Ireland and western Scotland.

Phytophthora ramorum is a heterothallic species requiring two mating types (A1 and A2) for sexual recombination. All NA1 and NA2 isolates so far have been found to be of the A2 mating type; whereas, the EU1 and EU2 isolates, with the exception of three Belgian isolates, belong to the A1 mating type (Brasier and Kirk 2004, Van Poucke et al. 2012, Werres and Kaminski 2005). However, there has been no evidence of natural sexual reproduction in population studies (Ivors et al. 2006, Vercauteren et al. 2010), and in *vitro* mating studies with *P. ramorum* have revealed slow and reduced production of gametangia (Brasier and Kirk 2004) and progeny showing reduced fitness (Boutet et al. 2010).

In Scotland, the history of *P. ramorum* can be split into two time periods. During the period from the first finding in April 2002 until the end of 2006, the disease was restricted to the ornamental nursery trade. Phytosanitary measures successfully reduced the number of outbreaks each year down to no outbreaks in 2006 (Schlenzig 2006). In the second period, from 2007 to the present, the disease was also found on ornamental plants in the managed environment, such as historic gardens, parks, and country estates, and the overall number of outbreaks increased dramatically, although the number of outbreaks in nurseries remained very low.

This paper reports the genotyping results of the Scottish *P. ramorum* population using the SSR markers described by Vercauteren et al 2010. It compares the population in the nursery trade with the population in the managed environment, and investigates the genetic diversity over the 10 years from the first outbreak until 2011.

Materials and Methods

Isolates and DNA Extraction

Samples of symptomatic material from host plants moving in trade or established plants in public gardens, parks, and country estates were collected by Scottish Government inspectors during official surveillance for the organism. The pathogen was isolated using semi-selective V8 medium as described by Jung et al. 1996. The government laboratory at Science and Advice for Scottish Agriculture had collected 228 isolates, comprising representative isolates from almost every outbreak site in Scotland from 2002 to December 2011. Depending on the size and duration of outbreak, 1 to 22 isolates were collected per site over the years. A total of 71 percent of the isolates had been isolated from *Rhododendron* spp., 17 percent from *Viburnum* spp., and the remainder from other hosts like *Magnolia* spp. or *Pieris* spp.

The Nucleo Spin Plant DNA extraction kit (Macherey & Nagel Inc.) was used to extract DNA. Mycelium from fungal cultures was transferred into 1.5 mL microcentrifuge tubes with 400 µL C1 extraction buffer (supplied with the kit) and was ground using micro-pestles with sterile sand as the grinding agent. Further extraction was performed in accordance with the manufacturer's instructions. The DNA from eight isolates from the Belgian study (Vercauteren et al. 2010) was included as reference.

SSR Genotyping

Seven SSR loci were genotyped that had previously shown variation among European *P. ramorum* isolates: 18, 64, 82a (Ivors et al. 2006), 82b, ILVOPrMS133, ILVOPrMS145a, and ILVOPrMS145c. All primer sequences and repeat motifs can be found in Vercauteren et al. 2010. Primers ILVOPrMS133 and ILVOPrMS145 were used without the M13reverse tag, but with an added 5'-GGGT-3' "pigtail" to the 5' end of the reverse primer to reduce stuttering. The resulting differences in allele sizes were taken into account for comparison with other European genotypes. Forward

primers were labelled with FAM (18, 64, ILVOPrMS133), NED (82), or VIC (ILVOPrMS145). All primers were combined in a multiplex PCR reaction. The reaction volume was 12 µl. Final PCR concentrations were 1x Type-it Microsatellite PCR Master Mix (Qiagen), 0.1 µM of primer pairs 82 and ILVOPrMS145 and 0.14 µM of primer pairs 18, 64 and ILVOPrMS133, and 10 ng template DNA. Amplification conditions were an initial denaturation at 95 ºC for 5 minutes, followed by 28 cycles of 30s at 95 ºC, 90s at 58 ºC, and 20s at 72 ºC followed by the final extension at 60 ºC for 30 min. Then 1 µl of each PCR product was mixed with 8.7 µl Hi-Di formamide loading buffer (Life Technologies) and 0.3 µl Gene Scan 500 LIZ size standard (Applied Biosystems), denatured for 5 min at 95 ºC, and run on a ABI 3130xl genetic analyzer (Applied Biosystems). Results were analyzed using Gene Mapper 4.0 (Applied Biosystems).

Where possible, genotypes were designated in accordance with a European SSR genotyping study conducted by the Institute for Agricultural and Fisheries Research (IVLO) in Belgium (2010 to 2011), in which over 1,300 isolates from throughout Europe had been genotyped (K. Heungens, IVLO, personal communication).

Results

All seven SSR markers were polymorphic in the Scottish population and distinguished 30 multilocus genotypes amongst the 228 Scottish isolates. The European main genotype (EU1MG1) was by far the most common genotype, with 51 percent of isolates found at 44 outbreak sites (fig. 1). It was consistently the most frequently found genotype throughout the 10 years of this study. Also relatively widespread were EU1MG5 (12 outbreak sites), EU1MG 18 (six outbreak sites), and EU1MG44 (four outbreak sites). EU1MG13 and EU1MG2, common genotypes on the European continent (K. Heungens, ILVO, personal communication), were found only on one occasion and not at all, respectively. A comparison with the ILVO study revealed that 13 of the detected genotypes were new to Europe (including the rest of the United Kingdom) and unique to Scotland (K. Heungens, IVLO, personal communication). Ten of those genotypes were site specific, often represented by single isolates. Almost 20 percent of isolates have a deletion of three repeats in marker 133. This mutation is unique to the United Kingdom and not found anywhere else in Europe (K. Heungens, IVLO, personal communication).

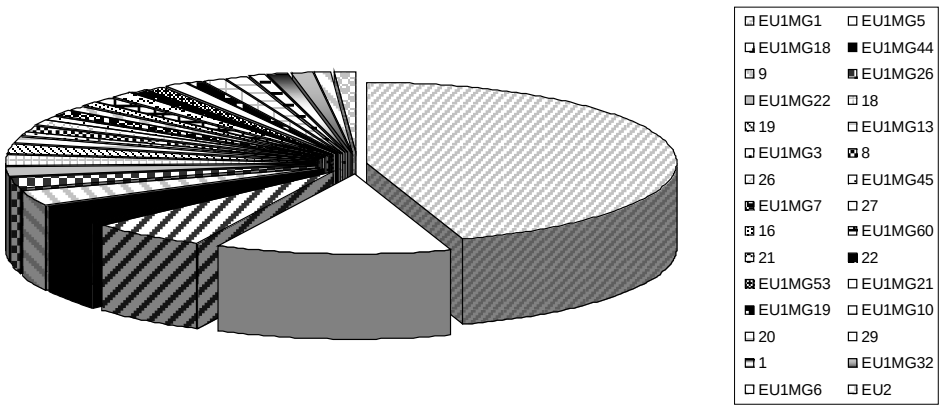

Figure 1—Multilocus genotypes found in Scotland from 2002 to 2011 according to the number of outbreak sites where they were present.

A total of 225 Scottish isolates belonged to the EU1 lineage. Three isolates did not match the EU1 or any of the other genetic lineages known at the time of the study. All three isolates were displaying the same SSR profile and have been collected in 2011 from the same garden location in the southwest of Scotland. The same deviating SSR profile had been found in three other isolates from Northern Ireland. Further analysis revealed that they belong to the new EU2 genetic lineage (Van Poucke et al. 2012; K. Heungens, IVLO, personal communication).

Six genotypes (68 percent of isolates) were found in both settings (horticultural trade and managed environment), and 25 genotypes were found at 39 sites in the managed environment compared to 11 genotypes found in 25 nurseries and garden centers (figs. 2 and 3). Five genotypes (2 percent of isolates) were present in nurseries and garden centers only. Nineteen genotypes (30 percent of isolates) were present only in the managed environment. The diversity of genotypes was therefore higher in the managed environment than it was in the horticultural trade.

Of the 13 outbreak sites from which at least five isolates had been collected, only two sites were infected by single genotypes. The number of genotypes in the other sites varied considerably, independently from the extent of the outbreak and the number of isolates collected. For example, from one garden outbreak site on the west coast of Scotland, 21 isolates had been collected over 4 years, all but one belonging to the same genotype. A park in Glasgow on the other hand yielded six different genotypes amongst only 11 isolates collected over 3 years.

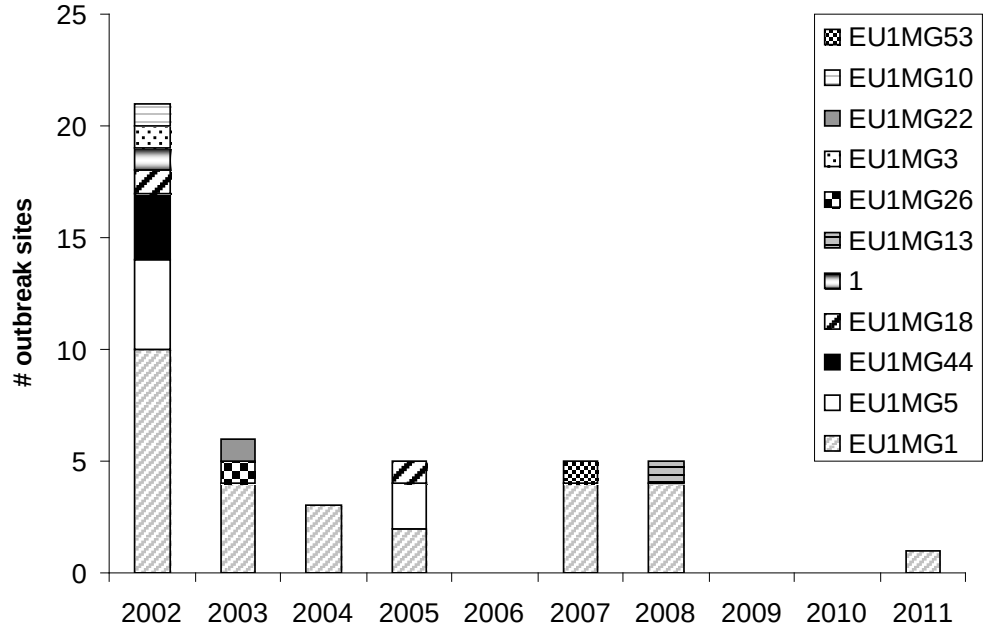

Figure 2—Genotypes detected in nurseries and garden centers. Genotypes unique to Scotland are indicated by simple numbers. Genotypes found elsewhere in Europe are named in accordance to European genotyping study as numbers with prefix “EU1MG” (K. Heungens, Institute for Agricultural and Fisheries Research, personal communication).

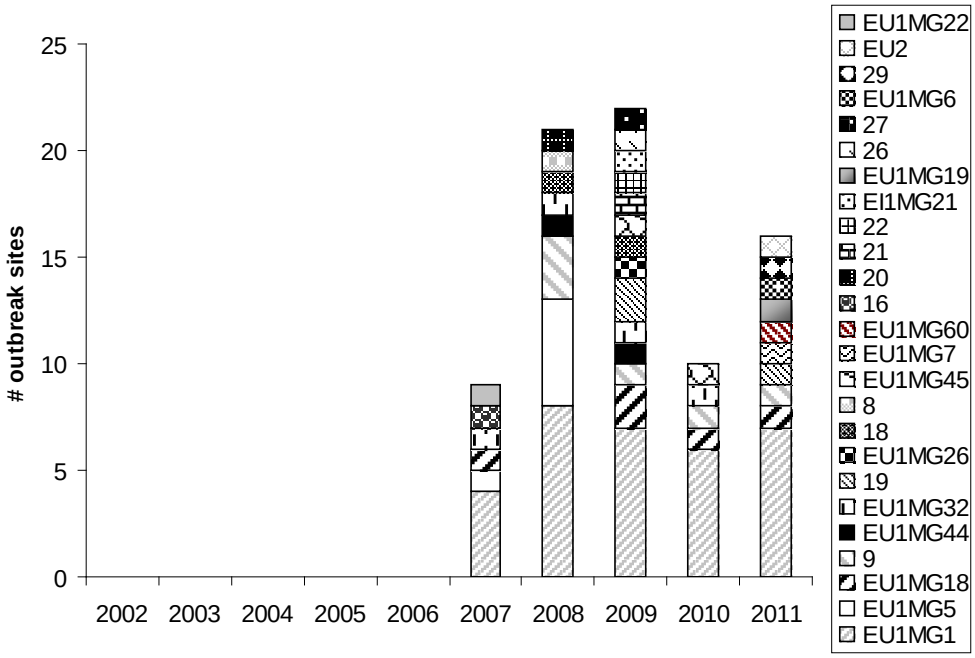

Figure 3—Genotypes detected in the managed environment. Genotypes unique to Scotland are indicated by simple numbers. Genotypes found elsewhere in Europe are named in accordance to European genotyping study as numbers with prefix "EU1MG" (K. Heungens, Institute for Agricultural and Fisheries Research, personal communication).

Discussion

All genotyping of European *P. ramorum* isolates so far has shown the limited diversity of a near clonal population (Ivors et al. 2006, Vercauteren et al. 2010), and the Scottish population is no exception. However, there are differences from the population in the rest of Europe. About half of all Scottish isolates belong to genotype EU1MG1. This is lower than on the European continent, where the percentage is 64 percent (K. Heungens, IVLO, personal communication). A few relatively common genotypes in Europe are very rare or absent in Scotland. On the other hand, 13 genotypes found in Scotland are not present in the rest of Europe. The Belgian study of the European *P. ramorum* population identified 66 genotypes amongst about 1,300 isolates (K. Heungens, IVLO, personal communication). However, approximately a sixth of the number of isolates resulted in the finding of 30 genotypes in Scotland. Taking the lower number of isolates into account, the genetic diversity appears higher in Scotland. The diversity of a population can be an indication of its age or a sign of lively exchange with other populations. The latter is unlikely in Scotland, situated on an island on the northern fringe of Europe and with limited horticultural trade. However, there are a number of historic gardens with rhododendron collections, and the area has a past tradition of plant hunting (and exchange). The apparently higher diversity might also be a consequence of the high sample density. The Scottish isolate collection encompassed isolates from almost every outbreak site in Scotland, with the exception of two or three minor sites. Moreover, depending on size and duration, multiple isolates were collected from each site. This nearly "complete" sampling increases the likelihood of finding rare genotypes, specifically the ones represented only by single isolates, which are easily missed when a smaller share of the population is sampled.

The limited distribution of the new EU2 lineage and the fact that, despite 10 years of surveys, it was only found in one site, suggests that it has only recently been introduced to Scotland. The origin of this new lineage and how it spread between Northern Ireland and Scotland is unclear and requires further research. The distance between the outbreak site in Scotland and the Northern Irish coast is

approximately 80 km. Rizzo et al. (2005) consider 3 km as the upper limit for natural dispersal, making human mediated spread more likely.

Long-distance dispersal of *P. ramorum* has been linked to the movement of infected plants in the horticultural trade, meaning a higher likelihood of the introduction of new genotypes into these businesses. Therefore, it might be expected that they have a higher genetic diversity than sites in the managed environment. That this is not the case in Scotland is probably thanks to the official eradication measures introduced immediately after the first findings of this disease. According to Vercauteren et al. (2010), "The human-induced bottleneck due to eradication efforts can lead to extinction of the least abundant genotypes." Many of the rare genotypes in nurseries appear to have been eradicated by phytosanitary measures. For example, Scottish genotypes 1, 6, and 15 found in 2002 were never detected again, neither in nurseries nor in the environment. Although rare genotypes are more susceptible to eradication and extinction, in some cases they can become the major genotype of a site, building a very specific local population. Scottish genotype 2, for example, is only present in one historic garden, but in this garden it has been found in 20 out of 21 isolates collected over 3 years.

The managed environment on the other hand shows a high degree of genotype diversity all throughout the years, with a high number of rare genotypes especially in the later years. Outbreaks in the environment are much harder to eradicate since the complete removal of infected plants often is not possible, giving rare genotypes a better chance of survival.

In general, there are two possible explanations when a variety of genotypes is found at the same outbreak site: they either are separate introductions of the pathogen or they have developed locally at the site. In this study, unique or very rare genotypes were found on some sites which were showing just one minor difference in the SSR profile to the main genotype at the same site (data not shown). The fact that these rare genotypes have not been found anywhere else can be taken as evidence that they developed locally within the site. Vercauteren et al. (2010) also found some evidence of local evolution of new genotypes in their study. Outbreaks in the semi-natural environment are often discovered later than outbreaks in the closely monitored plant trade and are much more difficult to eradicate. Often they last for years, providing more time for new genotypes to develop.

Acknowledgments

We thank Rowan Gray for providing excellent technical assistance. Special thanks go to Kurt Heungens, IVLO Belgium, for his valuable support in methodology and discussion of results.

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Genotypic Diversity of European *Phytophthora ramorum* Isolates Based on SSR Analysis[1]

Kris Van Poucke,[2] Annelies Vercauteren,[2] Martine Maes,[2] Sabine Werres,[3] and Kurt Heungens[2]

Abstract

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

In total, over 1,300 samples from 17 European countries were analyzed using seven EU1 polymorphic microsatellite loci. The majority of the samples were collected after 2001 from *Rhododendron* in nurseries. At least 66 EU1 genotypes were identified. Approximately 64 percent of the isolates belonged to multilocus genotype EU1MG1, which was present in all countries. Isolates with single repeat shifts in the most variable markers were the second most abundant and widespread, but at frequencies of less than 6 percent and in a maximum of 11 countries. As in the NA1 population, the structure of the genotype network is indicative of a clonal, expanding population that accumulated microsatellite mutations after a single introduction of the EU1MG1 genotype. The population structure and genetic diversity was similar in most of the countries represented by a sizeable number of samples. The population structure of the United Kingdom isolates is unique in that it is characterized by a large subpopulation with a unique mutation in one of the markers, a mutation that presumably arose relatively soon after the introduction of the pathogen in the United Kingdom. Based on the finding of unique genotypes in time and space, local evolution followed by national or international spread, was also observed for other countries. In general, however, the level of diversity was too small and the amount of international exchange was too extensive to draw detailed conclusions on the primary origin of specific isolates. There were no clear indications of sexual recombination within the EU1 lineage. Three isolates with highly deviating marker profiles were identified. Further analysis of these isolates revealed that they belong to a new (EU2) lineage, as detailed in a separate report (Van Poucke et al., EU2, a fourth evolutionary lineage of *Phytophthora ramorum*, this proceedings).

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Review Papers

Beyond ramorum - the Other Phytophthoras in Western Forests[1]

Everett Hansen[2]

Abstract

Little is known about indigenous *Phytophthora* species in natural ecosystems, although increasing evidence suggests that a diverse, trophically complex, *Phytophthora* community is important in many forests. In Oregon, 32+ *Phytophthora* species have been identified from forests. Globally, the number of described species has steadily increased, with a dramatic spike in recent years as new species have been split from old and other species have been discovered through exploration of new forest habitats triggered by the *P. ramorum* epidemics. In Oregon, almost two-thirds of the species were recently described. Forest soil, streams, and the upper canopies of trees are now being explored for *Phytophthora* diversity, and a new appreciation for the ecological amplitude of the genus is emerging. Three ecological assemblages, or guilds, of *Phytophthora* species can be distinguished in this accumulated work (fig. 1). Around the world, 10 to 15 species are regularly identified in surveys of temperate forest soils. Taxa in ITS clade 6 are especially numerous in forest streams and may be saprophytic in this habitat. In the upper canopies of trees in western tanoak forests, three or four *Phytophthora* species (including *P. ramorum*) with similar pathogenic behavior are encountered. Species from all three habitats—soil, streams, and upper canopies—occasionally cause lethal bole cankers on trees. A very few become invasive under circumstances we cannot yet predict.

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

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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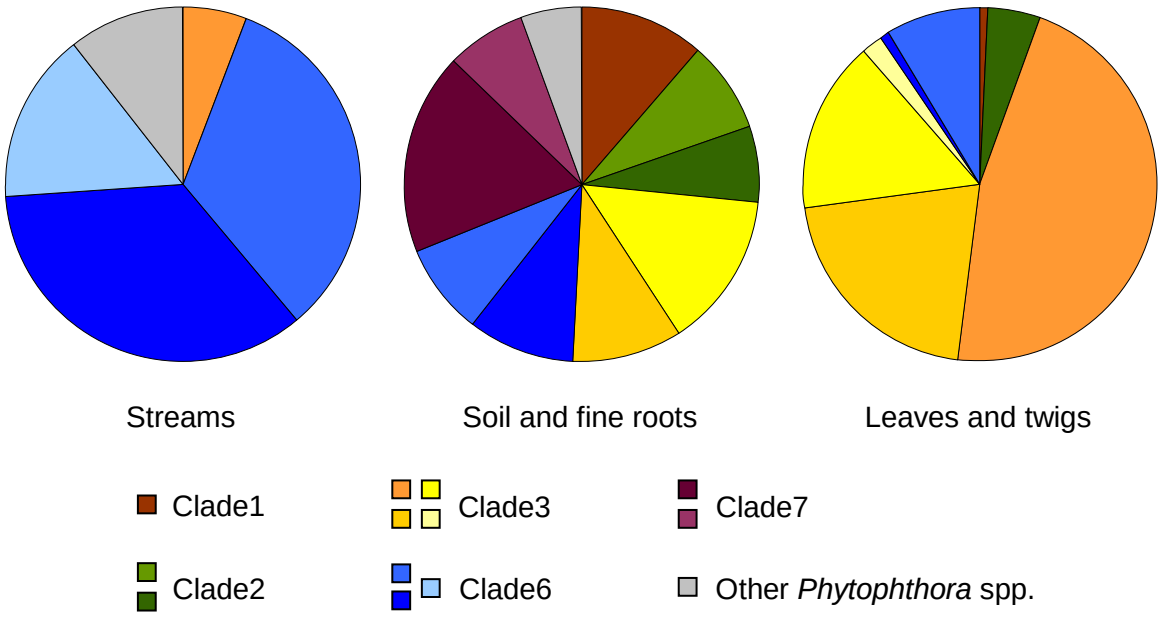

Figure 1—Clades associated with three ecological assemblages of *Phytophthora* species.

Landscape Epidemiology of Emerging Infectious Diseases in Natural and Human-Altered Ecosystems[1]

Ross K. Meentemeyer,[2] Sarah Haas,[2] and Tomáš Václavík[3]

Abstract

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

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Process and Pattern in the Emergence of *Phytophthora ramorum*[1]

Niklaus J. Grünwald,[2] Matteo Garbelotto,[3] Erica M. Goss,[4] Kurt Heungens,[5] and Simone Prospero[6]

The invasive sudden oak death pathogen, *Phytophthora ramorum*, has emerged repeatedly since its first detection in the 1990s in the United States and Europe. This paper will explore recent research by several groups published in the review by Grünwald et al. (Grünwald, N.J.; Garbelotto, M.; Goss, E.M.; Heungens, K.; Prospero, S. 2012. Emergence of the sudden oak death pathogen *Phytophthora ramorum*. Trends in Microbiology. 20: 131–138.), documenting the patterns observed and mechanisms inferred to explain these patterns. Briefly, four distinct clonal lineages are currently recognized, NA1, NA2, EU1, and EU2 named consecutively after the continent of origin on which they were first found, North America (NA) and Europe (EU). While the three clonal lineages NA1, NA2, and EU1 are found in Canada and the United States, Europe, to date, has the EU1 and EU2 clonal lineage. Detailed phylogeographic analysis has documented that the introduction of the NA1, NA2, and EU1 clonal lineages originated from separate populations. The status of the EU2 lineage has to date not been investigated using these evolutionary approaches, but the linage appears to be distinct and clearly evolutionarily diverged from the three other lineages (Van Poucke, K.; Franceschini, S.; Webber, J.F.; Vercauteren, A.; Turner, J.A.; McCracken, A.R.; Heungens, K.; Brasier, C.M. 2012. Discovery of a fourth evolutionary lineage of *Phytophthora ramorum*: EU2. Fungal Biology 116: 1178–1191.).

The NA1 lineage was introduced into California from an unknown source population, while the NA2 and EU1 lineages were introduced into the Pacific Northwest (either British Columbia or Washington). While the source population for the NA2 introduction remains to be established, coalescent analysis supported introduction of the EU1 lineage into North America from Europe. The EU2 lineage was first found in Northern Ireland and more recently in Western Scotland and the source population remains to be established (Van Poucke et al. 2012). Further emergence of *P. ramorum* lineages is likely, given the observed repeated emergence of *P. ramorum* over the last 2 decades, and appears to be driven by shipment of infested nursery material.

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Waterways and Monitoring

New Insights into the Ecology of *Phytophthora ramorum* in Streams[1]

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

Abstract

Many *Phytophthora* species, including *Phytophthora ramorum*, have been reported from surface waters such as canals, streams, rivers, ponds, and reservoirs, often in association with infested agricultural or natural landscapes (Hong and Moorman 2005). *Phytophthora* species are recovered with regularity and abundance from streams and rivers, in many cases in the absence of apparent terrestrial infestations (Hwang et al. 2010, Reeser et al. 2011, Sutton et al. 2009), or under conditions not conducive to sporulation in the landscape in the case of California summers. This strongly suggests that these organisms can complete their life cycle in aquatic environments, yet the biological underpinnings of this phenomenon remain effectively unknown. What *Phytophthora* propagules occur in surface waters? How frequent and widespread are they in such environments? From what substrates do they originate? What is their potential to infect plant material in this environment? To address these questions, we have undertaken experiments focusing on *P. ramorum* in both naturally infested California streams and controlled environments. The persistence of such plant pathogens in water bodies has significant implications for their spread and management in both agricultural and more natural contexts.

In the absence of terrestrial run-off, *Phytophthora* inoculum in waterways may originate from infections of leaf litter or aquatic plants. Though fresh leaf litter can serve as a transient substrate for growth and reproduction of *Phytophthora* spp. in streams, most vegetative litter quickly degrades in an aquatic environment. To determine the potential of leaf litter as a substrate for *P. ramorum* in streams, we exposed freshly picked rhododendron leaves, as well as those killed by drying or freezing, to natural inoculum in infested streams and additionally to laboratory-produced inoculum in controlled environment experiments. Baits were deployed monthly during peak pathogen activity from January to June in each of 2 years. *Phytophthora ramorum* was recovered by culturing from 62 percent of fresh leaves, but only 6 percent and 2 percent of frozen and dried leaves, respectively (n=298 per treatment). In laboratory tests, we incubated fresh, frozen, and dried leaves separately in cups of water inoculated with *P. ramorum* sporangia or zoospores for 7 days at 16 ^{o}C. Measuring the proportion colonized by sampling the surface of each leaf in a mosaic pattern, we found that *P. ramorum* colonized an average of 82 percent of fresh rhododendron leaf surfaces, but no more than an average of 30 percent for frozen and dried leaves (n=12 per treatment). Furthermore, when *P. ramorum* was incubated in similar laboratory experiments with spores of *Phytophthora gonapodyides*, a primarily saprobic, aquatic species, *P. ramorum* colonized an average of 68 percent, 10 percent, and 3 percent, while *P. gonapodyides* was recovered from an average of 0 percent, 78 percent, and 58 percent of fresh, frozen and dried leaves, respectively (n=12 per treatment), suggesting competition between the two species. These results indicated that *P. ramorum* may be limited biologically and ecologically from colonizing degraded leaf litter in aquatic environments.

We also examined colonization of naturally occurring leaf litter in an infested stream through mosaic sampling of individual leaves. *Phytophthora ramorum* was primarily recovered from bay laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.), and never from coast live oak (*Quercus agrifolia* Née) leaf litter. In one instance, it was recovered from alder *(Alnus* sp.) leaves from the stream. A portion of bay laurel litter leaves were sorted either as fresh (some green tissue, more supple) or degraded (mostly brown and thin). Both litter types were colonized by *P. ramorum* at similar levels. It is therefore possible that *P. ramorum* may persist on infected leaves as they degrade, even if it is not effective at colonizing already degraded leaves.

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[1] A version of the paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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To determine if *P. ramorum* propagules can adhere passively to substrates in streams, we incubated glass microscope slides in two naturally infested streams. Colonies of *P. ramorum* and *P. gonapodyides* were recovered from glass slides exposed in streams by submerging them into cooling selective media before it solidified, indicating that passive adherence of propagules to substrates in streams occurs.

Aquatic and riparian plants were evaluated as a potential source of *P. ramorum* inoculum. More than 100 specimens of aquatic plants representing more than 20 families were collected from within and near the edge of streams where *P. ramorum* had regularly been detected in the water, but not on the landscape. They included entire plants, submerged branches (sometimes rooting in the stream), or roots only. Both herbaceous and woody plants were sampled. Specimens were surface sterilized in 5 percent household bleach for 2 minutes, then kept in a few cm of water in partially sealed plastic bags, covered with a large plastic bag to keep in moisture, and incubated in a growth chamber at 16/12 ^{o}C, corresponding to 14/10 hours light/dark. We maintained plant material for 3 to 5 days with all but a little water drained from the containing bags to produce conditions conducive to sporulation prior to flooding and baiting them with rhododendron leaves. *P. gonapodyides* and other unidentified Pythiaceous species were baited from some plants, but *P. ramorum* was not recovered from any of the specimens.

Nonetheless, we found by direct isolation that leaves of chain fern (*Woodwardia fimbriata* Sm.) naturally submerged in an infested stream were infected with *P. ramorum* and *P. gonapodyides*. However, recovery from this previously unreported host was irregular and it is uncertain if such infections contribute significant inoculum to the stream.

These results suggest a complex ecological picture in regard to leaf litter and aquatic plants as potential substrates for the persistence and reproduction of *P. ramorum* in streams. While *P. ramorum* seems incapable of significant colonization of degraded leaf litter, it may persist on previously colonized leaves as they degrade further. The isolation of *P. ramorum* from a non-living substrate such as glass shows that spores are abundant and passively encounter and adhere to substrates. Further study is needed to determine the importance of infected leaf litter in dispersing and supporting spore loads downstream of infested terrestrial areas. It remains to be determined whether infections of typically non-host species, such as alder, can play a role in sustaining an active presence of *P. ramorum* in aquatic environments. None of the aquatic plants that were kept in growth chambers were infected by *P. ramorum* at a level detectable by baiting, though the detection of other *Phytophthora* spp. suggests that the method was effective. Further work is underway to test the specimens with direct inoculation. The isolation of *P. ramorum* and *P. gonapodyides* from chain fern shows that aquatic plants, like leaf litter or bait, can become infected in streams, but their significance as an inoculum source may be limited or transient. Nonetheless, the presence of such transitory substrates such as leaf litter and submerged leaves of aquatic plants may play a role in the seasonal fluctuation of spore loads observed in California streams. Bay laurel leaves are often shed in abundance in late spring and rising water levels often submerge drooping leaves of riparian vegetation. Such substrates may become mostly exhausted later in the summer by which time detection of *P. ramorum* in streams also declines.

Acknowledgments

For information and field work support, we thank Maia Beh, Radek Glebocki, Chris Lee, and Yana Valachovic at Humboldt County UC Cooperative Extension and Leonel Arguello at Redwood National Park. Thanks for technical support in the field and laboratory to Vitor Fontes, Ashley Hawkins, Michael Jones, and Heather Mehl. This work was funded by grants from the USDA Forest Service, Pacific Southwest Research Station, USDA Forest Service, Forest Health Protection, State and Private Forestry and the Gordon and Betty Moore Foundation.

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Eliminating *Phytophthora* spp. From Stream Water Throughout the Year With Algaecides[1]

Inga M. Meadows,[2] Jaesoon Hwang,[3] and Steven N. Jeffers[3]

Abstract

Due to the aquatic nature of oomycetes, *Phytophthora* spp. can be found in a wide variety of waterways in and around natural and agricultural ecosystems—including forest streams, urban streams, and irrigation ponds. They are disseminated effectively and efficiently in flowing water, so *Phytophthora* spp. can be moved readily from an infested area (e.g., a nursery) to nearby non-infested environments if waterways in the infested area become contaminated with propagules of the pathogen. This type of dissemination—particularly for exotic species like *P. ramorum*—poses a serious threat to plants in urban and natural environments that are in the vicinity of an infested area. General biocides such as chlorine, hydrogen dioxide, ozone, and UV radiation currently are used to eliminate pathogens (including *Phytophthora* spp.) from water. Different types of filtration systems utilizing both physical and biological barriers also are being used, but mainly for irrigation water. However, these options usually are not practical or feasible when an immediate eradication of a recently introduced pathogen is required. Oomycetes are closely related to brown algae. In previous research, we demonstrated that algaecides, particularly those with copper-based active ingredients, are very effective at eliminating *Phytophthora* spp. from artificially and naturally infested water samples. All of the algaecides tested are registered for use in a variety of waterways—including agricultural irrigation systems, fish ponds and hatcheries, fresh water lakes, and potable water reservoirs. Previous studies demonstrated that there was seasonal variation in the distribution of *Phytophthora* spp. and densities of these species in streams. Therefore, we examined the ability of commercial algaecides to eliminate *Phytophthora* spp. naturally occurring in two streams throughout 2010.

In January 2010, we initiated a year-long study to investigate the efficacy of four commercial algaecides with different active ingredients at eliminating naturally occurring propagules of *Phytophthora* spp.in two streams in western South Carolina at monthly intervals. The experiment was conducted in both streams each month, except in January, when it only was conducted in one stream. The four algaecides were applied at the highest recommended label rate: 1 ppm of copper hydroxide (K-Tea™; SePRO Corporation, Carmel, IN), 0.8 ppm of copper carbonate (Captain™; SePRO Corp.), 1 ppm of copper from chelates of copper gluconate and copper citrate (Algimycin®-PWF; Applied Biochemists, Germantown, WI), and 25 ppm of hydrogen dioxide (GreenClean® Liquid; BioSafe Systems, LLC, East Hartford, CT). Each month at each stream, 10 L of stream water was placed in each of fifteen 20-L buckets; three buckets were treated with each of the four algaecides, and three buckets were not treated (controls). All buckets remained in the stream to maintain ambient temperature of the stream water (fig. 1). Before the water was treated (0 hours) and at 2 and 4 hours after treatment, three aliquots of 200 ml of water were removed from each bucket, and each aliquot was passed through a Durapore® membrane filter with 5 µm pores (fig. 2). Membranes were inverted onto PARPH-V8 selective medium to detect propagules of *Phytophthora* spp. Isolation plates with membranes were returned to the laboratory and kept in the dark at 20°C for 3 days; then mean numbers of colony-forming units (cfu) in the 200-ml aliquots (i.e., propagule densities) were determined.

In a supplemental study, we evaluated the efficacy of each algaecide at two lower rates to determine the minimum amount of copper needed to eliminate propagules of *Phytophthora* spp. Reduced rates were: Algimycin®, 0.25 ppm (low) and 0.5 ppm (medium); Captain™, 0.2 ppm (low) and 0.4 ppm (medium); K-Tea™, 0.25 ppm (low) and 0.5 ppm (medium); and GreenClean® Liquid, 1 ppm (low) and 10 ppm (medium). The non-treated and high-rate treatments were those used in the study described above. At each sampling period from April to August, an additional six 20-L buckets were filled with 10 L of stream water (fig. 1); three buckets were treated with one algaecide at the low rate; and three buckets were treated with the same algaecide at the medium rate. Three 200-ml

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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aliquots were assayed from each bucket at 0, 2, and 4 hours after treatment as described above. Each reduced rate was tested twice during the year, once in each stream.

Propagules of *Phytophthora* spp. were detected in both streams in all months and in all non-treated water samples. In one stream, mean propagule densities in non-treated water at 0 hours ranged from 1.1 to 30 cfu (mean 18.6 cfu); 30 cfu was the maximum number that could be counted with accuracy, so actual densities may have been greater. In the other stream, mean propagule densities ranged from 1.2 to 24.8 cfu (mean 8.3 cfu). The three copper-based algaecides completely eliminated propagules of *Phytophthora* spp. from water at 2 hours after treatment, so no propagules were detected at either 2 or 4 hours. GreenClean® Liquid, an algaecide that does not have a copper-based active ingredient, was not as consistent and somewhat less effective. In one stream, propagules were detected in March (0.7 cfu) and May (2.6 cfu) at 2 hours after treatment and in May (1.6 cfu) and June (0.1 cfu) at 4 hours after treatment. In the other stream, propagules were detected in five monthly samples (0.8 to 2.5 cfu) at 2 hours after treatment and in four monthly samples (0.1 to 0.6 cfu) at 4 hours after treatment. Propagules were not detected in any other water sample treated with GreenClean® Liquid.

In the supplemental study, reduced rates of the copper-based algaecides were as effective as the maximum rate at eliminating propagules of *Phytophthora* spp. from naturally infested stream water; no propagules were detected after treatment with the low and medium rates of the three copper-based algaecides. However, GreenClean® Liquid was not as effective; propagules were not completely eliminated by reduced rates, and propagule density was reduced significantly only by the medium rate. Therefore, lower rates of the copper-based algaecides appear to be effective at eliminating propagules of *Phytophthora* spp. from water, but GreenClean® Liquid was not effective when applied at the reduced rates used in this study. In summary, algaecides continue to show promise as management tools for eliminating propagules of *Phytophthora* spp. from naturally infested water.

Figure 1—Twenty-one buckets, each containing 10 L of stream water treated with an algaecide or not treated (control), sitting in a stream to maintain ambient water temperature. Fifteen buckets were used to compare efficacy of four algaecides and a non-treated control, and six buckets were used to evaluate reduced rates of one algaecide. Three replicate buckets were used for each treatment, and buckets were arranged in a completely randomized design.

Figure 2—Each month, all water sub-samples were processed at the stream site to maintain sample integrity and prevent potential propagule degradation during storage and transit.

Comparison of *In Situ* and *In Vitro* Baiting Assays for *Phytophthora ramorum* Survey of Waterways in the Southeastern United States[1]

Steven Oak,[2] Jaesoon Hwang,[3] and Steven Jeffers[3]

Abstract

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

In situ baiting was conducted according to the established national survey protocol, with three baiting periods during each of the spring and autumn seasons (six in all). Initiation of spring baiting varied with latitude and surveyor scheduling, ranging from February 14 to March 23, and was concluded by May 2. Fall baiting was conducted between September 21 and December 2. Eight collections of water samples were made for the *in vitro* assay—at the same time leaves were deployed or retrieved for *in situ* baiting. For the *in vitro* assay, two 800 ml water samples were collected in 100 ml aliquots, and each sample was placed in a 1 L Nalgene screw-top bottle. Each sample was baited immediately with 20 freshly cut leaf pieces and one whole, asymptomatic, non-wounded leaf of forest-grown *Rhododendron maximum* L. Bottles were capped, placed on their sides, and held for 3 days at 18 to 22 °C in the dark. Baits then were removed, rinsed in distilled water, and blotted dry. Leaf pieces were processed immediately for detection, while whole leaves were placed in moist chambers for up to 14 days to allow lesion development. Two detection methods were used for both assays—isolation on selective PARPH-V8 medium and nested or real-time PCR. Relative assay performance was determined by comparing *P. ramorum* detection results for sample sets collected at the same time.

There were 72 total cases for comparison of relative assay performance possible (12 sites x six baiting periods). However, site F1 was available for sampling only in the first spring baiting period, leaving 67 comparable cases. *Phytophthora ramorum* was recovered by one or both assays at least once during the year in 11 of 12 waterways surveyed (fig. 1), with more than double the detections occurring during spring (31) than autumn (15). *Phytophthora ramorum* was recovered by one or both assays in 32 of these cases (48 percent). Out of the 32 positive cases, the pathogen was recovered by both assays in 14 cases (44 percent), while each assay alone recovered the pathogen in nine cases (28 percent).

Pathogen recovery by each baiting assay differed considerably by season. During spring, there were 20 comparable cases in which *P. ramorum* was recovered by one or both assays. *In vitro* baiting recovered the pathogen in seven of these cases without corroboration by the *in situ* assay, while *in situ* baiting recovered the pathogen in two cases without *in vitro* corroboration. The pathogen was recovered by both assays in 11 additional cases. Relative performance of the two assays was exactly the inverse during autumn: two pathogen

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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recoveries by *in vitro* baiting only, seven recoveries by *in situ* baiting only, and three recoveries by both methods.

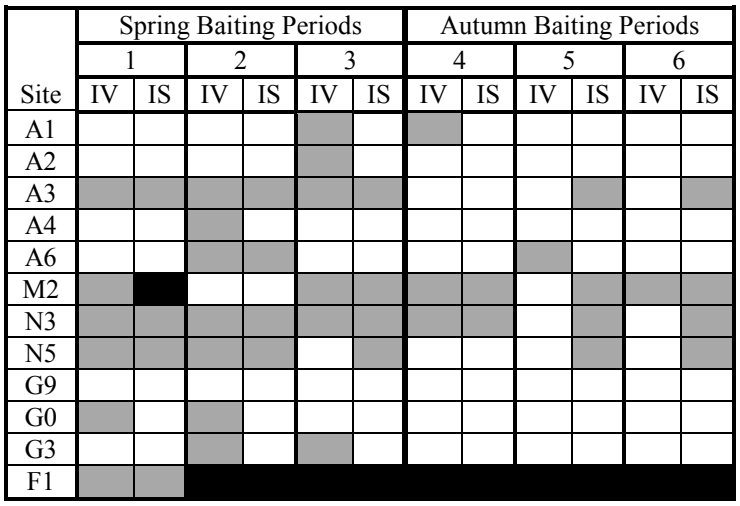

Figure 1—*Phytophthora ramorum* recovery for 12 waterways in the southeastern United States by baiting season and assay method (IV = *in vitro*; IS = *in situ*). Shaded cells indicate *P. ramorum* positive; blackened cells indicate baits unavailable.

Despite seasonal differences, *in vi*tro baiting was equal to *in situ* baiting for detection of *P. ramorum* in water during the 2011 baiting year, and demonstrated superiority in several instances. The detection of the pathogen by *in vitro* baiting was the first ever at site A1, despite 29 *in situ* baiting periods over 5 years. *In situ* baits were deployed, but lost due to flooding, during the first spring baiting period at site M2. However, *in vitro* samples could be safely collected and *P. ramorum* was recovered. Most importantly, *P. ramorum* would have escaped detection altogether at five sites had the *in vitro* assay not been used (A1, A2, A4, G0, and G3). There were no sites where *in situ* baiting demonstrated this advantage.

In situ baiting has proven effective for recovery of *P. ramorum* from water since 2006. Even though the *in vitro* assay samples a very small volume of water at only one point in time relative to *in situ* baiting, this did not prove to be disadvantageous for pathogen recovery in this survey. This fact suggests that waters draining infested areas in the eastern United States contain inoculum at detectable densities most of the time, at least during our spring baiting season. *In vitro* methods allow the sampling of intermittent waters, such as ephemeral drainages and puddled irrigation water in ornamental crop nurseries suspected of containing infested plants, as well as perennial streams in a variety of settings where *P. ramorum* has been introduced. Plans are in place to repeat this comparison survey at these sites in 2012, and expand it to include west coast forest and nursery sites. Changes to the national survey protocol will be considered if results prove repeatable.

Nurseries

Pathways of Spread of *Phytophthora ramorum* in a Simulated Nursery Setting: An Update[1]

Kurt Heungens,[2] Bjorn Gehesquière,[2] Kris Van Poucke,[2] Annelies Vercauteren,[2] and Martine Maes[2]

Abstract

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

Experiments were conducted at a mock nursery plot under specific biosafety conditions. The plot was lined with an impermeable film, a common practice in potted plant production in Europe. Rhododendron was chosen as the test plant due to its susceptibility and its prevalence as a nursery host for *P. ramorum* in Europe. Pathogen dispersal was monitored from individual infected potted plants placed in the middle of a circle of healthy detector plants. The rate at which the disease spread onto the detector plants was monitored in replicated experiments. Indirect splash dispersal (via the water film on the plastic ground cover and back to the leaves), as well as direct aboveground plant-to-plant dispersal (via air or via leaf-to-leaf splashing), were investigated by selective physical blocking of such pathways.

Indirect dispersal via the drain water film was at least as important as direct dispersal. Contamination of the drain water film was confirmed using leaf baits and direct PCR-mediated detection at significant distances from the source plants. This demonstrated that indirect spread via the water film could take place over larger distances than direct dispersal. Movement of the pathogen from the water film into the root ball of detector plants was demonstrated using leaf baiting of the root balls, combined with physical blocking of this pathway.

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

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Effect of Oomatistatic Compounds and Biological Control Agents on Production of Inoculum and Root Colonization of Plants Infected With *Phytophthora ramorum*[1]

Nina Shishkoff[2]

Abstract

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

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Determining the Risk of *Phytophthora ramorum* Spread From Nurseries Via Waterways[1]

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

Abstract

Phytophthora ramorum, the fungus-like pathogen which causes sudden oak death, is a threat to the Pacific Northwest nursery industry. Because this is a quarantine organism, the destruction of plants and mitigation treatments resulting from a positive *P. ramorum* detection has caused millions of dollars in losses to the commercial nursery industry in California, Oregon, and Washington. There is concern about movement of the pathogen to nurseries and forests in the eastern United States. An increase has been seen in the NA2 and EU1 lineages from nursery samples in Washington in recent years, so a study of the relative fitness of *P. ramorum* isolates in the Washington State University culture collection was undertaken. Eighty-five isolates were screened for sensitivity to the fungicide mefenoxam and for relative pathogenicity on detached rhododendron leaves. Most isolates of *P. ramorum* were sensitive to the fungicide with the exception of some EU1 isolates from one nursery and its trace-forwards. A strong relationship between phenotypic characteristics such as fungicide sensitivity and pathogenicity, and the originating nursery, was seen. Since *P. ramorum* is moving from nurseries into streams, a method for exposing plants to contaminated stream water was tested. Further studies will include measuring inoculum levels in irrigation water from streams to determine whether this pathway is of importance in disease spread.

Keywords: *Phytophthora ramorum*, nursery industry, mefenoxam sensitivity

Introduction

Long-distance spread of *Phytophthora ramorum* occurs mainly by movement of infected nursery stock, and some countries have imposed quarantines to reduce further spread (EPPO 2006). *Phytophthora ramorum* was first detected in a California nursery in 2001, and in nurseries in California, Oregon, Washington, and British Columbia in 2003. In 2004, a shipment of camellias from a California nursery resulted in the spread of *P. ramorum* to nurseries across the United States and in British Columbia, Canada. Since then, infected ornamental nursery stock has been detected in 48 nurseries in Washington State, as well as throughout the United States and British Columbia. Three of the four genetic lineages of *P. ramorum* have been found in Washington nurseries. When *P. ramorum* is detected in a nursery, measures are taken to eradicate the pathogen. This can result in a financial burden to the nursery and some have gone out of business (Dart and Chastagner 2007).

In some cases, *P. ramorum* is not completely eradicated from a nursery, and soil and water becomes contaminated. The pathogen has spread from nurseries to adjacent waterways in several locations in Washington. Once infested, streams remain positive for *P. ramorum*, even after mitigation steps have been taken at the nursery and the pathogen can no longer be detected at the nursery site. The movement of an isolate from the NA2 lineage of *P. ramorum* to salal (*Gaultheria shallon* Pursh) and soil outside of a Washington nursery via contaminated water in 2009 and 2010 illustrates the importance of this pathway as a means of spreading *Phytophthora* pathogens from nurseries to plants and soil in the landscape. In another case, *P. ramorum* was detected once on riparian willow plants outside of a nursery in Mississippi, although subsequent sampling failed to recover the pathogen (Jeffers 2011). Other than the single incidents in Washington and Mississippi,

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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the pathogen has not spread from streams to vegetation, even in areas with forest infestations in California and Oregon.

Mefenoxam (Metalaxyl-M®, Subdue MAXX®) is one of the most commonly used chemicals for controlling *Phytophthora* species on ornamentals and other crops. It has been shown to be effective when used preventively against *P. ramorum* and is inhibitory to both mycelial growth and sporulation. Unfortunately, resistance to this chemical can develop in *Phytophthora* spp., including *P. ramorum* (Wagner et al. 2007). In some studies, it has been shown that metalaxyl resistant (MR) isolates of *Phytophthora* are more fit than metalaxyl sensitive (MS) isolates. These isolates tended to be more aggressive on host material, have a higher infection rate, and increased sporangia and oospore production than MS isolates (Chycoski and Punja 1996, Hu et al. 2008, Mukalazi et al. 2001). Exposure to sublethal doses of mefenoxam and other fungicides induces a switch in mating type, which could lead to sexual recombination and the production of new genotypes (Groves and Ristaino 2000).

In recent years, the EU1 and NA2 lineages of *P. ramorum* have been found more frequently during Washington nursery inspections, and the NA1 lineage less often. In this study, we examined some isolates of *P. ramorum* to determine whether there was a difference in fitness among isolates that might explain this trend. We also tested a method for determining whether inoculum levels in streams were sufficient to cause infection on plants irrigated with stream water.

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

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

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

Isolates were considered to be sensitive to the fungicide if there was scant or no growth at 1 ppm active ingredient and resistant if there was significant growth at 1 ppm. Only a few of the isolates tested showed resistance to mefenoxam, and these belonged to the EU1 lineage and originated from Nursery #41 and its trace forwards.

When the relationship between pathogenicity and fungicide sensitivity was examined, three groups were observed: isolates showing some resistance to the fungicide, isolates with low pathogenicity, and the remaining isolates not having characteristics of the other two groups (fig. 1). The fungicide-resistant group was composed entirely of isolates from the EU1 lineage and originated from Nursery #41. The isolates of low pathogenicity were mostly of the NA1 lineage, with two from the co-mingled EU1/NA2 samples and one EU1 isolate from Nursery #44. Most of the weak NA1 isolates originated from Nursery #35. A positive relationship between pathogenicity and fungicide sensitivity was seen when the first two groups were removed from the analysis ($R^2 = 0.3877$), suggesting that there may be some trade-offs between fungicide resistance and pathogenicity. However, the EU1 isolates showing resistance were some of the most aggressive. Preliminary spore counts show that the NA2 isolate taken from the salal plant outside of Nursery #45 and an EU1 isolate from Nursery #41 are the most prolific sporulators, and this may explain their persistence in soil and water and ability to

spread. Likewise, there has not been spread from Nursery #35, although it has been found positive multiple times. NA1 isolates from this nursery were less fit than NA1 isolates collected from other nurseries.

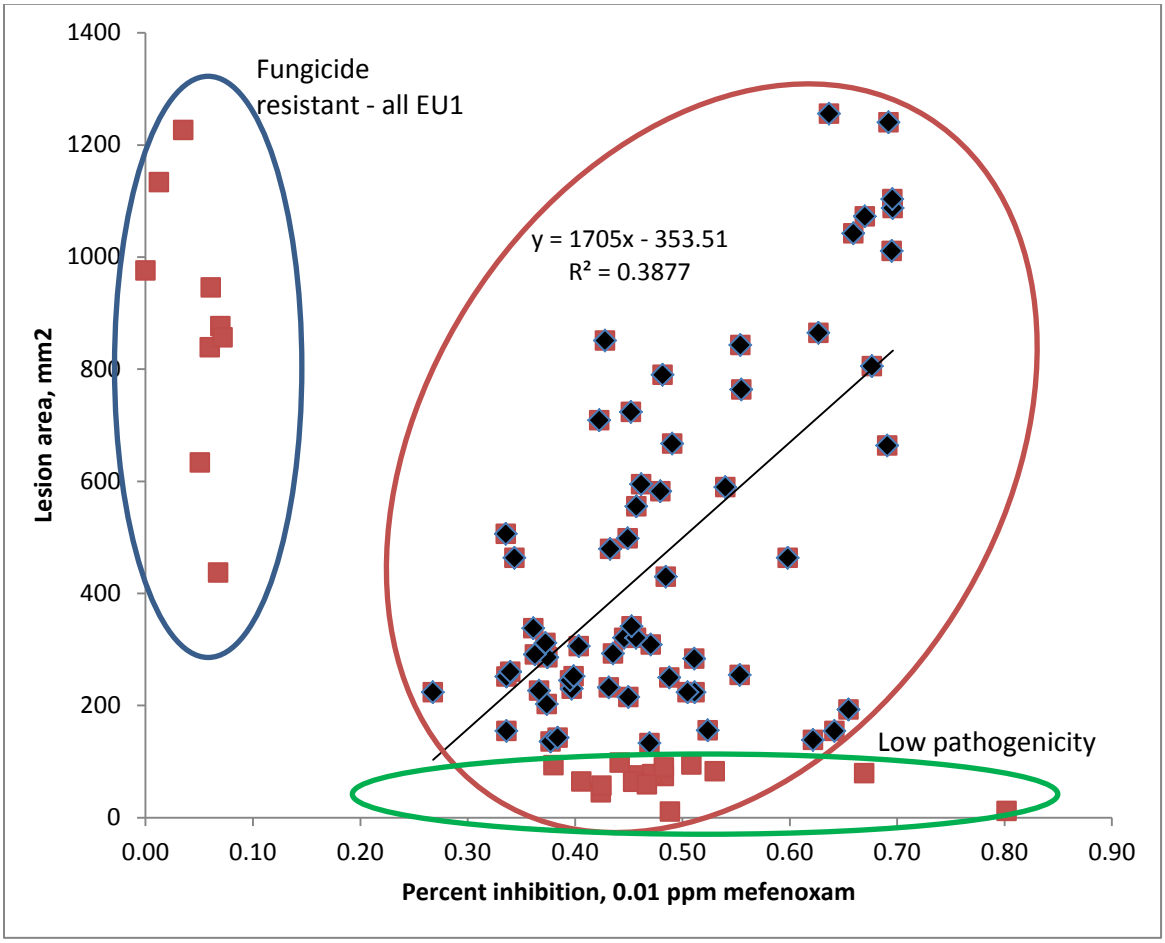

Figure 1—Scatter plot showing relationship between pathogenicity (lesion area on rhododendron leaves) and fungicide tolerance (percent inhibition on 0.01 percent mefenoxam) for 85 isolates of *Phytophthora ramorum* collected from Washington nurseries and other locations.

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

Nationally, there is concern about the potential risk of spreading *P. ramorum* via the irrigation of plants with infested water. Although research in California and Europe has shown that plants in nurseries can be infected when overhead irrigated with water infested with *P. ramorum*, it is unknown what levels of inoculum are present in streams in Washington and how much is needed for infection of plants or infestation of soil to occur. Being able to quantify inoculum levels in waterways and understand the inoculum threshold necessary for infection will assist the nursery industry and regulatory agencies in making decisions about the level of risk in using *P. ramorum* infested water for irrigation in Washington State.

To test whether plants overhead-irrigated with contaminated stream water would become infected, a "shower" apparatus was constructed (fig. 2). A bilge pump was attached to a floating platform and pumped water through a sprinkler onto potted rhododendron plants. Humidity was maintained by

enclosing the plants in the fiberglass "shower stall." A timer was included to allow for irrigation at predetermined intervals, and the apparatus was powered using a car battery.

The "shower" was first tested in the biocontainment unit at WSU-Puyallup. Potted rhododendron seedlings were placed in the chamber and overhead-irrigated with a zoospore suspension of *P. ramorum* at a concentration of 1 x 10^4 zoospores/ml. After 4 weeks, the plants were sampled for *P. ramorum* by culturing and by qPCR. A small amount of *P. ramorum* DNA was present on three samples, one sample was positive for *P. ramorum* in culture, and only one plant had symptoms of *P. ramorum* infection. More than 80 percent of soil and root baits were positive for *P. ramorum*. In this small study, soil and roots had very high levels of colonization by *P. ramorum* after overhead irrigation. Plants had very low levels of infection. The apparatus was then deployed at a site where leaf baiting has been positive for *P. ramorum* in 2009 and 2010. Rhododendron plants were placed into the shower and bait bags were deployed upstream from the pump for three, 1-week intervals in June 2011. After exposure, plants were taken to WSU-Puyallup and placed in the biocontainment unit for 4 to 6 weeks. All samples were negative for *P. ramorum* using culturing methods and qPCR. *Phytophthora ramorum* was found in the stream earlier in the year by the Washington State Department of Natural Resources, but possibly June was too late for detection. Several other Oomycetes were isolated from leaf baits and identified using DNA sequence analysis. Based on these preliminary results, it appears that inoculum levels of *P. ramorum* were very low or nonexistent in the stream that was sampled, or that it was too late in the season to detect it. Water temperature in the ditch was 15 ˚C at deployment of the shower, which is in the range for *P. ramorum* growth. However, other Oomycete species present may be more competitive than *P. ramorum* under these conditions. Plans are underway to use the shower apparatus in addition to baiting and PCR methods for quantifying *P. ramorum* inoculum in streams and to determine whether these inoculum levels are sufficient to cause disease on plants.

Figure 2—"Shower" apparatus for exposing plants to *Phytophthora ramorum* inoculum in stream water. Potted rhododendron plants were placed inside the shower and overhead irrigated with water, which was pumped from the stream at three 1-minute intervals programmed into the timer. Plants were exposed for 1 week, after which time they were taken to the biocontainment facility at Washington State University-Puyallup for incubation.

Conclusions

From this study, we have determined that there are considerable differences in fitness of *P. ramorum* isolates both within and between clonal lineages. Pathogenicity and fungicide screening of additional isolates collected from Washington nurseries is ongoing, as are further tests of fitness, including sporulation potential of selected isolates.

Determining the critical level of *P. ramorum* inoculum to cause infection from irrigation water is also underway. In the laboratory study, very little aboveground infection was seen at the high-inoculum level used, but root and soil baiting was almost 100 percent positive. The lack of *P. ramorum* found in the small field test could be due to a number of factors, such as absence or extremely low inoculum concentration, competition by other organisms, and water quality parameters.

Acknowledgments

The authors wish to thank Washington State Department of Agriculture (WSDA), Washington State Department of Natural Resources (WADNR), and the USDA Forest Service for their cooperation and financial support; and our students, volunteers, and technicians for their help in the lab and field.

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How Does *Phytophthora ramorum* Infect Rhododendron Leaves?[1]

Sabine Werres,[2] Marko Riedel[3]

Abstract

In most parts of Europe, rhododendron is the most important host for the spread of *Phytophthora ramorum*. To get a better knowledge of leaf infection and capacity for sporulation, infection studies were carried out. Detached leaves of the *Rhododendron* cultivar 'Catawbiense Grandiflorum' (CG) and the *R. insigne* hybrid `Brigitte´ (B) were inoculated with a zoospore suspension of a *P. ramorum* isolate of mating type A1 and A2, respectively. The CG developed much greater leaf necrosis than B (average after 55 days 106.5 mm^2 versus 0.12 mm^2). The trichomes on the B leaves seemed to prevent the germ tubes from detecting the stomata. *Phytophthora ramorum* zoospore germ tubes invaded the leaf tissue via the stomata. Appressoria-like structures could be observed. On the infected leaves, new hyphae grew out of the stomata. Sporangia and chlamydospores that developed on the mycelium originated from the applied zoospores as well as on the hyphae growing out of the stomata after infection. They could be observed mainly on the necrotic leaf areas. Only single oospores developed within 12 days on the 'Brigitte' leaves.

The study was funded by the U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. The detailed data from this study, and additional studies from K. McKeever, M. Elliott, and S.F. Shamoun, are published at http://journals.oregondigital.org/ForestPhytophthora/article/view/3036/2721.

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Fire Ecology

Fire Behavioral Changes as a Result of Sudden Oak Death in Coastal California Forests[1]

Y. Valachovic,[2] C. Lee,[2] H. Scanlon,[3] J.M. Varner,[4] R. Glebocki,[2] B.D. Graham,[2] and D.M. Rizzo[5]

Abstract

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

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

Fuel loadings were greater in diseased than in non-diseased stands, yet great variability was observed and differences were not significant. However, fuel loads observed in herbicide-treated stands were significantly greater than in control stands ($P < 0.001$); total weight of downed woody debris (all size classes) approximately doubled with the herbicide treatment ($\bar{x}$ = 106.3 Mg ha^{-1}) over the control condition ($\bar{x}$ = 58.1 Mg ha^{-1}). The increasing trends in herbicide and diseased plots were similar, suggesting that fuel loading in diseased plots may continue to increase relative to control plots over a longer time horizon than observed.

Fuel models based on the observed surface fuel accumulations in herbicide-treated and diseased plots predict that for some early-to-mid-phase (2 to 8 years) herbicide-treated forests, and for late-phase (8 years plus) diseased forests, rates of spread, flame lengths, and fireline intensities could increase significantly over the baseline, challenging effective firefighter response and requiring alternative approaches to fire suppression. These results, in addition to the relatively high background surface fuels observed in the control stands, highlight the need for fuels treatments and effective disease management strategies in infested stands and as sudden oak death expands throughout a broader region.

For the full paper, please see Valachovic, Y.S.; Lee, C.A.; Scanlon, H.; Varner, J.M.; Glebocki, R.; Graham, B.D.; Rizzo, D.M. 2011. Sudden oak death-caused changes to surface fuel loading and potential fire behavior in Douglas-fir-tanoak forests. Forest Ecology and Management. 261: 1973–1986.

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Survival of *Phytophthora ramorum* Following Wildfires in the Sudden Oak Death-Impacted Forests of the Big Sur Region[1]

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Introduction

The summer of 2008 brought the first wildfires to occur in known *Phytophthora ramorum*-infested forests in California, with the largest individual fire burning in the Big Sur region of the central coast (Monterey County) (Metz et al. 2011). More than 100,000 ha in Big Sur were ultimately burned that summer, providing a natural experiment to examine the feedbacks between a destructive, invasive forest pathogen and wildfire. Big Sur is one of the most botanically and ecologically diverse areas in California, and its forests were among the earliest infested and most impacted by sudden oak death (SOD) in the state (Mascheretti et al. 2008, Meentemeyer et al. 2008). In 2006 and 2007, we established a network of 280 long-term forest plots in Big Sur to study the feedbacks between *P. ramorum,* its various hosts, and the physical environment (Haas et al. 2011, Metz et al. 2011). This plot network provided important pre-fire data on pathogen distribution, tree mortality, and host density levels, and a post-fire survey of burn severity indicators quantified forest impacts immediately post-fire in a subset of the plot network.

The pre- and post-fire data from the Big Sur plot network allowed for a rare opportunity to study the interactions between *P. ramorum* and wildfire. Metz et al. (2011) found that, while burn severity was not greater in *P. ramorum*-infested areas compared to uninfested areas despite greater fuel loads, the stage of the disease invasion impacted burn severity in different forest strata. In this study, we examined the direct and indirect impacts of wildfire on the persistence of *P. ramorum* across the burned landscape of Big Sur (Beh et al. 2012). Specifically, we addressed three questions: (1) Did the 2008 wildfires eradicate *P. ramorum* from areas known to have been infested prior to the fires? (2) If the wildfires did not eradicate the pathogen, under what conditions was *P. ramorum* able to persist in forest stands despite fire? (3) What are the likely reservoirs for pathogen persistence and re-invasion?

Methods and Materials

To accomplish the research objectives of this study, we completed intensive *P. ramorum* surveys in 2009 and 2010—1 and 2 years post-fire, respectively—within 63 plots in the Big Sur network that were known to contain *P. ramorum*-infected trees at the time of plot establishment. Of these plots, 45 were located within the areas burned in the wildfires of 2008 and 18 were outside of the fire perimeters; the plots in each burn status group were evenly divided between redwood-tanoak and mixed-evergreen forest types. Our surveys consisted of sampling *P. ramorum*-symptomatic vegetation in the plots, nearly all of which was collected from California bay laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.) and tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H. Oh). Because these *P. ramorum* host species sprout prolifically following injury or fire, many of the samples collected from burned plots originated from post-fire regenerative growth, including leaves and twigs from basal and epicormic sprouts. All plant materials collected during the surveys were processed using standardized isolation techniques for culturing *P. ramorum* (Davidson

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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et al. 2003). Following the determination of *P. ramorum* presence in each plot, we examined the conditions under which *P. ramorum* persisted on the Big Sur landscape: we used over 20 plot-based variables consisting of burn status, habitat type, pre-fire host abundance, pre-fire disease prevalence, and post-fire host mortality measurements as predictors in the analyses of *P. ramorum* recovery following the wildfires.

Results

The Big Sur wildfires of 2008 suppressed, but failed to eradicate, *P. ramorum* from areas of the landscape that were previously infested with the invasive forest pathogen. We were able to recover the pathogen 1 and 2 years following the fires in burned plots of both forest types, and in some cases, with no difference in frequency than in unburned plots. However, *P. ramorum* recovery 1 year after the wildfires tended to take place in plots with the lowest burn severities, while pathogen recovery 2 years post-fire occurred in plots with greater burn severities and was largely influenced by high levels of pre-fire disease prevalence and low levels of post-fire bay laurel mortality. In plots from which *P. ramorum* was not recovered even 2 years post-fire, burn severities and levels of post-fire bay laurel mortality tended to be high. In sum, multiple interacting biotic and abiotic factors were responsible for the persistence or lack thereof of *P. ramorum* in burned, previously infested plots (Beh et al. 2012).

Discussion

Our results indicate that while wildfire is not a panacea for the control of *P. ramorum*, it may at least temporarily reduce the abundance of the pathogen. Destruction and mortality of hosts of *P. ramorum*, especially bay laurel, was likely the most significant impact of the wildfires on the pathogen's survival, as there is little chance that *P. ramorum* would be able to subsist in dead hosts, regardless of whether the pathogen could have survived the high temperatures produced in the fires. Our findings that the recovery of *P. ramorum* in burned plots was positively correlated with the number of bay laurels expressing symptoms of *P. ramorum*-infection prior to the fires further highlights the importance of this sporulating host to the establishment, spread, and persistence of the pathogen. Patchy burn patterns, typical of mixed-severity fires, which left green, *P. ramorum*-infected bay laurels amidst the charred landscape, may have allowed these trees to serve as inoculum reservoirs that could infest newly sprouting vegetation (Perry et al. 2011). One unexpected result from this study was that two other *Phytophthora* spp., *P. pseudosyringae* and *P. nemorosa*, were frequently isolated from new vegetative growth in burned plots that were not known to contain these pathogens prior to the fires. Continued and ongoing surveys in Big Sur will provide additional information on *P. ramorum* re-establishment following fires, host mortality trends, and the effects of competing invasive *Phytophthora* species in the post-fire landscape.

Acknowledgments

We thank H. Mehl, K. Aram, A. Oguchi, C. DeLong, R. Cobb, and E. Fichtner for providing field and laboratory support for this research. We also thank R. Meentemeyer and the members of his lab for partnership in the Big Sur plot network. We are grateful to California State Parks, Los Padres National Forest, Landels-Hill Big Creek Reserve, Monterey Peninsula Regional Park District, and numerous private landowners in the Big Sur area for allowing research on their properties. This research was funded by the USDA Forest Service Pacific Southwest Research Station, USDA Forest Service State and Private Forestry, NSF Ecology of Infectious Diseases grant EF-0622770, and the Gordon and Betty Moore Foundation.

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Collateral Damage: Fire and *Phytophthora ramorum* Interact to Increase Mortality in Coast Redwood[1]

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Abstract

Invading species can alter ecosystems by impacting the frequency, severity, and consequences of endemic disturbance regimes (Mack and D'Antonio 1998). *Phytophthora ramorum*, the causal agent of the emergent disease sudden oak death (SOD), is an invasive pathogen causing widespread tree mortality in coastal forests of California and Oregon. In the absence of the pathogen, species composition in these forests is shaped by a number of biotic and abiotic factors, including wildfire. Large wildfires in California in 2008 provided an opportunity to test the interactions between *P. ramorum* and wildfire and their separate or joint impacts on forest composition. Here we ask whether the interacting effects of *P. ramorum* and wildfire on three dominant species in redwood forests were predictable from effects of either disturbance alone or whether there were synergies that occurred where both disturbances were present.

The 2008 Basin Complex and Chalk fires in Big Sur, California, burned more than 40 percent of the 280 forest monitoring plots established throughout the region in 2006-2007 (Metz et al. 2011). In 2009, we surveyed every stem that had been alive at plot establishment to determine mortality 1 year following the fire. In particular, we analyzed survival of tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H. Oh), California bay laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.), and coast redwood (*Sequoia sempervirens* (D. Don) Endl.) in 61 redwood forest plots, half of which were infested with *P. ramorum*. These species differ in susceptibility to mortality from the pathogen and wildfire. Tanoak and bay laurel are both competent sporulating hosts for the pathogen and sensitive to wildfire, but only tanoak suffers lethal bole canker infections. Redwood may become infected with the pathogen, but is an epidemiologically unimportant host in the spread or impacts of the disease and is generally resistant to wildfire. To understand potential increases to fire-caused mortality caused by the pathogen, we analyzed stem survival in stands with and without the presence of *P. ramorum* using generalized linear mixed effects models with binomial errors and stem size (diameter at breast height, DBH) as a covariate. At the stand level, we compared the percentage of basal area that died between 2006-2007 and 2009 among plots in all combinations of burned/unburned and with/without SOD.

In the absence of fire, all three species had very little mortality between 2006-2007 and 2009, except for tanoak stems in pathogen-infested plots, as was expected from the asymmetric disease impacts that occur among hosts. All three species had increased mortality in burned plots, but mortality in tanoak and bay laurel was much higher than in redwood. Redwood mortality varied greatly by tree diameter: for stems >60 cm DBH there was negligible mortality, and below 10 cm DBH, there was > 80 percent mortality, regardless of pathogen presence. Surprisingly, mortality risk increased dramatically for redwood stems 20 to 80 cm DBH in infested, burned plots relative to uninfested plots. This increase in mortality was driven almost entirely by plots that were in an intermediate stage of disease progression where a mixture of standing and surface fuels occurred. For example, mortality of 40 cm DBH stems was over 65 percent in these stands compared to approximately 5 percent in uninfested stands.

At the stand level, loss of tanoak basal area was highest in plots that experienced both SOD and fire, but the amount appeared predictable from the additive amount of biomass lost to either disturbance alone. Loss of bay laurel basal area was not dependent on the disease status of a plot, but rather reflected the high fire-caused

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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mortality of this species. Overall, basal area loss of redwood was low because the largest diameter redwoods had very low mortality regardless of disease status in the stand. Nevertheless, twice as much basal area died in infested, burned plots relative to disease-free, burned plots, a result that was not predictable from the impacts of either disturbance on redwood alone.

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

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Sudden Oak Death Effects on the Dynamics of Dead Wood[1]

Richard C. Cobb,[2] João A.N. Filipe,[3] Margaret R. Metz,[2] Ross K. Meentemeyer,[4] and David M. Rizzo[2]

Abstract

Sudden oak death has impacted forests notable for high-fire risk and contiguous host communities in California and Oregon coastal forest ecosystems. The disease continues to emerge in stands and landscapes with a large biomass of tanoak (*Notholithocarpus densiflorus* (Hook.&Arn.) Manos, Cannon & S.H.Oh), and we show that woody debris also increases with the progression of disease. This increase in fuels and the possibility of increased carbon release may impact wildfire intensity, wildlife, and regional carbon management goals (Cobb et al. 2012a, Lamsal et al. 2011, Valachovic et al. 2011).

We measured infection, mortality, and tree/snag fall rates in a set of plots in redwood- (*Sequoia sempervirens* (D. Don) Endl.) dominated stands initially established to measure the spread and impacts of sudden oak death (Cobb et al. 2010, Maloney et al. 2005). We combined these measurements with a one-time measurement of live tanoak biomass using allometric equations and a census of course woody debris (dead wood >10 cm diameter; Cobb et al. 2012a). Log and snag mass were calculated by estimating wood volumes using diameter and length measurements and measured wood density in approximately 25 percent of sampled logs. Tanoak wood decomposition rates were also estimated using measured changes in wood density over time and differences in average un-decomposed tanoak wood density with measured wood density in trees where the year of mortality was known. Lastly, we combined these field measurements with a linked epidemiological and ecosystem model to understand the rates and duration of wood debris accumulation. We combined a stand-level spatial spread and mortality model (Cobb et al. 2012b, Filipe et al. 2012) with a simple model of snag fall and host biomass decomposition (from snags and logs). Our future goal is to combine these models and measurements with regional spread models (Meentemeyer et al. 2011) to understand the spatial extent, rates, and magnitude of altered C cycling from woody debris in California forests impacted by sudden oak death.

In pathogen-invaded stands, snag mass was 22.4 Mg ha^{-1} while mean log mass was 11.5 Mg ha^{-1}. In comparison, dead-tree masses in un-invaded stands were 0.27 and 1.16 Mg ha^{-1} for snags and logs respectively (Cobb et al. 2012a). Woody debris mass and accumulation rates are principally driven by the amount of pre-disease tanoak biomass and the prevalence of infection in tanoak and California bay laurel (*Umbellularia californica* (Hook. &Arn.)Nutt.). In an associated study, we showed that tanoak infection and mortality rates are determined largely by tanoak size distribution and the local prevalence of infection in sporulation-supporting hosts (Cobb et al. 2012b). These are the same drivers of woody debris amounts in the geographically broad and diverse host landscape of our study plot network (Maloney et al. 2005). Overall, sudden oak death generates lower maximum amounts of woody debris and these materials accumulate at slower rates compared with better studied disturbances such as harvesting and wildfire. We identify two broad results critical to the management of disease-impacted forests. First, the maximum amount and duration of woody debris can be predicted by our model based on relatively simple stand-level measurements of tanoak density, tanoak tree size, and density of bay laurel. Second, sudden oak death is likely to result in a longer period of increased woody debris levels compared to other disturbances such as wildfire. While tanoak decomposes relatively quickly compared to true oaks and redwood, considerable amounts of woody debris are likely to accumulate in stands dominated by tanoak and bay laurel or in stands with high pre-disease biomass. Woody debris accumulation is sufficient to

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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impact fire intensity (Valachovic et al. 2011) suggesting that fuels reduction treatments may be necessary in regions at risk of sudden oak death emergence or those already impacted by the disease.

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Biology I

Combining Field Observations and Genetic Data to Reconstruct the Invasion of *Phytophthora ramorum* in California[1]

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Abstract

Although it has been convincingly shown that forest populations of the pathogen *Phytophthora ramorum* have undergone a significant bottleneck and reproduce exclusively asexually (Ivors et al. 2004, 2006; Mascheretti et al. 2008), objective results showing that nurseries were the original source of the introduction remain elusive (Mascheretti et al. 2008). A previous attempt to define routes of pathogen movement resulted in a largely unresolved network (Mascheretti et al. 2009), showing at best that populations from Santa Cruz and Marin Counties were important sources within California. Previous attempts at reconstructing the entire history of the sudden oak death (SOD) epidemic in California were limited by: 1) incomplete sampling; 2) the inability to include singleton samples; and 3) over-collapsing of non-spatially contiguous, yet genetically similar, samples into large meta-samples that confounded the coalescent analyses. Here, we employ a complete sampling coverage of 832 isolates of *P. ramorum* (the causative agent of SOD) from 60 California forests, genotyped at nine microsatellite loci.

The following microsatellite loci were genotyped: PrMS39a, PrMS39b, PrMS43a, PrMS43b, PrMS45 (Prospero et al. 2007), locus 18, locus 64 (Ivors et al. 2006), and loci ILVO145PrMS145 (a and c) (Vercauteren et al. 2010). Rather than using data simply based on number of microsatellite repeats, we employed Bruvo's distances as in previous studies (Bruvo et al. 2004). This metric is appropriate for analyses of populations that comprise closely related genotypes originated from the same founder genotypes because larger shifts in the number of repeats are weighed, not proportionally, but in terms of likelihood. Analysis of molecular variance (AMOVA) (Excoffier et al. 1992), as implemented by ARLEQUIN v.3.5 (Excoffier et al. 2005), was employed to generate pair-wise estimates of Φ_{ST} among all 62 *P. ramorum* forest and nursery populations. The Bruvo distance among each pair of unique multilocus genotypes (MGs) was estimated and fed to ARLEQUIN as an external file as the basis for the evolutionary distance in the AMOVA calculations.

Many pair-wise estimates of Φ_{ST} were low and not significantly different from zero (as evaluated by permuting individuals across locations 10,000 times). Populations were therefore recursively clustered by pooling the pair of populations or clusters that yielded the minimum Φ_{ST} at each round until no further insignificant clustering (i.e., minimum Φ_{ST} $P > 0.05$) was possible (Mascheretti et al. 2008, Mascheretti et al. 2009, Roewer et al. 2005). The algorithm was supervised by applying it only to populations from within the same county. Following the county-based clustering, the algorithm was continued to completion, permitting collapses among counties. Additionally, we re-ran the algorithm without supervision. The results were evaluated based upon knowledge from previous analyses of *P. ramorum* populations (Mascheretti et al. 2008, Mascheretti et al. 2009) and by subjecting the final set of populations from each run (by county, overall, and unsupervised) to a traditional hierarchical AMOVA: evaluating the Φ_{CT} value, which provides an unbiased way to judge population groupings (i.e., by maximizing the 'among group' variation whilst minimizing the 'within group variation') (Dupanloup et al. 2002). Examination of Φ_{CT} values indicated that the supervised clustering (by county $\Phi_{CT} = 0.3083$, $P < 0.001$) was superior to the unsupervised algorithm (unsupervised $\Phi_{CT} = 0.2879$, $P < 0.001$), but that allowing collapsing among counties offered little further improvement (overall $\Phi_{CT} = 0.3099$, $P < 0.001$). Since collapses among counties may reflect recent shared source populations, rather than direct migration between these populations, the 'by county' set of populations was conservatively chosen as the basis for subsequent analyses.

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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The genetic relationships among the final set of populations (post-clustering) were visualized by estimating the matrix of average pair-wise Bruvo genetic distances (Bruvo et al. 2004) among all populations with five or more samples and constructing the shortest neighbor-joining (NJ) tree using FASTME v.2.07 (Desper and Gascuel 2002). Bootstrap values (1000) were generated similarly and summarized using CONSENSE (PHYLIP package v.3.6) (Felsenstein 2005). All individuals and populations were also subject to genetic clustering analysis using STRUCTURE (Pritchard et al. 2000), and the posterior probability of membership in each of the identified genetic clusters was then examined for each population, singleton, and 'historical' isolate.

We have previously (Mascheretti et al. 2009) attempted to infer infection routes by estimating all bidirectional migration rates *M* among populations using MIGRATE-N (Beerli 2006, Beerli and Felsenstein 2001), but results were far from showing a clear pattern of spread, possibly due to the overcollapsing of all contiguous genetically similar populations into the same metapopulation. Additionally, it is very unlikely that most *P. ramorum* populations approach genetic equilibrium, violating the assumptions of MIGRATE-N and rendering estimates of *M* unreliable. Here, rather than estimate *M*, we used the output *ln* marginal likelihoods (*ln*(*ml*)) to choose among different migration models (Beerli and Palczewski 2010) and reconstruct the minimal, most probable, set of unidirectional migration routes among the new and highly resolved population dataset. Twenty-nine populations ($n \geq 5$) yield a computationally intractable number of possible models, and we therefore took a stepwise approach (Croucher et al. 2012). The number of possible models was limited by incorporating "epidemiological" data, disallowing models that included migration from a younger to an older population.

A total of 224 MGs were defined, more than half of the MGs (139 (62.1 percent); 66 (57.4 percent) were defined. The most frequent genotypes were MG38 ($n = 95$; 11.4 percent); MG42 ($n = 113$; 13.5 percent); and MG46 ($n = 122$; 14.6 percent); corresponding to the three frequent and possible founder MGs (#13, #14, and #15; respectively) originally identified in Mascheretti et al. (2008).

Iterative collapsing reduced the initial set of 43 populations ($n > 5$) to 29 (table 1). AMOVA results are given in table 1. Although the proportion of genetic variation within individual populations (68 percent) and among groups (31 percent) was high, variation among populations within groups (the final populations) was extremely low (1 percent) when clustering populations only within, but not between, counties. The NJ tree, rooted through the Nursery_SC1 sample, is shown in fig. 1. The tree accurately reflects the age of each infestation, with the oldest infestations (except SO4) all close to the root and the youngest infestations furthest from the root. Although bootstrap values were low (expected given few loci), the remarkable congruence between age and topology indicates that the tree accurately reflects the genetic relationships among populations and that these genetic relationships reflect the progression of the infestation from older to younger populations.

Table 1—Analysis of molecular variance results for the 29 populations defined by "within-county" collapsing

Group	df	SS	Variance Components	% Variation
Among Groups	28	17.975	0.0214	30.83
Among Populations Within Groups	15	0.871	0.0472	1.17
Within Populations	767	36.236	0.0695	68.00
SUM	810	55.081		
Φ_{SC}	0.0170^{NS}			
Φ_{ST}	0.3200***			
Φ_{CT}	0.3083***			

STRUCTURE (Pritchard et al. 2000) was used to analyze all populations. The *ln* probability of the data, for each replicate at each value of *k*, was summarized using STRUCTURE HARVESTER (Earl and vonHoldt 2011). Ln Pr(X|*k*) increased with each value of *k* and did not have a clear maximum. The method of Evanno et al. (2005) indicated an optimal ΔK at $k = 2$. This method cannot evaluate $k = 1$ and it is therefore probable that the 'true' $k = 1$ – suggesting that the entire epidemic may result from single introduced MG. A second maximum ΔK was observed at $k = 4$, and this value was therefore examined as an indicator of possible *sub-structure* in the data. The genetic clusters map to the tree by age, with cluster 1 representing the oldest infestations, cluster 3 the next oldest and the majority of populations, then cluster 4, and finally cluster 2 mapping to the youngest populations.

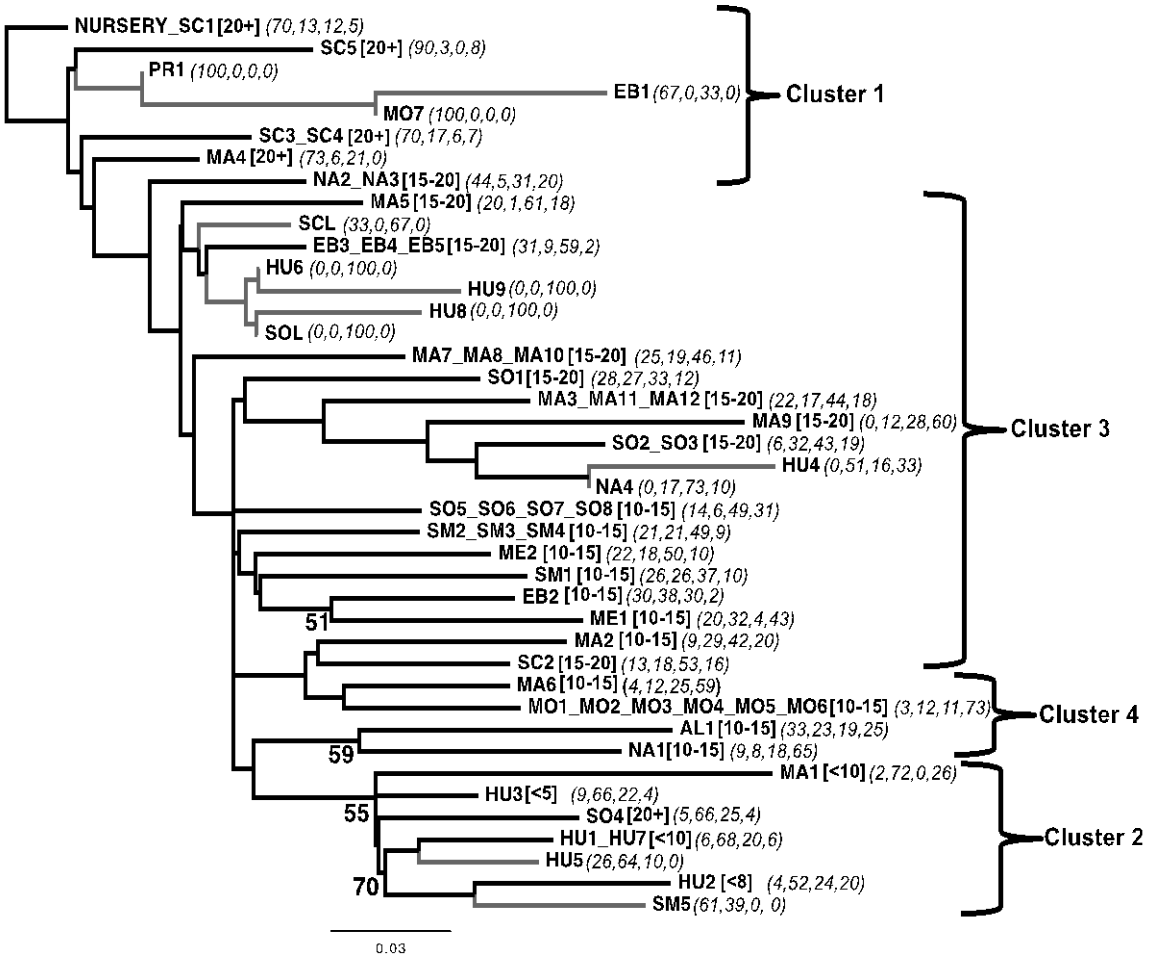

Figure 1—Neighbor-joining representations of the genetic relationships among *Phytophthora ramorum* populations. The numbers in parenthesis, following the years since infestation, indicate the percent posterior probability of membership in each of the genetic clusters (1,2,3,4) identified by STRUCTURE. Populations can be broadly identified with each of these clusters as indicated by the brackets. EB= East Bay, HU= Humboldt, MO= Monterey, NA= Napa, MA= Marin, SCL= Santa Clara, SC= Santa Cruz, AL= Southern Alameda, SM= San Mateo, PR= Presidio National Park, SO= Sonoma. Cluster 1 is consistently the oldest, while Cluster 2 is the youngest, based on field work.

The coalescent approach to migration model choice (Beerli and Palczewski 2010) identified for the first time, unambiguously and without subjectivity, that the Nursery_SC1 population was the original source population in the initial pair-wise analyses and prior to incorporating the historical data, thus corroborating independently that the SOD epidemic in California started in the Santa Cruz area and originally came from Nursery infestations (results not shown). The network shows a classical epidemic pattern with early spread to a few key localities - the forests around Santa Cruz, the San Francisco East Bay region, and the Golden Gate National Recreational Area in Marin County - followed by a multitude of infestation routes from these focal points. The analysis also revealed that, in several cases, multiple infestation routes involving different sources have affected the same counties, proving that many counties were each infested more than once during the brief history of the disease.

Until this study, there has been no clear understanding of the presence of four distinct clusters in California forests. The Nursery-associated cluster 1 appears in all analyses as the most ancestral one of the four (data not shown), with clusters 3 and 4 derived from it by a single repeat change at one locus, while cluster 2 is more closely related to cluster 3. Interestingly, the founder cluster 1 remains associated almost exclusively with nurseries and with forests neighboring Santa Cruz nurseries, where multiple lines of evidence indicate the original outbreak started. The other three clusters are much more widespread than cluster 1 (fig. 2). It is possible that clusters other than 1 may be better adapted to colonize forests. With the ability to differentiate the four clusters, this hypothesis can now be tested by further research.

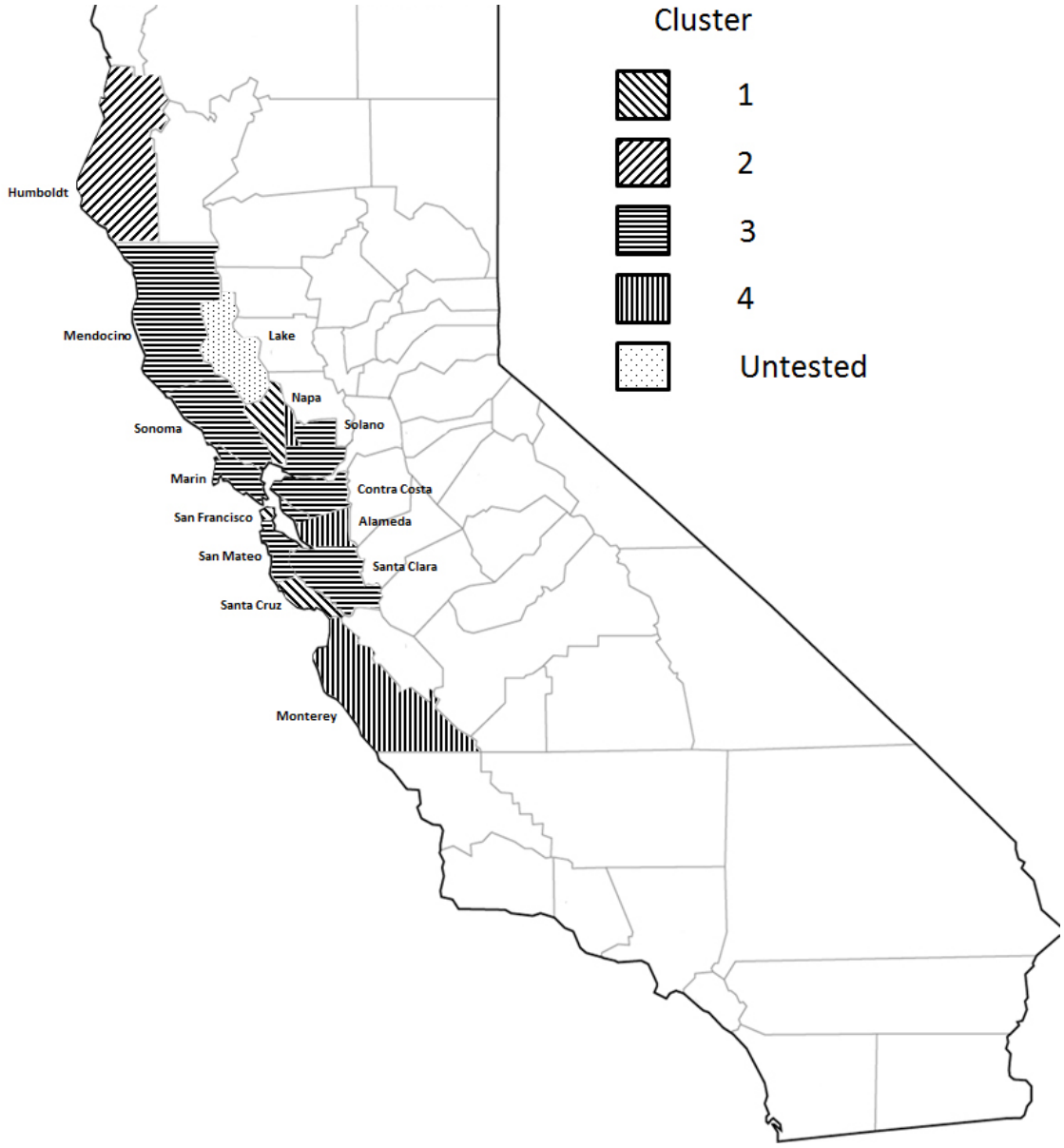

Figure 2—Distribution of the four genetic clusters of *Phytophthora ramorum* in California wildlands. Cluster 1 is the most ancestral cluster and is associated with nurseries; however, it is not as widespread as the other clusters, suggesting a microevolutionary trajectory possibly driven by adaptation.

Acknowledgments

This study was funded by an Ecology of Infectious Diseases grant (EF-062654), provided jointly by NSF and NIH, and by the Gordon and Betty Moore Foundation. The collection of samples predating the NSF-NIH grant was made possible through funding from USDA-APHIS and by the USDA FS, State and Private Forestry.

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Host-Induced Phenotypic Diversification in *Phytophthora ramorum*[1]

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Abstract

Forestry, agriculture, and native ecosystems face ever-increasing threats by invasive species. Not all introduced species are, however, invasive. In order to establish and persist in a non-native land, introduced species have to adapt to different environments, unfamiliar food, and predators. There are a number of examples where invasive species evolved quickly in non-native regions (Brasier 1995, Davis 2009), such as leg size of cane toad (Phillips et al. 2006) and leaf size of St John's wart (Maron et al. 2004). Large phenotypic variation has been observed in clonal lineages of the generalist plant pathogen *Phytophthora cinnamomi* (Hüberli et al. 2001). Rapid phenotypic changes in invasive animals and plants are believed to result from selection of existing genetic variation; in a non-native location, under different selective pressure, different traits from their native range may be selected. Contribution of *de-novo* mutations for adaptation is probably not sufficient to explain rapid phenotypic changes observed in invasive species. *De-novo* DNA mutations are rare: spontaneous mutation rate estimated for a plant is only one base substitution per genome per generation (Becker et al. 2011) and non-neutral mutation rate in eukaryotes is approximately 1/300 per genome per generation (Drake 1999). Hence, it is unclear how an invasive clonal pathogen, which is devoid of genetic variation, will adapt to new hosts and a new environment. It may be that, in response to environmental stress, invasive clones may generate variation on which selection acts.

There are several mechanisms proposed for the generation of *de novo* variation in response to stress. For instance, adaptive mutation proposes that DNA mutation rates may increase as an immediate and direct response to selective pressures (Cairns et al. 1988). Environmental stress may also induce epigenetic variation (Eichten et al. 2011), which is a heritable variation in gene regulation that cannot be attributed to DNA sequence variation. Transposable elements (TEs), which are mobile genetic elements and are highly mutagenic, may also be involved in the generation of *de novo* variation. A typical eukaryotic genome harbors thousands of TEs; however, their activities are epigenetically suppressed. Stress is known to activate TEs, which may contribute to genome diversification (Madlung and Comai 2004).

Isolates of *Phytophthora ramorum* belonging to the NA1clonal lineage display a large variation in colony morphology and growth rate. In culture, *P. ramorum* grows in a circular mycelial mat, which is defined as wild type (*wt*) (Brasier et al. 2006). However, some NA1 isolates show irregular and fluffy colony types, which are referred to non-wild type (*nwt*). Some isolates stop growing upon subculturing, which is referred to early senescence. Isolates showing *nwt* morphology are less aggressive on *Rhododendron* leaves (Elliott et al. 2011). We have recently shown that dramatic phenotypic variation among *P. ramorum* isolates is associated with the host species from which the microbe was originally cultured, but not with multilocus genotypes (Kasuga et al. 2012). Isolates originating from oaks (*Quercus* spp.) are less virulent on oak seedlings than those originating from California bay laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.). Furthermore, oak isolates are more likely to show *nwt* phenotype and early senescent phenotype than bay laurel isolates (Kasuga et al. 2012). Microarray mRNA profiling was conducted to detect gene regulation differences between oak and California bay laurel isolates. As a result, derepression of hundreds of transposable elements (TEs) and down-regulation of Crinkler effector homologs were observed in the majority of oak isolates, but this expression pattern was rare in

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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isolates from California bay laurel. In some instances, oak and California bay laurel isolates originating from the same geographic location had identical multilocus genotypes, but had different phenotypes. Because (1) no genetic subdivision has been detected between California bay laurel and oak isolates, and (2) oak is a dead-end host and infection is initiated from asexual propagules produced on nearby foliage host such as California bay laurel, we hypothesized that *P. ramorum* derived from a foliar host undergoes host-dependent genetic/epigenetic alterations inside an oak host. The observed phenomenon may be described as host-induced phenotypic diversification (HIPD); heritable phenotypic changes of the pathogen can occur inside host plants and the rate of change is host species dependent.

Direct evidence of HIPD and associated genetic/epigenetic alteration need to be established. We examined phenotypes of re-isolates from artificially inoculated canyon live oak (*Quercus chrysolepis* Liebm.) with an isolate displaying *wt* colony type typical of California bay laurel isolates. Nine months post inoculation, 20 percent of re-isolates (n=67) showed *nwt* colony phenotype. Both *wt* and *nwt* colony types were frequently recovered from the periphery of a single lesion in the cambium, which indicates a stochastic nature of the phenotypic conversion. In contrast, the vast majority of isolates obtained from California bay laurel consistently showed wild type (*wt*) colony morphology. Global mRNA profiling confirmed that some of the re-isolates growing in culture showed expression patterns typical for those isolated from oak trunks (i.e., derepression of TEs and repression of genes belonging to Crinkler effector family).

We have demonstrated that *P. ramorum* growing in oak undergoes phenotypic conversion; colony phenotypes and gene expression patterns of California bay laurel isolates were drastically changed after 9 months under the bark of canyon live oak. Although actual TE transposition and genome diversification are yet to be experimentally validated, the observed TE activity in re-isolates strongly indicates the occurrence of TE-mediated genome diversification in *P. ramorum* growing in oak hosts. According to an epi-transposon hypothesis, which is a theory of genome evolution to explain punctuated equilibria observed in the fossil records of animals, physiological stress, associated with major climatic change or invasion of new habitats, disrupts epigenetic silencing of TEs, which results in TE reactivation and genome diversification (Zeh et al. 2009). Brasier (1986, 1995) proposed a theory of episodic selection to explain rapid evolution observed in invasive plant pathogens: species will undergo episodic selection under sudden environmental disturbances such as geographical transposition, alterations in resource availability, exposure to a new host, and sudden climatic changes, which may result in the emergence of a highly fitted pathogenic clone or allopatric hybrid. Abiotic stress as well as interspecific hybridization proposed in the theory of episodic selection are known to cause transcriptional activation of TEs that may subsequently lead to restructuring of the genome (Madlung and Comai 2004). The generated genome diversity may then be served as raw material on which episodic selection can act. The epi-transposon hypothesis provides a molecular mechanism for accelerated evolution and hence complements the theory of episodic selection. Activation of TEs in suboptimal hosts may thus be a widespread strategy among invasive pathogens for adaptation and host shifts.

Acknowledgments

We thank R. Jiang and S. Tripathy for making *P. ramorum* gene models available for microarray construction and data analysis, and Marianne Elliott for sharing unpublished data.

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Susceptibility of Larch, Hemlock, Sitka Spruce, and Douglas-fir to *Phytophthora ramorum*[1]

Gary Chastagner,[2] Kathy Riley,[2] and Marianne Elliott[2]

Introduction

The recent determination that *Phytophthora ramorum* is causing bleeding stem cankers on Japanese larch (*Larix kaempferi* (Lam.) Carrière) in the United Kingdom (Forestry Commission 2012, Webber et al. 2010), and that inoculum from this host appears to have resulted in disease and canker development on other conifers, including western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), grand fir (*Abies grandis* (Douglas ex D. Don) Lindl.), and Sitka spruce (*Picea sitchensis* (Bong.) Carrière), potentially has profound implications for the timber industry and forests in the United States Pacific Northwest (PNW). A clearer understanding of the susceptibility of these conifers to *P. ramorum* is needed to assess the risk of this occurring in the PNW.

Methods

An experiment was conducted to examine the susceptibility of new growth on European (*L. decidua* Mill.), Japanese, eastern (*L. laricina* (Du Roi) K. Koch), and western larch (*L. occidentalis* Nutt.); western and eastern hemlock (*T. canadensis* (L.) Carrière); Sitka spruce; and a coastal seed source of Douglas-fir to three genotypes (NA1, NA2, and EU1) of *P. ramorum* in 2011. In 2012, a similar experiment was conducted using only the four larch species.

Container-grown seedlings or saplings were used in all experiments. Five trees or branches of each species were inoculated with a single isolate of the three genotypes by spraying the foliage with a suspension of zoospores (10^5/ml). Seedlings or branches that were sprayed with water alone served as the control. Trees or branches were encased in plastic bags for 5 to 6 days and placed in a biocontainment unit maintained at 19 to 20 °C with 24-hr light. In 2011, symptoms were monitored and isolations were made from all symptomatic trees 8 to 11 days after inoculation, and also repeated on certain symptomatic trees at later dates, up to 38 days after inoculation. The trees used had few, if any branches, so lesion data are based on the lesions that developed on the stem. In 2012, the trees used had an additional year of growth and branch development, so lesion length data were based on lesions that developed on both the stem and branches. Isolations were made at 11 days for trees that were almost totally dead and at 21 days for the remainder of the trees. Isolations were repeated on some trees at 55 days after inoculation.

Due to the discovery in the United Kingdom that *P. ramorum*-infected Japanese larch needles can sporulate heavily in the fall, we wanted to determine if infection could take place on larch seedlings at that time of year. We conducted an experiment during 2011 using European, Japanese, eastern, and western larch and Douglas-fir seedlings from the same nursery stock used in the spring 2011 inoculation trial that had been maintained outdoors. In early October, five trees of each species were inoculated as described above. Seedlings sprayed with water alone served as controls. After 5 days, the plastic bags were removed and the seedlings were maintained in the

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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biocontainment chamber and monitored for symptom development for 8 weeks. A sampling of needles with the variety of symptoms observed were collected from both the control and inoculated trees, surface sterilized, and plated onto CARP medium.

Results

The data presented below is only for inoculated seedlings where *P. ramorum* was isolated from symptomatic tissue. No *P. ramorum* was isolated from any of the non-inoculated controls.

Symptoms observed on the seedlings inoculated in the spring ranged from those occurring on individual needles or needle bundles to shoot dieback that extended into the previous year's stems in the form of lesions. Seedlings were killed when lesions girdled the stems. In the 2011 spring inoculation trial, the number of infected seedlings varied by genotype of the pathogen and host (table 1). The four larch species had the highest number of infected seedlings. Western larch tended to have the largest lesions. No *P. ramorum* was recovered from any of the eastern hemlock, which only exhibited minor needle symptoms. Of the isolates used, the NA2 isolate infected all of the conifer species except eastern hemlock. Overall, the NA2 isolate was isolated from 27 of the 40 inoculated seedlings. In comparison, the EU1 and NA1 isolates were isolated from 14 and 10 seedlings, respectively. Although the number of seedlings that became infected varied by genotype, the average size of the lesions the isolates caused after 8 to 11 days only ranged from 5.3 cm for the NA1 and EU1 isolates to 5.7 cm for the NA2 isolate.

Table 1—Number of *Phytophthora ramorum*-positive inoculated seedlings and sizes of lesions that developed, spring 2011

	No. of *P. ramorum*-positive seedlings[a]				Lesion length (cm)	
Host	Total	NA1	NA2	EU1	Max.	Avg.
Western larch	13	4	5	4	28.5[b]	12.6
Japanese larch	11	2	5	2	4.5	3.6
Eastern larch	10	2	5	3	4.2	2.4
European larch	9	2	5	2	5.5	3.7
Sitka spruce	6	0	4	2	4.0	2.7
Douglas-fir	3	0	2	1	2.5	2.5
Western hemlock	1	0	1	0	2.5	2.5
Eastern hemlock	0	0	0	0	0	0

[a] Total of five seedlings were inoculated with each genotype. Data was collected 8 to 11 days after inoculation.
[b] Probably the result of multiple lesions coalescing.

In 2012, there was no significant (ANOVA) difference among the three isolates of *P. ramorum* with respect to the overall percentage of symptomatic bundles, number of lesions per seedling, lesion size, and mortality on the four spring-inoculated larch species (table 2).

Table 2—Effect of isolate on symptom development on four larch species, spring 2012

Isolate genotype	% sym. bundles[1]	Avg. no. of lesions/seedling[a]	Avg. lesion length (cm)[a]	% mortality[b]
NA1	35.6	4.6	6.5	35.0
NA2	45.4	4.9	7.3	35.0
EU1	52.5	4.8	7.8	40.0

[a] Data collected 21 days after inoculation.
[b] Data collected 55 days after inoculation.

Except for the limited symptom development that occurred on the eastern larch, the data from the 2012 spring inoculated trial confirmed the susceptibility of western, European, and Japanese larch to isolates of the three genotypes of *P. ramorum* used in this test (table 3). Although not significantly different, western larch tended to have the highest percentage of symptomatic bundles, number of lesions per seedling, lesion length, and mortality.

Table 3—Differences in symptom development on four spring-inoculated larch species in 2012[a]

Larch species	% sym. bundles[b]	Avg. no. of lesions/seedling[b]	Avg. lesion length (cm)[b]	% mortality[c]
Western	60.1	6.0	8.3	53.3
European	44.0	4.3	6.1	46.7
Japanese	32.7	4.3	8.0	33.3
Eastern	5.3	2.7	3.2	0.0

[a] Five trees of each species were inoculated with a NA1, NA2, and EU1 isolate of *P. ramorum*.
[b] Data collected 21 days after inoculation.
[c] Data collected 55 days after inoculation.

When the plastic bags were removed from the seedlings in the fall 2011 inoculation test, minimal symptoms (a few gray needles, yellow/brown needle tips, a faint lighter-colored needle banding, single yellow needles) were evident from any of the inoculated and control seedlings. During the 8-week incubation period, no additional symptoms developed. Isolations from symptomatic needles 8 weeks after inoculation resulted in a single positive recovery of *P. ramorum* from the proximal end and center of a single, partially gray needle from one Japanese larch seedling inoculated with the NA2 isolate.

Results from these trials indicate that all of the conifer species tested, except eastern hemlock, exhibited some level of susceptibility to *P. ramorum*. The highest recovery of *P. ramorum* and level of symptom severity occurred on the larch species, especially western larch. These experiments also indicate that the NA1, and especially the NA2, genotypes of *P. ramorum* have as much potential as the EU1 genotype to cause disease on newly emerging growth of larch. Virtually no infection occurred on any of the larch inoculated in the fall. Additional studies are needed to determine the potential sporulation of NA1, NA2, and EU1 isolates of *P. ramorum* on susceptible conifers, especially western larch.

Acknowledgments

The authors wish to thank Don Sherry, WSU, for assistance in the lab and Weyerhaeuser, Drakes Crossing and Lawyer Nursery, for providing seedlings. Funding for this project was provided by USDA Forest Service, Pacific Southwest Research Station agreement 10-JV-11272138-081.

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Diagnosis and Management of *Phytophthora ramorum* Canker in Canyon Live Oak, an Atypical Bole Canker Host[1]

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Abstract

Diagnosis of sudden oak death (SOD) in tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H. Oh) and susceptible red/black oak species (coast live oak, *Quercus agrifolia* Née; Shreve oak, *Q. parvula* Greene var. *shrevei* (C.H. Mull.) Nixon; California black oak, *Q. kelloggii* Newb.) is facilitated by the fact that infected trees commonly develop obvious bleeding bark cankers. In contrast, canyon live oak (*Q. chrysolepis* Liebm.) exhibits a different pattern of symptom expression, which has made it difficult to identify and document *Phytophthora ramorum* bole cankers in this species.

In 2002, *P. ramorum* was shown to cause dieback in small (<2 cm diameter) branches of canyon live oak, but no bole cankers in mature canyon live oaks were observed (Murphy and Rizzo 2003). In 2006, we observed a cluster of recently-killed mature canyon live oaks on Bolinas Ridge in Marin County. The affected trees were amid *P. ramorum*-infected California bay laurel (*Umbellularia californica* (Hook. & Arn.) Nutt. The overall pattern of disease was consistent with SOD, but affected trees had been dead too long to attempt pathogen isolation.

In 2008, mortality and large trunk cankers were seen in large, mature canyon live oaks in the Los Trancos Open Space Preserve in Santa Clara County. Symptomatic canyon live oaks had bole cankers that were extensively colonized by flat-headed borers and ambrosia beetles and had *Annulohypoxylon thouarsianum* sporulation. *Phytophthora ramorum* symptoms were common on foliage of California bay laurel, which was abundant in the vicinity of affected trees. However, we were unable to recover *P. ramorum* from symptomatic canyon live oak in several rounds of sampling of multiple trees. After ruling out other possible causes of tree decline, we surveyed the affected stand to determine the spatial pattern of disease in the stand. We found that the likelihood of canker symptoms and mortality in canyon live oak were highly associated with proximity to California bay laurel (fig. 1) and the amount of California bay laurel cover within 2.5 to 5 m. This relationship was nearly identical to that seen in SOD-affected coast live oak stands (Swiecki and Bernhardt 2008). We conducted another intensive round of sampling in the stand and obtained positive PCR detections of *P. ramorum* from trunk cankers on two symptomatic canyon live oaks, but no positive cultures.

[1] A version of the paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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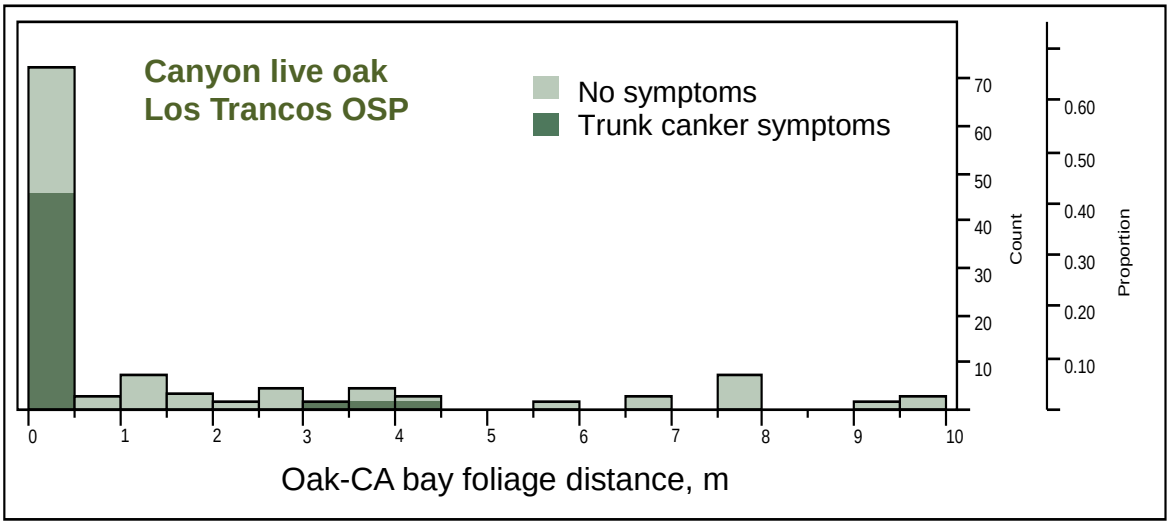

Figure 1—Distribution of distances between oak trunks and nearest California bay laurel foliage for canyon live oaks with and without SOD-like trunk canker symptoms at Los Trancos Open Space Preserve.

We subsequently tested pathogenicity of *P. ramorum* on detached large-diameter canyon live oak logs under lab conditions using established protocols (Kirk and Brasier, n.d.). Large, dark-brown cankers developed in the phloem of inoculated canyon live oak logs within 2 weeks after inoculation, and *P. ramorum* was readily re-isolated from canker margins (Aram et al. 2011). This provided the first clear indication about the internal appearance of early stage *P. ramorum* cankers on this species. However, the detached log assay does not provide reliable information about the appearance of external symptoms that develop in the field.

In July 2010, we initiated a field test by inoculating 18 canyon live oaks (25 cm average DBH) and two Shreve oaks, which served as positive controls, at a site in San Mateo County. Trees were inoculated with agar plugs taken from the margins of actively growing *P. ramorum* cultures, following procedures previously used for coast live oak (Rizzo et al 2002). Each tree was inoculated with two different local *P. ramorum* isolates and a control mock inoculation (sterile agar only).

Nine of the inoculated canyon live oaks were located in a natural closed canopy stand on a northeast-facing slope that included some intermixed tanoak and California bay laurel. The canyon live oaks in this lower plot were relatively tall with small crowns. Some of the trees were partially overtopped and many had evident thinning. The other nine inoculated canyon live oaks and two Shreve oaks were in an adjacent hilltop stand (upper plot) that had been planted about 25 years earlier. The seed source(s) are unknown, but likely included trees from well beyond the lower plot area. Although the upper plot area also had a closed canopy, trees were much shorter and had wider crowns than in the lower plot. Most canopies were relatively dense, which has resulted in extensive dieback of the lower branches due to shading. The planting included a few tanoaks along with Shreve and canyon live oaks.

We evaluated symptoms periodically through June 2012, nearly 2 years after inoculation. Bleeding was seen in six of the 18 inoculated canyon live oaks. However, periods of active bleeding were short, so relatively few trees had visible bleeding at any given time (fig 2.). Only 17 percent (6/36) of the *P. ramorum* inoculation points developed any bleeding prior to sampling. The amount of bleeding was usually limited to a few drops up to about 2 mm in diameter. Only one tree, which developed a large canker within 4 months, had easily visible amounts of bleeding. Another tree that never showed bleeding developed large cankers which were detected by the presence of extensive ambrosia beetle boring dust about 1 year after inoculation and *A. thouarsianum* sporulation by 70 weeks after inoculation. No other canyon live oaks were colonized by these secondary organisms by June 2012. Both inoculated Shreve oaks developed large girdling cankers that overgrew the mock inoculation site. Bleeding was seen in one of these trees 19 weeks after inoculation.

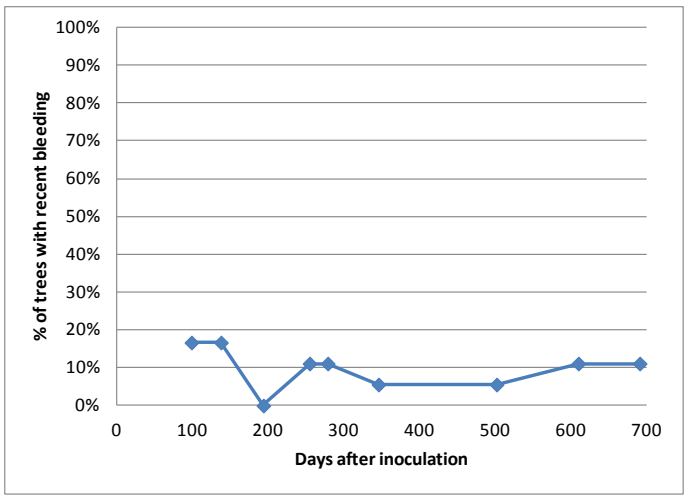

Figure 2—Percent of inoculated canyon live oak with recent bleeding symptoms at various times after inoculation with *Phytophthora ramorum*.

We sampled at least two canyon live oaks from both the upper and lower plots at 20 and 40 weeks after inoculation. One Shreve oak, which did not show bleeding symptoms, was sampled at 20 weeks. Cankers were exposed by removing outer bark around the inoculation point with a draw knife. Cankers developed at all sampled *P. ramorum* inoculation points, but varied widely in size, extending from a few to about 30 cm from the inoculation point (fig. 3). Trees in the lower plot were more likely to have small cankers. We re-isolated *P. ramorum* from all sampled cankers at both sampling times, with no clear trends relative to plot or sampling depth in the cankers. However, the pathogen was recovered at a higher efficiency from Shreve oak than from canyon live oak.

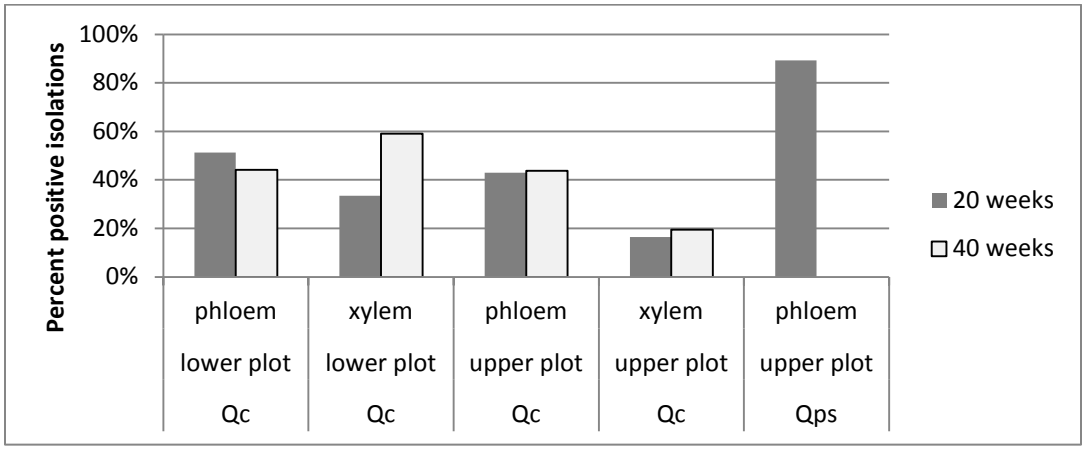

Figure 3—Percent positive *Phytophthora ramorum* isolations from cankers of inoculated trees 20 and 40 weeks after inoculation (Qc = canyon live oak; Qps = Shreve oak).

Using symptom information gleaned from inoculation studies, we have successfully isolated *P. ramorum* from several naturally infected canyon live oaks, but isolation efficiency has remained very low. Successful isolations came from relatively recent cankers that were identified by small amounts of bleeding. However, our results suggest that many infected trees do not bleed and cankers in these cryptically infected trees cannot be seen until they are colonized by secondary organisms. This has complicated ongoing management studies to prevent *P. ramorum* infection in canyon live oak. Our results also show that bole cankers caused by *P. ramorum* or other *Phytophthora* species in hosts that develop little or no bleeding may be very difficult to detect and could remain unrecognized or attributed to other causes.

Acknowledgments

This research was supported by cost-share grants from the USDA Forest Service, State and Private Forestry, Forest Health Protection, and Midpeninsula Regional Open Space District (MROSD). We thank Lydia Baker of the Matteo Garbelotto lab at UC Berkeley for PCR test results, and Elizabeth Fichtner for assistance with field work. We thank Cindy Roessler of MROSD for facilitating and encouraging this project.

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Roads Are Not Significant Pathways for SOD Spread, in Oregon At Least[1]

Everett Hansen,[2] Joe Hulbert,[2] and Ebba Peterson[2]

Introduction

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

Methods

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

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

Road segments were surveyed during the rainy season in 2011 and again in 2012 in a series of transects, on foot. The road segments were in heavily trafficked areas passing through concentrations of SOD sites. Water was collected from mud puddles on the roads and baited for *P. ramorum*. Symptomatic roadside vegetation was also collected and tested for *P. ramorum* infection. A total of 108 puddles and 92 vegetation transects were sampled.

Results

The spatial analysis showed no association between roads and SOD sites. Some sites were adjacent to roads, but others were far from roads (up to 600 m). The median measured distance from SOD centroids to roads (101 m) was not significantly different (pseudo-p=0.47) from the median distance expected under randomness (fig. 1).

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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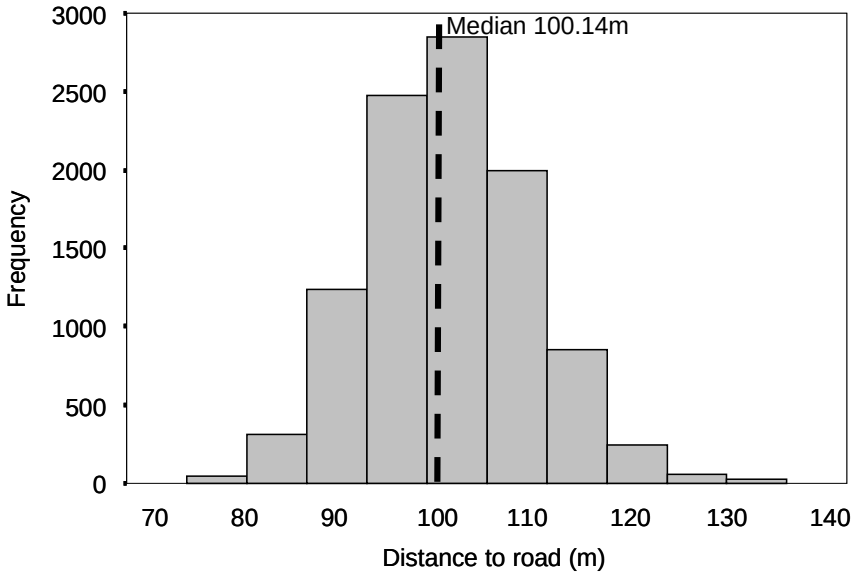

Figure 1—Median measured distance to nearest road (dashed vertical line) compared to median distances generated through a restricted randomization test with 10,000 reiterations of the random dataset (bar graph).

In the 2 years of road sampling, *P. ramorum* was not recovered from road puddles except for single puddles where SOD sites spanned the road (Duley Creek) or run off from an adjacent site (Thousand Line) crossed the road. *Phytophthora ramorum* was isolated from roadside vegetation in seven instances (of 92, 100 m transects) in two areas, but both situations were in an area of general infestation with roadside host plants growing immediately beneath an infected over story tree. *Phytophthora ramorum* was not recovered from puddles or vegetation further along the roads in any case (table 1).

Table 1—Recovery of *Phytophthora ramorum* from puddles on roads by baiting and from roadside vegetation subject to splash from the roads (100 m transects) by direct isolation

Site	Puddles		Transects	
	Sampled	*P. ramorum*	Sampled	*P. ramorum*
Lewis Creek	10	0	10	0
Thousand Line	42	1	34	0
Duley Creek	10	1	12	5
Mountain View Drive	8	0	4	0
Bean Creek	14	0	8	0
Ostenburg Road	14	0	6	2
Bravo Creek	4	0	8	0
Ransom Ridge	6	0	10	0

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

Acknowledgments

This work was funded by the USDA Forest Service, PSW Research Station, and PNW Region, Forest Health Protection.

The Effect of *Phytophthora ramorum* on the Physiology and Xylem Function of Young Tanoak Trees[1]

Elizabeth Stamm[2] and Jennifer Parke[2]

Abstract

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

In experiments with 2-year-old tanoak saplings, stems of 60 trees were wound inoculated with one of three treatments, including a high-elicitin expressing *P. ramorum* isolate (PR-07-058[NA2]), a low-elicitin expressing *P. ramorum* isolate (4353[NA1]), or a noninoculated wounded control. Physiological parameters including photosynthesis and water usage were measured weekly for 5 weeks. Sets of trees from each treatment were sampled destructively twice monthly to measure the conductive properties of stem xylem tissue and to examine for the presence of tyloses. There was a large difference between the high- and low-elicitin treatments in stem hydraulic conductivity as early as week 2 of the experiment. Significant treatment differences were also observed in tree mortality. Trees inoculated with the high-elicitin expressing isolate died sooner and at a higher rate than trees inoculated with the low-elicitin expressing isolate. Photosynthesis and stomatal conductance began to decline 3 weeks after inoculation. This experiment was potentially compromised by differences in growth rate of the two isolates, so the experiment was repeated with high- and low-expressing NA2 isolates with similar growth rates.

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

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

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Screening Gulf Coast Forest Species for Susceptibility to *Phytophthora ramorum*[1]

Jason Preuett,[2] Daniel Collins,[2] Douglas Luster,[3] and Timothy Widmer[3]

Abstract

Phytophthora ramorum, the causal agent of sudden oak death in California oak woodlands, poses a threat to woody plants in the rest of the United States, including the Gulf Coast area, which is regarded as a high-risk location. Several plant species native to Gulf Coast forests were tested for susceptibility to *P. ramorum*, including yaupon (*Ilex vomitoria* Aiton), spice bush (*Lindera benzoin* (L.) Blume), southern magnolia (*Magnolia grandiflora* L.), sweetbay magnolia (*Magnolia virginiana* L.), black willow (*Salix nigra* Marsh.), baldcypress (*Taxodium distichum* L.), Virginia creeper (*Parthenocissus quinquefolia* (L.) Planch.) from two sources (Louisiana and Maryland), and eastern baccharis (*Baccharis halmifolia* L.). This study was conducted at the U.S. Department of Agriculture Agricultural Research Station biosafety containment greenhouse facility at Ft. Detrick, Maryland. Foliage of four plants for each species tested was inoculated with a suspension of 50,000 zoospores per ml until run-off. Inoculated plants were placed in a dew chamber at 20 ºC for 5 days. After incubation period, leaf lesion areas were assessed for necrosis. Representative samples of necrosis infection areas were plated on *Phytophthora*-selective medium to confirm *P. ramorum* infection. The average percentage of leaf area necrosis was 5.0, 0.2, 8.6, 1.5, 1.1, 0.2, 32.1, 4.9, and 27.9 percent for inoculated baldcypress, black willow, sweetbay magnolia, Virginia creeper (Louisiana and Maryland genotypes), eastern baccharis, southern magnolia, spicebush, and yaupon, respectively; and 4.2, 0.3, 0.3, 3.1, 1.1, 0.4, 0.6, 1.2, and 0.1 percent for non-inoculated control plants, respectively. Comparison of inoculated versus non-inoculated plants showed significant differences ($P \leq 0.05$) for yaupon ($P=0.0008$), southern magnolia ($P=0.001$), and sweetbay magnolia ($P=0.0009$).

An additional study comparing asymptomatic infection of both tested genotypes of Virginia creeper was completed using the same procedure described above. The study was limited due to the available plants after the previous described study. The average percentages of leaflet infection for the two sources were similar for Louisiana (10.0 percent) and Maryland (13.6 percent) genotypes. Further study is needed on Virginia creeper to better understand its potential role in the Gulf Coast region as a potential inoculum source for *P. ramorum*.

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[1] A version of the paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Phytophthora ramorum in Coast Live Oak: Search for Resistance and Mechanisms[1]

B.A. McPherson,[2] D.L. Wood,[2] S.R. Mori,[3] A. Conrad,[4] and P. Bonello[4]

Abstract

Despite the presence of *Phytophthora ramorum* in northern and central California forests since at least 1994, asymptomatic coast live oaks (*Quercus agrifolia* Née) still remain in heavily infested stands. Coast live oak infection and mortality rates of 5 percent y^{-1} and 3 percent y^{-1}, respectively, observed in long-term monitoring plots in Marin County, California give no indication that individual trees appear to evade infection. In some study plots, more than 50 percent of the mature coast live oaks have died since 2000.

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

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

The Marin and Santa Cruz County studies were conducted in areas where *P. ramorum* had been established and were, therefore, 'prescreened' for susceptibility. Despite the presence of the pathogen in Briones Regional Park, East Bay Regional Park District, Contra Costa County, California, extensive coast live oak stands are still not affected. We initiated a study in 2010 to investigate the relationship between phloem chemistry, canker size, and survival. The responses of two coast live oak populations to inoculation and to natural infection will be monitored to determine if phloem chemistry can be a reliable predictor of survival.

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Metabolite Profiling to Predict Resistance to *Phytophthora ramorum* in Natural Populations of Coast Live Oak[1]

A. Conrad,[2] B. McPherson,[3] D. Wood,[3] S. Opiyo,[4] S. Mori,[5] and P. Bonello[2]

Abstract

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

Though the mechanisms of persistence are not known, putative phenolic biomarkers of oak resistance to the pathogen have been identified and include tyrosol hexoside pentoside, ellagic acid, and total phenolics. To test the accuracy of these biomarkers in predicting resistance within natural populations of CLO, we quantified biomarkers in 148 mature trees from two naïve stands in Briones Regional Park, East Bay Regional Park District, Contra Costa County, California. Those trees were then inoculated with *P. ramorum* to assess their resistance by measuring resulting cankers approximately 10 months after inoculation. We applied a predictive model to estimate survival based on canker lengths. This model predicted that 18 percent of inoculated trees have an 80 percent probability or greater of being resistant, with 85 percent of those trees containing one or more biomarkers at or above resistant threshold levels. Current efforts are focused on identifying novel biomarkers, which may better predict resistance in naïve populations of CLO. Additionally, to account for phenology as a potential source of variation in CLO phenolics, we measured biomarkers every season from December 2010 to November 2011 in 14 randomly selected control trees. We found no significant differences among seasons in the putative phenolic biomarkers tyrosol hexoside pentoside, ellagic acid, and total phenolics.

Mitigation of disease, via exploitation of host resistance, is one of the most effective and economically feasible management strategies for keystone tree species in forested ecosystems. The ability to identify resistant trees in natural populations of CLO, before they are attacked by the pathogen, through development of relatively easily measureable biomarkers, could be incorporated into disease management plans aimed at preserving resistant trees which could otherwise be lost to urban encroachment, fire, or other destructive disturbances.

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Biology II

The Importance of Understory Infection by *Phytophthora ramorum* as a Means of Primary Disease Establishment in Oregon Forests[1]

E.K. Peterson[2]

Abstract

Phytophthora ramorum-infested soils have been implicated as a source of primary inoculum in natural ecosystems. Implicit in this pathway is the need for infection of understory vegetation during pathogen establishment, preceding infection of bole hosts. In support of soil dispersal, studies using artificially-inoculated soils have shown that understory inoculum can infect low-lying foliage of tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H. Oh) and California bay laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.) (Fichtner et al. 2009). The lack of association between sudden oak death (SOD) and roads in Oregon, however, is inconsistent with a soil-mediated, long-distance dispersal mechanism. Regardless, *P. ramorum* can be recovered from soils at sites treated as part of the SOD eradication program (Goheen et al. 2008), as well as from streams within infested watersheds during all seasons of the year (Sutton et al. 2009). It is unknown to what extent these understory inoculum sources are responsible for establishing new disease foci, posing a risk for the continued spread of *P. ramorum* in Oregon.

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

Despite the abundance of understory hosts and other *Phytophthora* spp., *P. ramorum* was not recovered from foliage along streams bearing inoculum, except when associated with overstory mortality. California bay laurel was the most common host at all locations, although understory and overstory tanoak were present at all sites. *Phytophthora nemorosa* was the most common *Phytophthora* spp. recovered, and was equally abundant directly adjacent to streams as out of the splash and flood line. *Phytophthora ramorum* was isolated from only four sites. We preferentially recovered *P. ramorum* from tanoak and out of the splash and flood line. All samples positive for *P. ramorum* were directly downhill from overstory mortality. Immediately downstream from overstory mortality we failed to recover *P. ramorum* from streamside vegetation.

A strong disease gradient was detected around SOD-positive overstory tanoaks, indicating spatial dependence upon overstory sources. Understory hosts were abundant at all sites. There was a significant, negative relationship between pathogen recovery and distance from the center of each site, lending evidence that secondary inoculum originated from overstory canopies.

We found no evidence that soil or stream-borne inoculum is causing significant infection in understory vegetation, at least during the conditions of the eradication program practiced in Oregon. Despite a decade of inoculum presence in some waterways, stream-borne inoculum is not resulting in significant stream-side infection. Rather, the majority of understory infection is associated with overstory mortality. The lack of understory infection independent of these overstory sources implies that the movement of soil-borne inoculum has not contributed to the dispersal of *P. ramorum* in Oregon. Most likely infection is establishing in overstory

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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tanoak via aerial means and is dispersing locally in rain splash. Importantly, our results support the use of aerial surveys and the detection of deceased, overstory tanoak as a means to describe the distribution of *P. ramorum* in Oregon.

Acknowledgments

Thanks to the USDA Forest Service, Pacific Northwest Region (Region 6) Forest Health Monitoring Program and the Pacific Southwest Research Station for funding.

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Determining Landscape-Scale Changes in Forest Structure and Possible Management Responses to *Phytophthora ramorum* in the Mt. Tamalpais Watershed, Marin County, California[1]

Janet Klein,[2] Andrea Williams,[2] and John Menke[3]

Abstract

The Marin Municipal Water District's (MMWD) 7487 ha Mt. Tamalpais watershed in Marin County, California has the dubious distinction of being one of the earliest and most extensive areas impacted by *Phytophthora ramorum* in California. Rapid die off of tanoaks (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H. Oh) were first documented in 1995.With funding support from the U.S. Department of Agriculture Forest Service, MMWD initiated an assessment of landscape-scale changes in forest structure and understory floristics relative to *P. ramorum* spread in the Mt Tamalpais watershed. The assessment looked at changes in the extent and severity of diseased stands over a 5-year period as well as changes in understory vegetation. Three specific questions were addressed to support the development of a response strategy: (1) What sudden oak death (SOD)-related changes have already occurred? (2)What future SOD-related impacts are likely, or where is SOD likely to spread? (3) What is the status of natural regeneration in SOD-impacted stands? An additional benefit of this project was revision of the SOD-impacted portions of the 2004 vegetation map for the Mt. Tamalpais watershed to more accurately reflect stand conditions in 2009.

Analysis of true color aerial imagery of the watershed indicated that the spatial extent and severity of SOD-related tree mortality expanded between 2004 and 2009 from 3541 ha to 4330 ha. This represents 83 percent of all habitat on the watershed with a principal component of tanoaks, coast live oak (*Quercus agrifolia* Nee), black oak (*Q. kelloggii* Newb.), canyon live oak (*Q. chrysolepis* Liebm.), or Shreve's oak (*Q. parvula* var. *shrevei* (C. H. Muller) Nixon). The largest un-impacted stand in 2009 was 61 ha in size. In both 2004 and 2009, canopy mortality was most pronounced in tanoak-dominant assemblages in the western Bolinas Ridge portion of the watershed. Much of the expansion between 2004 and 2009 was due to a spatial increase in coast live oak mortality in the southern and eastern portions of the watershed. Type conversions from one recognized vegetation association to another were detected for 840 ha of habitat where tanoak ceased to be a primary component.

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

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

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Temporal Fluctuations and the Role of Disturbance in Disease Progression of the Sudden Oak Death Epidemic[1]

Melina Kozanitas,[2] Todd W. Osmundson,[2] and Matteo Garbelotto[2]

Abstract

With its high host mortality and ability to cause landscape-scale alterations in forest cover and composition, sudden oak death (SOD) (etiological agent *Phytophthora ramorum*, Stramenopila, Oomycota) mirrors past forest disease epidemics such as Chestnut Blight and Dutch Elm Disease. In contrast with these past epidemics, however, the appearance of SOD converges with a time of significant advancement in the development of molecular genetic tools that allow the movement of individuals (or individual genotypes) to be tracked and the role of evolutionary processes in disease progression to be assessed. Such methods have been instrumental in reconstructing the likely origins and geographic pathways of spread of the pathogen. However, little is yet known about the local-scale processes contributing to disease maintenance and progression. Since 2008, we have surveyed a network of sampling plots in the San Francisco Bay Area multiple times per year in order to examine seasonal patterns in the incidence and population genetic structure of infection foci. A period of severe drought in 2007-2009, followed by a period of normal to above-normal precipitation in 2010-2011, provide a significant opportunity to examine the effects of abiotic disturbance on disease progression. Here, we present recent and ongoing research focused on assessing seasonal patterns in genotypic diversity of viable infections that can act as reservoirs of new infectious propagules, assessing the effect of drought as a potential agent of selection on pathogen genotypes, examining the relationship between infections on dead-end (*Quercus*) and amplifying (California bay laurel, *Umbellularia californica* (Hook. & Arn.) Nutt.) hosts, and assessing the possible role of competitive interactions with sympatric *Phytophthora* species (*P. pseudosyringae*, *P. nemorosa*) using culture-based surveys, culture-independent (qPCR) assays, and analyses of population genetic structure (based on variable microsatellite loci) to infer the underlying processes of pathogen demographic expansion, contraction, and spread.

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Dynamics of Aerial and Terrestrial Populations of *Phytophthora ramorum* in a California Watershed Under Different Climatic Conditions[1]

Catherine A. Eyre,[2] Melina Kozanitas,[2] and Matteo Garbelotto[2]

Abstract

We present a study of the epidemiology of sudden oak death (SOD) in California within a watershed based on temporally and spatially replicated surveys of symptoms, viability of the pathogen from symptomatic leaves, and genetic analyses using polymorphic SSR markers.

Phytophthora ramorum is sensitive to climate; its optimal growth and transmission conditions are in the spring with warm rainy weather, and although it can survive and persist in harsh conditions, its capacity for transmission and dispersal are significantly reduced in drought conditions. Its main method of transmission is via rainsplash. The San Francisco Bay Area experienced a period of drought for several years prior to, and including, 2009, where conditions for *P. ramorum* were suboptimal and SOD outbreaks were notably fewer. However, 2010 saw a return to wetter conditions. We studied the population dynamics of *P. ramorum* resident in two different substrates (leaves and soil) during that climatic transition to study the effects on diversity and isolation success. Population genetics have been used to reconstruct the global history and migration of the pathogen to understand its origins and emergence as a significant pathogen in North America and Europe. Our study is one of the first to address the population dynamics of *P. ramorum* at a local micro-evolutionary scale and to compare the populations in different substrates.

Intense sampling of soil and leaf populations was carried out over a period of 2 years which spanned a climatic transition from drought in 2009 to a wetter climate in 2010. The survey was conducted in the San Francisco Public Utilities Commission watershed district, near San Mateo, California. The area has been infested for over 10 years and is subject to minimal management, so it is relatively undisturbed. Six survey plots were set up in two drainages within the watershed in areas of coast live oak (*Quercus agrifolia* Née), tanoak (*Notholithocarpus densiflorus* Manos, Cannon & S.H. Oh) and California bay laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.). Leaves were sampled five times during the 2-year study period for leaves: Early (March/April), Peak (June), and Late (September) 2009; and Early and Peak 2010. Soil was sampled twice during Peak time each year. Each plot consisted of six 100 m transects radiating out from a central point. Symptomatic leaves were sampled from the canopy of California bay laurel trees at 10 m intervals along each transect. Simultaneously, soil was sampled from three areas around the base of the tree and amalgamated. Leaves were plated onto PARP selective growth medium (pimaricin; 400µl/L, ampicillin; 250 mg/L, rifampicin; 10 mg/L, PCNB; 25 mg/L), incubated, and *P. ramorum* growth identified and subcultured. Soil samples were flooded with dH_2O and baited with leaf discs taken from uninfected *Rhododendron* var Cunningham's White leaves for 5 to 7 days, then leaf discs plated onto PARP+H (as PARP above but with addition of 25 mg/L hymexazol). *Phytophthora ramorum* growth was subcultured onto PARP.

Mycelial cultures were grown in liquid pea broth and DNA extracted with NaOH extraction (Wang et al. 1993). DNA was amplified using fluorophore labeled primers for six different microsatellite loci: MS18, MS64 (Ivors et al. 2006), MS38, MS43, MS45 (Prospero et al. 2007), MSILVO145 (Vercauteren et al. 2010). Multilocus genotypes (MLG) were assigned using Gimlet software. Genetic diversity indices were calculated: Stoddart & Taylor's G; G^ percentage of maximum diversity i.e., G/N, where N is sample size; R, clonal genotype diversity where R=(G-1)/(N-1) where G is the number of MLGs present in a sample and N is the sample size. Bruvo distances were calculated between MLGs and populations analyzed for genetic structuring by Analysis of Molecular Variance (AMOVA) analysis using Arlequin v3.1.5.2.

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Symptoms of SOD on leaves of the transmissive host, California bay laurel, increased significantly from 15 to 39 percent in six survey plots between dry and wet conditions, while levels of identical symptoms caused by other foliar pathogens were highest (69 percent) in dry conditions. This suggests that *P. ramorum* and other pathogens, while occupying the same niche, are favored by different climatic conditions. *Phytophthora ramorum* isolation success from leaves was lowest during the cold dry fall/Late season in 2009, and the greatest number of isolations was made during the Peak 2010 when conditions were rainy and warm. Isolation from soil was very similar in both years, with isolation success decreasing slightly in 2010. The soilborne population seems less labile with respect to climate; the soil environment probably provides a better buffer to climatic change than the tree canopy.

The populations sampled were very diverse. In leaves, 22 MLGs were detected in Peak 2009, and the number detected more than doubled to 49 MLGs in Peak 2010. In soil, the number was more consistent between years; 20 MLGs were detected in 2009, 23 MLGs in 2010. Many of the genotypes detected were singletons (i.e., only found in one individual). Diversity indices were calculated using the multilocus genotypes of isolates from these times. Using Stoddart and Taylor's *G* ,which is an absolute measure, the diversity increased throughout the sampling period, tracking the increasing numbers of MLGs detected, and was greatest in Peak 2010 for both substrates with diversity greater in leaves than in soil (Leaf: G=14.687, Soil: G=12.27) (fig. 1). Indices *G^* and *R*, which take sample size into account, followed very similar patterns to each other and showed an almost inverse relationship to *G*. According to *G^* and *R*, diversity was lowest during the Peaks of 2009 and 2010 as compared with other sampling seasons. These low values for diversity indices at the times of year when the most isolations and most MLGs were detected indicates that the populations were dominated by a few highly abundant genotypes. This effect was strongest in 2010, when the lowest values for *G^* and *R* were recorded, coinciding with when the climate had transitioned to wetter conditions. In these favorable conditions for *P. ramorum* growth and sporulation, there is more inoculum present leading to greater competition for space and resources between individuals. It may be that certain individuals have some selective advantage and those phenotypes are selected for and become dominant within the population. Although microsatellite markers are selectively neutral and do not confer any advantage on an individual, the advantageous phenotypes may carry the MLGs to dominance by association.

Analysis of genetic structuring of populations showed that there was no structure between the two drainages, but there was structure within the drainages between the individual plots. This is likely to have arisen due to local evolution of genotypes from small founding populations and restricted movement of the pathogen, especially in the drought years. This structuring was more pronounced in leaf populations in 2010, probably as a reflection of the more favorable conditions and proliferation of the pathogen-enhancing founder effects. There was significant structuring between soil and foliar populations, but soil and leaf genotypes were generally intermixed and closely related, indicating they are part of the same source population. It may be that there is a different ability of genotypes to adapt to different substrates. Very few genotypes were shared between substrates, and where they did overlap, the relative proportions in each substrate were significantly different. Finally, leaf populations were similar between sampling times, but soil populations showed some structure, suggesting that there is turnover between years in the soil population. Rarefaction was used to ensure that sample sizes were sufficient to capture a good representation of the diversity present to ensure that turnover was not an artifact of insufficient sampling of a very diverse population.

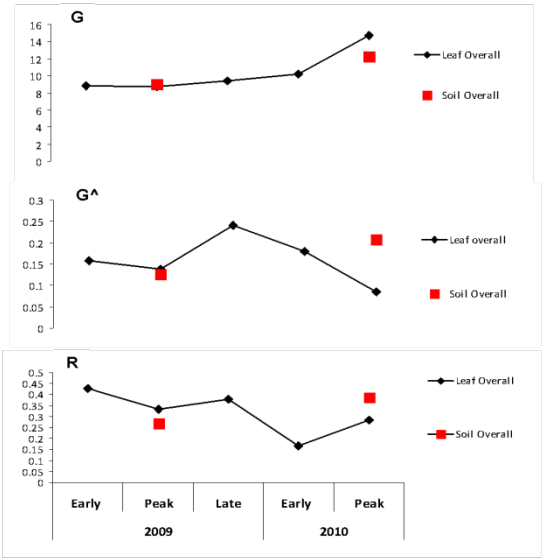

Figure 1—Genetic diversity indices for soil and leaf populations of *Phytophthora ramorum* sampled in 2009 and 2010.

Acknowledgments

This study was funded by the San Francisco Public Utilities Commission, the U.S. Department of Agriculture, USDA Forest Service, Pacific Southwest Research Station and by the National Science Foundation-Ecology of Infectious Disease Grant 1115607. We are very grateful to all our volunteers who assisted with data collection in the field and lab.

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Management

Novel Approaches to SOD Management in California Wildlands: A Case Study of "Eradication" and Collaboration in Redwood Valley[1]

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Abstract

In California, sudden oak death (SOD) treatment efforts have been localized, often targeting specific trees or properties. The widespread nature of SOD establishment and spread in coastal mountains of California has mostly precluded use of broader eradication strategies, which are more applicable in isolated infestations like those in Oregon. However, the 2010 detection of a new infestation in Redwood Valley, California—more than 80 km from the nearest known infestation, and the northernmost known occurrence in the state—presented an opportunity for the first attempt at containment and potential "eradication" in California. The infestation was isolated to a relatively small geographic area and was of high priority, effectively located at the gateway to Redwood National Park, Yurok and Hoopa tribal lands, Bureau of Land Management (BLM) and US Department of Agriculture Forest Service (USDA FS) lands, and the dense tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H. Oh) forests of the Klamath watershed.

Given the nature of the Redwood Valley infestation and its proximity to important ecological and cultural landscapes, spread of the disease from this area is highly undesirable. However, the context for the management in this area was complex, requiring careful collaboration from the beginning. The pathogen was initially detected through stream sampling near Orick in May 2010, many miles downstream from Redwood Valley and near the mouth of the 80,937 ha (200,000 ac) Redwood Creek watershed. Only through extensive sampling and the targeted engagement of large landowners throughout the watershed was the source of the infestation narrowed to Redwood Valley. Even then, the infestation spanned a number of private properties, including small residential landholdings and large private timberlands, and necessitated the cooperation and commitment of diverse stakeholders. Likewise, the project required a prompt, creative funding strategy and ultimately involved the support of the USDA FS, the Natural Resources Conservation Service (NRCS), California Department of Forestry and Fire Protection (CAL FIRE), the University of California, local contractors, and private landowners. The multi-tiered collaboration required by this project is unique for SOD management efforts in California, where treatments have previously been limited in size and scope.

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

Keywords: *Phytophthora ramorum*, sudden oak death, eradication, management, collaboration

Introduction

In California, sudden oak death (SOD) treatment efforts have been localized, often targeting specific trees or properties. The widespread nature of *Phytophthora ramorum* establishment and spread in

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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California has mostly precluded use of broader control strategies, which are more applicable in isolated infestations like those in Oregon. However, in the last decade, the pathogen has spread north to previously uninfested Humboldt County, and patchy, discontinuous infestations there have presented new opportunities for treatment, with less than one percent of the at-risk habitat infested to date.

This paper will focus on recent treatment efforts in Redwood Valley (northern Humboldt County), which were based on early experimental efforts in southern Humboldt County (Valachovic et al., Suppression of *P. ramorum* infestations through silvicultural treatment in California's north coast, this proceedings) and on treatment projects in Oregon (Goheen et al. 2010). The Redwood Valley management efforts will require sustained and continued attention, but its development and its outcomes are notable in a number of ways. First, the discovery of the Redwood Valley infestation is testament to the efficacy of stream monitoring efforts taking place in California's north coast and beyond. Second, the project is an example of the careful collaboration necessary for rapid disease response, especially in California's complex social and ecological landscape. Third, the Redwood Valley project has made enduring contributions to the regulatory and funding structure around SOD; the newness of the disease and the consequent lack of institutional infrastructure for SOD treatment in Redwood Valley necessitated the development of new, SOD-specific regulatory categories and funding options, which are now available for other similar treatment efforts. In these ways, the Redwood Valley project has not only advanced the collective understanding of what it takes to treat SOD, but it has also laid important foundations for future collaboration and action in California's wildlands.

Finding *Phytophthora ramorum* in Redwood Valley

In the California north coast region, stream monitoring is the primary method used for early detection of *P. ramorum*, the pathogen that causes SOD. Mesh bags containing fresh, disease free rhododendron leaves are placed at strategic locations in streams and rivers—effectively baiting the pathogen—and follow-up laboratory tests determine whether or not leaves became infected with *P. ramorum* while they were in the stream. If samples test positive for the pathogen, further efforts are needed to determine the terrestrial source of the inoculum present in that watercourse. A broad network of north coast streams has been monitored since the early 2000s, and though these monitoring efforts do not cover all of the watersheds in the region—they are limited by access issues and available resources—they remain one of the most critical tools for SOD detection in the area.

In May 2010, a sample collected near the mouth of Redwood Creek (near Orick) tested positive for *P. ramorum* for the first time in its 7-year monitoring history. This positive sample was significant not only because the site had previously been free of the pathogen, but also because it was the first detection in northern Humboldt County—more than 80 km north of the nearest known infestation in California and at the gateway to Redwood National Park, Yurok and Hoopa tribal lands, Bureau of Land Management (BLM) and U.S. Department of Agriculture Forest Service (USDA FS) lands, and the dense tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H. Oh) forests of the Klamath watershed (fig. 1).

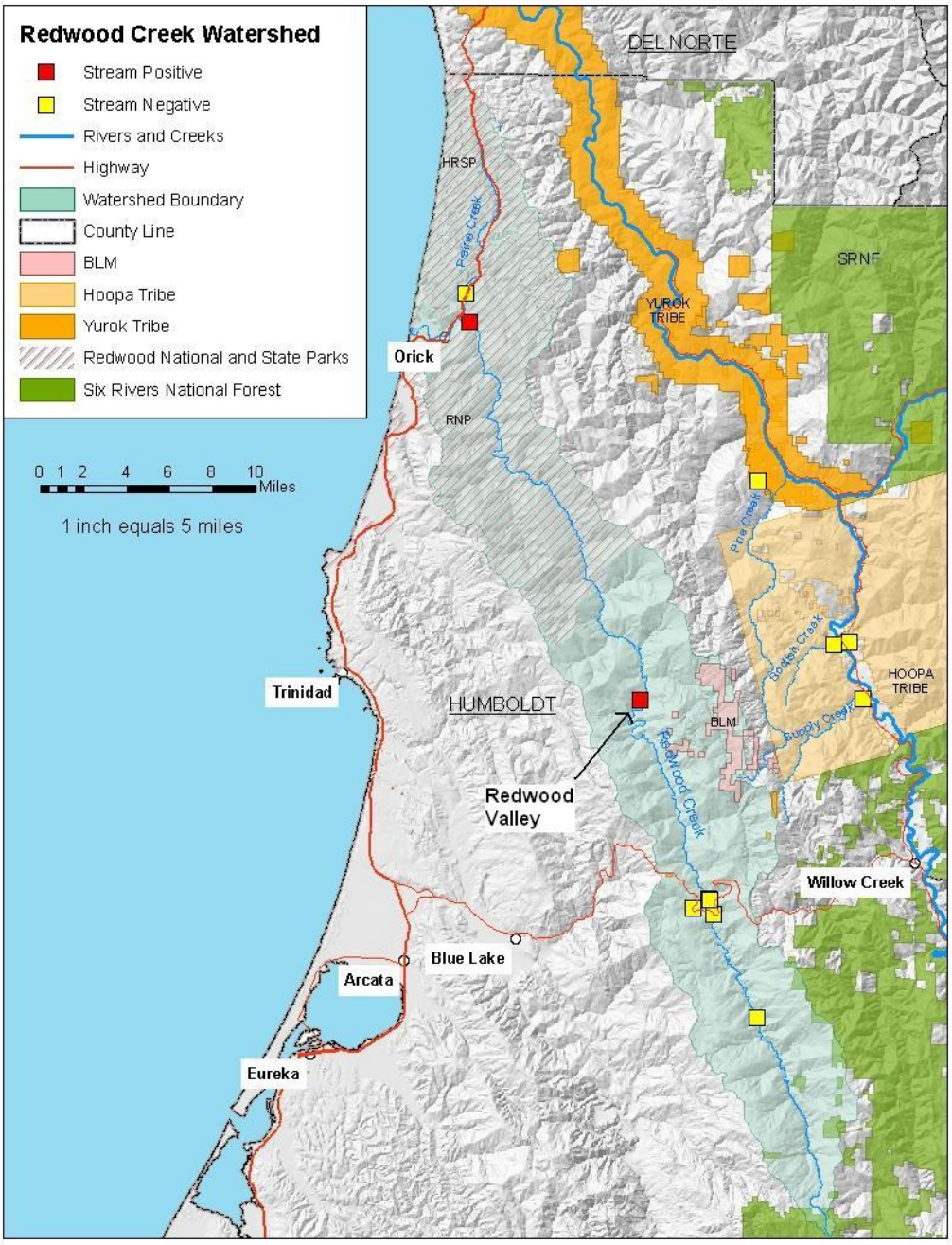

Figure 1—The Redwood Creek watershed, with location of stream positives at Orick and in Redwood Valley. Map also shows adjacent public and tribal lands. The Redwood Valley infestation was more than 80 km north of the nearest known infestation in California.

The positive sample in Redwood Creek was only the beginning of a much larger effort; the next task was to identify the source of the pathogen, which was somewhere in the 80,937 ha (200,000 ac) watershed. University of California Cooperative Extension (UCCE) staff, who facilitate much of the *P. ramorum* monitoring in the north coast region and had collaborated with UC Davis and Redwood National Park in the monitoring of Redwood Creek, took a lead role in follow-up efforts associated with the Redwood Creek sample. They held a meeting with the major landowners in the watershed to inform them of the positive test result and gain access for further surveying. They also expanded the local stream monitoring network to include as many Redwood Creek tributaries as possible, hoping to

narrow the infestation to a more manageable sub-watershed. In spite of these efforts, the pinpointing of the infestation in Redwood Valley—a residential area in the middle of the largely unpopulated watershed—was somewhat coincidental. In July 2010, a UCCE staff person who was walking from the road to the creek to pick up a stream monitoring bag recognized the symptoms in the immediate area. Using aerial reconnaissance by USDA FS flight teams and substantial ground surveys, the team confirmed that the infestation was small, limited geographically to Redwood Valley.

Treatment in Redwood Valley

Deciding to Treat

After years of working on SOD in the north coast region, UCCE was well positioned to advance discussion and action in Redwood Valley. Long before the discovery of the Redwood Valley infestation, UCCE was working on SOD-related outreach and education across the county and had formed a coordination group to help guide efforts. They were also engaged with the larger disease management community in California, with strong ties to the California Oak Mortality Task Force (COMTF), scientists within the University of California (UC) and Oregon State University, scientists and pathologists with the USDA FS, California Department of Forestry and Fire Protection (CAL FIRE), and other federal and state agencies. In approaching the Redwood Valley infestation, UCCE brought together these partners and found strong support from scientists, funders, and affected landowners for management.

Given the sensitive location of the Redwood Valley infestation, there was widespread interest in treatment from the beginning. Redwood Valley is close to many important public lands, including Redwood National Park and BLM areas, and it is also adjacent to tribal and private forest lands. The forests surrounding Redwood Valley—especially to the east—are thick with tanoak, and the potential for SOD-related mortality is very high. Redwood Valley is also one of the few areas in the watershed that is accessible for crews and equipment; if the pathogen were to spread down river or into the upper watershed, there would be no practical way to treat it. Given these shared concerns, public, private, and tribal land managers in the area were supportive of swift, comprehensive treatment.

Project Collaborators

Many agencies and organizations were involved in early planning efforts. Staff from UCCE brought forward their experience with various *P. ramorum* treatment approaches in southern Humboldt County, and funding from the American Recovery and Reinvestment Act (ARRA) allowed them to devote consistent time and energy to the project. Researchers from UC Davis's Rizzo lab were instrumental in this early phase of the project; they were primary collaborators on the stream monitoring efforts that first revealed the new infestation, and they continued to offer critical scientific input, fieldwork, and lab work as the project moved forward. The Garbelotto lab at UC Berkeley also supported the project, conducting genetic testing of *P. ramorum* in Redwood Valley and offering insight on the origin of the pathogen, which appeared more related to the infestations in southern Humboldt County than to the nursery positives in the nearby McKinleyville area. Other early collaborators included CAL FIRE, the Vegetation Management staff at Redwood National Park, industrial and non-industrial private landowners in Redwood Valley, scientists and forest pathologists from the USDA FS Forest Health Protection Program and the Pacific Southwest Research Station, staff from the BLM, and more. As the project became better developed, other important collaborators stepped in to provide funding and labor, including the Natural Resources Conservation Service (NRCS) and CAL FIRE.

Treatment Methods

The treatment in Redwood Valley was based on the experimental treatments that were implemented in southern Humboldt County (Valachovic et al. 2010; Valachovic et al., Suppression of *Phytophthora ramorum* infestations through silvicultural treatment in California's north coast, this proceedings) and on the experiences from the southern Oregon management efforts (Goheen et al. 2010). The prescription included the removal of all tanoak and California bay laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.) within 100 m of infected trees (fig. 2). The cut material was either piled and burned on site or securely trucked off site and donated to a local power company, or, when neither of those options was feasible, the material was lopped and scattered on site. In two situations, landowners had special (often large diameter) trees that they did not want removed, so those trees

Figure 2—Before and after treatment in Redwood Valley, spring 2012. (Photos by L. Quinn-Davidson)

were limbed to reduce the total live crown of potentially infected foliage, decrease tree-to-tree connectivity, and increase airflow. One of the major landowners in the valley—a private timber company—took responsibility for treatment work on their land by using an herbicide to treat standing California bay laurel and tanoak trees within 100 m of infected trees. The UCCE team was responsible for surveying the extent of the infestation and providing crews with maps of infected trees.

Because tanoak and California bay laurel are both prolific basal sprouters, herbicides were used where possible for post-treatment stump control. Originally, imazapyr was the primary chemical used for both tree species; it worked well on tanoak stumps, but close observations of California bay laurel stumps indicated that imazapyr alone would be slower to control California bay laurel sprouts. Staff from UCCE found similar results on standing trees in earlier experiments in southern Humboldt County, but had not yet used imazapyr to control stumps. A combination of imazapyr and glyphosate, which they found effective for standing California bay laurel trees, should be a more effective treatment for California bay laurel stumps, and has been used in the second round of stump control in Redwood Valley.

Funding

The Redwood Valley project involved financial and other contributions from a number of collaborators. As mentioned previously, ARRA funding supported UCCE staff in coordinating the project. The USDA FS contributed funds for treatment and monitoring, and NRCS contributed funds for treatment through their Forest Stand Improvement program. Forester time for surveying and significant crew time for project implementation were contributed by CAL FIRE, and private landowners also donated labor and money to the project.

The cost per hectare of treatment in Redwood Valley varied greatly from property to property. Some of the treated areas were residential, requiring careful work by highly skilled contractors. In some cases, tree removal around houses was performed with a boom lift, and trees were removed piece by piece. In these areas, it was also very important for cut material to either be piled and burned or trucked off site. This residential work was complex and slow and was significantly more costly than the work performed in wildland settings. Wildland work was conducted primarily by CAL FIRE crews or private contractors, who moved relatively quickly and were able to lop and scatter much of the cut material on site.

Project Outcomes

Treatment

The first phase of treatment in Redwood Valley was completed in spring 2012 and covered approximately 150 ha on almost 20 different properties. Follow-up stump control work occurred in the late fall of 2012, and more treatment work may take place in the future, depending on the results of current project monitoring activities.

However, the outcomes of the Redwood Valley project extend beyond the physical boundaries of the treatment. The project necessitated the development of important new regulatory and funding structures for SOD treatment work in California, and those options are now in place to enable future efforts.

New “Component” for NRCS Forest Stand Improvement Program

The NRCS supports forest management actions on private lands through its Forest Stand Improvement (FSI) program, NRCS Practice Code 666. Typically, projects that are funded through the FSI Practice fulfill one or more of the “components” listed in the code, including prescribed burning, firebreaks, tree/shrub pruning, erosion control, and more. When NRCS stepped forward to support the efforts in Redwood Valley, they were lacking an adequate component for SOD treatment activities, which require sanitation and urban-interface considerations not common in typical FSI

activities. Staff from NRCS worked with UCCE and other project collaborators to develop a new FSI component for SOD treatment, which included strict, SOD-appropriate requirements for equipment sanitation and handling of infected plant material, and technical and legal requirements appropriate for tree removal work near homes. The per-acre value of the new SOD treatment component (i.e., the amount NRCS is willing to pay landowners per acre of treatment) was based on estimates given by arborists for work in Redwood Valley, and it was significantly higher than any existing component under the FSI Practice. This component is now established and available for use in future SOD treatment efforts. It could also be adapted for use in different NRCS programs or initiatives, if necessary. For example, the new component was developed specifically for the urban-interface setting in Redwood Valley, but NRCS may develop a lower cost alternative for similar work in wildland settings.

New Regulatory Language in California Forest Practice Rules

In January 2011, the California Board of Forestry approved a temporary emergency regulation for control of *P. ramorum* in California's privately owned forestlands. This emergency rule was drafted and adopted in response to the Redwood Valley infestation, which was one of first California infestations to include commercial timberlands and called attention to the need for regulatory options that enable and encourage swift silvicultural treatments in SOD-infested areas. In March 2012, the Board voted to permanently adopt the new rule, regulation 14 CCR § 1052.5, Emergency Notice for Outbreaks of Sudden Oak Death Disease.

The new regulation, which went permanently into effect in January 2013, allows use of an Emergency Notice to manage infected forestlands through harvest of infected trees. Using this permit, landowners may be able to offset some of the costs of treatment work through commercial utilization of diseased trees. Although landowners and foresters have the ability to take some of these actions without the new regulation (under existing rules), they are not allowed to commercially utilize any of the harvested materials without filing a Timber Harvest Plan, which is a much slower permit to prepare and receive approval for. Additionally, the new regulation allows harvesting and commercial utilization of infected or symptomatic trees near streams and wet areas, where most of the California bay laurel is found. This new regulatory language will increase the options available to affected landowners, offset some of the costs of treatment activities on private forestlands, and, most importantly, help control the spread of *P. ramorum* in California.

Lessons Learned

It's been 2 years since *P. ramorum* was discovered in Redwood Valley. In that time, a wide range of collaborators have worked together to secure funds and implement treatments in an effort to protect and conserve the vulnerable forests of California's north coast. Over 150 ha have been treated, and project partners have invented creative new funding and regulatory options to enable efficient, appropriate action. The scale of the treatment and the level of collaboration—both in funding and in implementation—is unprecedented for this type of work in California, and in these ways, the project has been a major success.

However, the project has also faced a number of challenges, from social to climatic to biological. In a residential area like Redwood Valley, it takes significant time and effort to garner landowner support and coordinate the legal agreements and access necessary to survey and treat the project area. In this case, most landowners were supportive of the project, but some landowners had special requests and two (of 22) opted not to participate at all; luckily, the non-participating landowners were geographically central to the project and their untreated properties were buffered by treatment, yet the pockets of infested forest remain a threat to the integrity of the project.

Even more concerning has been the weather in recent years. The last two springs have been some of the wettest on record, and late, warm rains have supported rapid spread of the pathogen. The project boundary expanded several times during the treatment period due to the discovery of newly

infested trees, and post-treatment monitoring has recently revealing new pathogen detection outside of the treatment boundary. These weather concerns are compounded by the dearth of information on the lag time between infection and symptoms; treatment activities are based on surveys, but surveys rely on disease symptoms, and it is unclear what size treatment buffer will be sufficient to capture trees that are infected but not yet showing symptoms.

The challenges to treatment in Redwood Valley highlight important considerations for future efforts, both in the valley and beyond. For one, it appears that the 100 m buffer around infected trees is insufficient to contain spread of the pathogen, especially in wet years. This finding is corroborated by the treatment efforts in Oregon, which have employed a 100 m buffer (Goheen et al. 2010), but have recently scaled up to use a 300 m buffer where feasible following observations of annual distances between diseased trees (Hansen 2008). Second, landscape-scale treatment efforts for *P. ramorum* require monitoring that is consistent, frequent, and conducted at a scale that matches the scale of pathogen invasion, allowing for detection of the cryptic spread beyond the borders of infestation. This may require skilled monitoring teams dedicated solely to following up on sudden oak death treatment efforts, something that has been absent on treated private property in California because of funding constraints--although Redwood National Park hired a team of seasonal technicians to monitor the lower reaches of Redwood Creek (downstream of the project) and conduct comprehensive surveys throughout the park. Third, early and consistent outreach to area landowners is essential to engage them in educational efforts and enlist their assistance in monitoring; this is especially important for landowners at the periphery of the treatment area. Fourth, using only a grant-funded approach to disease management, especially when many view the disease as an "emergency," will always have limitations in being able to rapidly deploy crews, allow for release time for staff to work on the disease and find sufficient funds to complete the work.

Conclusions

The Redwood Valley project is a pioneering effort in California; it is the first large-scale *P. ramorum* treatment project in the state, and it has involved an impressively diverse array of agencies, organizations, and landowners. However, the collaborators in the Redwood Valley project have faced difficult questions, and they have had to weigh the risks of managing the disease against the risks of not managing. This remains an ongoing challenge, especially in the face of continued pathogen spread in the area, but the project has engendered a strong partnership, one that will likely serve as a model for disease management in the future.

Beyond the social realm, the Redwood Valley project is also providing practical tools to encourage and inform future efforts. The project has already resulted in regulatory and funding options for *P. ramorum* treatment in California, giving landowners new incentives and opportunities for controlling the spread of the pathogen. And now, through further monitoring and critical consideration of the approach and techniques employed in Redwood Valley, the project will continue to contribute to the collective understanding of what it takes to manage and contain the pathogen. The insights gleaned from this project, in concert with the institutional changes that it inspired, will set a strong foundation for future collaboration and action within California.

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Suppression of *Phytophthora ramorum* Infestations Through Silvicultural Treatment in California's North Coast[1]

Yana Valachovic,[2] Chris Lee,[2] Brendan Twieg,[2] David Rizzo,[3] Richard Cobb,[3] and Radoslaw Glebocki[2]

Abstract

In 2006, three forested sites infested with *Phytophthora ramorum* in Humboldt County, California were subjected to different combinations of treatments designed to reduce inoculum and control spread. One treatment, consisting of removal of all California bay laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.) and tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H. Oh) trees, was applied at all three sites, and other treatments were applied as case studies at single sites. The sites were monitored for 6 years. Results to date suggest that the treatments that involved the cutting of California bay laurel and tanoak substantially reduced *P. ramorum* inoculum levels. However, in treatment areas where scattered California bay laurel trees were inadvertently missed because of a restricted time window for operations, a relatively minor component of residual California bay laurel trees may have become infected following treatment and/or harbored prior cryptic infections and subsequently spread *P. ramorum* to regenerating California bay laurel and tanoak. The data suggest that pathogen reestablishment in these sites was driven by both incomplete treatment application and spread from adjacent, untreated stands.

Keywords: *Phytophthora ramorum*, silvicultural treatment, infestation suppression

Introduction

One major goal of *Phytophthora ramorum* research is to provide scientifically tested and effective management strategies to help land managers and landowners control the pathogen and its effects on properties and landscapes of varying sizes (Valachovic et al. 2010). Various large-scale management projects have been undertaken in Oregon (Goheen et al. 2004, Kanaskie et al. 2009), the United Kingdom (Webber et al. 2010), and northwestern California (Valachovic et al., Novel approaches to SOD management in California wildlands: A case study of eradication and collaboration in Redwood Valley, this Proceedings). These projects have yielded varying levels of success, but much useful knowledge. Similarly, researchers have identified several effective approaches for protecting individual trees from *P. ramorum* infection (Garbelotto et al. 2007, Lee et al. 2011, Swiecki and Bernhardt 2010), including application of phosphite systemic fungicide to individual trees to prevent infection and removal of California bay laurel, the main wildland carrier host that supports high sporulation of the pathogen.

The field experiments described in this paper were designed to help understand the efficacy of a range of treatment techniques for controlling or containing *P. ramorum* at the scale of the small- to medium-sized individual property in order to minimize pathogen impacts and protect other stands of trees or other properties from pathogen invasion. At this scale, protection of individual trees by phosphite may be prohibitively expensive. Similarly, some silvicultural techniques may also be too costly while others may not fit all landowners, such as the use of herbicides to kill host trees or

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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control stump sprouting. The properties in this study await finer scale data collection in summer 2012, but the preliminary results presented here suggest some fruitful information for *P. ramorum* management.

Methods

Study Sites

Southern Humboldt County lies within a Mediterranean climate zone (warm, dry summers and cool, wet winters), and although our study sites range from ~13 to ~18 mi (21 to 29 km) inland from the ocean, they are considerably influenced by fog and maritime wind because of several southeast-to-northwest flowing rivers that serve as corridors to the sea. The area receives an average of 1700 mm of precipitation per year, almost all coming between November and May; average maximum temperature is 19.8 ˚C, while average minimum temperature is 6.6 ˚C (WorldClimate 2012). The underlying geology of most of the region consists of sedimentary marine deposits of the Franciscan Formation, which have contributed to the formation of inceptisols and ultisols that are slightly to moderately acidic, gravelly to loamy in texture, and well suited to timber production (Natural Resources Conservation Service 2012a, b). Vegetation types within the study areas fall within the redwood (*Sequoia sempervirens* (D. Don) Endl.) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco)-tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H. Oh) alliances (Sawyer et al. 2009); these two forest types intergrade into each other at varying elevations and on varying slope positions depending on local topography.

Treatments took place on three sites in southern Humboldt County, California: "Connick Creek," "Jay Smith," and "Salmon Creek." The Jay Smith site is owned by California State Parks and, while not developed for recreation, is managed for resource values such as old-growth redwood habitat. The other two sites are privately owned parcels and are managed for a variety of uses. The extent of detectable *P. ramorum* infestations at each site was delineated by ground surveys in 2005, and treatment areas were then outlined by creating a ~100 m buffer from all locations of symptomatic hosts.

Treatments

Because of landowner objectives, funding, and practical constraints, only one treatment (complete removal or cutting of all California bay laurel and tanoak) was replicated across sites. A few patches of bay laurel were inadvertently left within the treatment area at Jay Smith because of harvest restrictions associated with marbled murrelet (*Brachyramphus marmoratus*) nesting season prevented continued operations and a final clean-up of the site. A list of experimental treatments follows:

1. Complete California bay laurel and tanoak removal by chainsaw (Connick Creek and Jay Smith) or by herbicide of standing trees without cutting (Salmon Creek only).
2. Same as (1), but with subsequent broadcast burning (Jay Smith only).
3. Removal of California bay laurel alone by chainsaw (Connick Creek only).
4. Girdling of large California bay laurel along with fuel hazard reduction (FHR) thinning (Connick Creek only).

Data Collection

Treatment units varied in size within and among sites. For each unit, enough 0.04 ha (0.1 ac) randomly located circular plots were established to yield a 5 percent sample of its area, and plots were also established in adjacent untreated areas. Within plots, all trees >12.7 cm (5 in) diameter at breast height (DBH) were tagged and the trees and their basal sprouts were examined for *P. ramorum* symptoms. Sprout clumps and saplings were enumerated and examined in one 0.02 ha (0.05 ac) circular subplot within each of these plots, and seedlings were enumerated and examined within four 0.004 ha (0.01 ac) subplots per plot. Plots were established in January through May of 2006; the first

plot surveys were conducted pre-treatment in 2006, and similar surveys were conducted annually in 2007 through 2011. In post-treatment surveys, California bay laurel and tanoak sprouts from stumps of treated (removed or herbicide-treated) trees were also examined. Where symptoms were found, at least one tissue sample was taken from each symptomatic cohort (trees, saplings) where possible, excluding tissue from bole cankers.

A series of transects was also surveyed at Jay Smith in 2011. Transects were 6 m (18 ft) wide and spaced 80 m (262 ft) apart running through the treatment units and from 0 to 150 m (492 ft) outside the treated area (depending on land ownership). Within each transect, symptomatic hosts were recorded, and one to several tissue samples were collected where symptoms were present; multiple samples were taken where multiple symptomatic individuals occurred within a ~3 m radius. Each time a sample was collected from symptomatic tissue(s) in a ~3 m radius, surveyors then moved 15 m (50 ft) further along the transect and resumed surveying for symptoms. All tissue samples were plated on PARP medium for identification of *Phytophthora* spp. To complete fine-scale data collection at these sites, similar transects will be installed at Connick Creek and Salmon Creek in 2012. Along these transects and those at Jay Smith, details on growth type, size, and symptoms of all California bay laurel and tanoak individuals will be recorded, and tissues will be collected from every symptomatic individual encountered.

Data Analysis

Data analyses were conducted in SAS® version 9.3 (SAS Institute, Carey, SC). For logistic regression, the LOGISTIC procedure was used where data representing overall infection response (yes/no) across the entire study were modeled. Individual survey year infection data were modeled with survey year as an effect, and plots were considered repeated measure subjects, using the REPEATED option in the GENMOD procedure. Only predictor variables satisfying an alpha level of 0.05 in chi-square tests are presented. The LOGISTIC procedure was used to calculate the area under receivership operator curves (ROC scores) of model predictions.

Results

Based on logistic regression in which the response variable was whether or not a plot had *P. ramorum* detected at any time during the study, the effect of treatment (yes/no) had a significant negative relationship to infection probability (fig. 1). Probability of plot infection ranged from 14 to 36 percent for the different treatment types, as predicted by logistic regression (model ROC score = 0.74). Addition of a site by survey year interaction term to the treated (yes/no) predictor resulted in a model with good discriminatory power (ROC score of 0.81 vs. 0.64 for model shown in fig. 1) and reflects that infection levels increased strongly in 2010 and 2011 and varied among sites in some survey years (fig. 2). Logistic regression of 2011 Jay Smith transect data added to cumulative plot sample data showed that having uncut California bay laurel trees within 50 m of a sample significantly increased its probability of becoming infected—by about 20 percent (n=271 samples). By summer 2012, it had been confirmed that the vast majority of patches of residual California bay laurel trees within the treatment units and those examined outside of the treatment area (up to ~100 m outside treatment unit boundaries) were infected with *P. ramorum*.

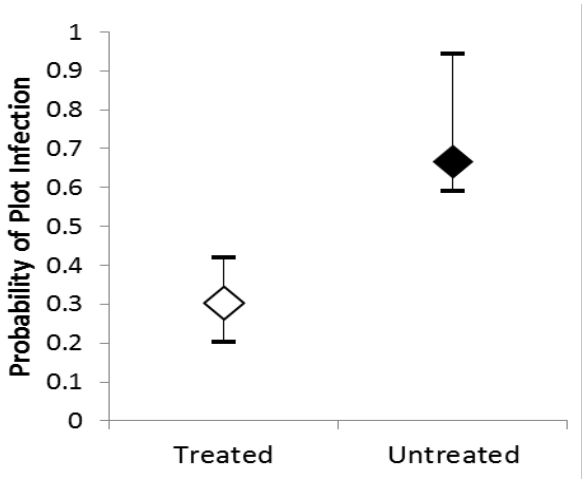

Figure 1—Probability of *Phytophthora ramorum* detection in plots at any time during the survey period, as predicted by logistic regression model. Error bars represent 95 percent profile likelihood intervals.

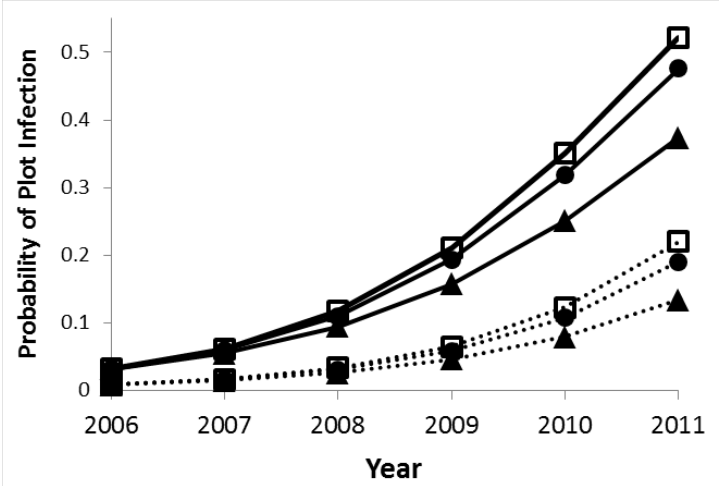

Figure 2—Probability of *Phytophthora ramorum* detection in plots by survey year and site, as predicted by logistic regression model. Squares: Jay Smith, Circles: Salmon Creek, Triangles: Connick Creek. Solid lines: untreated plots, dotted lines: treated plots.

Tanoak mortality was substantial in plots in untreated areas, averaging from 20-24 percent of tanoak trees at the three sites. In the California bay laurel removal and FHR treatments at Connick Creek, the probability of *P. ramorum* detection was not higher than in California bay laurel-tanoak removal treatments; tanoak mortality was 12 percent and 7 percent in these treatments, respectively. Tanoak trees showed many symptoms consistent with the pathogen, but retrieval of viable symptomatic tissues was rarely possible. Surprisingly, none of the California bay laurel trees girdled in the FHR treatment died.

Discussion

While the treatments did not fully control the pathogen, it is promising that they reduced the probability of plot-level infection by an average of 55 percent. Complete pathogen control in these study sites was confounded by several factors. First, it became obvious in 2007 that the pathogen had already become established in the untreated areas surrounding the treatment units that were believed

to be free of the pathogen in 2006. Infestations in these areas may have been present in 2006, but were cryptic in nature, and inoculum from more distant sources continued to arrive at the sites. Second, the few residual, uncut California bay laurel trees in treatment units at Jay Smith, along with the significant number of California bay laurel outside the treatment area, likely became infected from outside sources after treatment and may have had prior cryptic infections and/or facilitated subsequent local spread to regenerating California bay laurel and tanoak seedlings and stump sprouts in treatment units. Lastly, the herbicides used at Salmon Creek did not kill trees quickly; it took until 2009 before even half of the trees had died. This left a strong inoculum source within the treatment units to directly infect regenerating hosts in the understory. Subsequent herbicide trials have been completed, and effective approaches have been established to rapidly kill standing bay and tanoak trees (see Valachovic et al., Novel approaches to SOD management in California wildlands: A case study of eradication and collaboration in Redwood Valley, this Proceedings).

Results presented here are preliminary because transect surveys have not been completed at all sites; plot-based surveys were less effective in detecting pathogen reinvasion, likely due to spatial patchiness of infections within and outside the treatment units. This is evidenced, for example, by the fact that 2011 transect surveys at Jay Smith detected many infected individuals in one section of a treatment unit that, from permanent plot data, appeared to have very low infection levels. Due to the randomization of plots within treatment units, the infested section of the treatment unit did not include any permanent plots by chance. The converse was also true, as another unit at Jay Smith contained several plots clustered near residual bay laurel trees that became infected, and understory individuals in these plots also became infected. Further transect-based surveys in 2012 will add to this currently incomplete picture. These transect data will support more complete spatial analyses and will provide better understanding of the relationship between proximity to untreated California bay laurel trees and pathogen reestablishment in treatment areas.

Due to the fairly regular distribution and frequency of tanoak in areas adjacent to the treatments and within treatment units that did not include removal of all California bay laurel and tanoak, it was not feasible in this study to examine effects of proximity to live tanoak trees on infection probability. However, the high tanoak mortality in these areas, along with the apparent spatial dependence of understory infections on diseased canopy tanoak trees in Oregon (Peterson, Testing the importance of understory *Phytophthora ramorum* as a means of primary disease establishment in Oregon forests, this Proceedings), suggests that in the absence of tanoak removal, a substantial proportion of tanoak trees will be lost in infested zones and that the disease will be further spread to understory hosts.

Even with limitations in the data presented, results from the Jay Smith site, in particular, reinforce the importance of California bay laurel as the important driver of pathogen spread and establishment (Davidson et al. 2008) and of the need for *P. ramorum* management to include thorough treatment of California bay laurel. In southern Oregon, the pathogen has spread more than 100 to 300 m from the nearest known infection-related mortality in the previous year (Hansen 2008). These results suggest treatments should extend more than 100 m from the nearest infected tree to be most efficient in reducing pathogen populations and continued spread. The exact size of the area to be treated outside of the boundaries of observed infestations (e.g. 100, 200, 300 m buffers) necessitates further field and modeling research.

Acknowledgments

We thank property owners in southern Humboldt County as well as California State Parks for permission to conduct experiments on the properties. Individuals and agencies that assisted with project implementation or data collection include the following: J. Marshall, H. Scanlon, S. Underwood, California Department of Forestry and Fire Protection, California Department of Corrections, California State Parks, California Department of Transportation, Institute for Sustainable Forestry, T. Metz, J. Ficklin, J. Breton, H. Vaughn, B. Eastwood, D. Kahn, Elk Ridge Forestry, and Northwest Forestry and Marine. The list of collaborators is large, and we apologize for any unintentional omissions. This project was supported with funds from USDA Forest

Service, Forest Health Protection; USDA Forest Service, Pacific Southwest Research Station; and American Recovery and Reinvestment Act.

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Is Stump Sprout Treatment Necessary to Effectively Control *Phytophthora ramorum* in California's Wildlands?[1]

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Abstract

In California, wildland hosts that support sporulation of *Phytophthora ramorum*, such as California bay laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.) and tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H. Oh), also develop prolific basal sprouts following mortality, injury, or tree harvest. Assessing long-term silvicultural treatment effectiveness for *P. ramorum* control is complicated by this stimulation of basal sprouting following tree removals. To better design *P. ramorum* treatments, we need to know how sprouts regenerating from cut host tree stumps are involved in local persistence of *P. ramorum*. These sprouts could act as reservoirs to maintain inoculum levels as forests regenerate and/or serve as points of re-invasion from vegetation surrounding treatment areas.

Following host tree removal treatments of infested stands in 2006, stump sprouts showed little infection for at least 3 years, suggesting that younger sprouts were less likely to become infected, or that climate was perhaps simply not suitable for the pathogen during these initial years, or both. To help clarify these issues and determine whether manual removal of sprouts after host tree removal is necessary to control pathogen persistence and reestablishment, we established two different sprout cohorts alongside each other in 2011 in areas at three sites where hosts were removed in 2006.

One year after we established this study, it appears that California bay laurel sprouts that were manually cut in 2011 were less likely to be infected than nearby untreated sprouts that had grown for 7 years. Tanoak sprouts manually cut in 2011, on the other hand, show similar infection rates to nearby tanoak sprouts that were left uncut. At two of the sites, infection rates on both treated and untreated tanoak stump sprouts in 2012 have remained low, similar to pre-treatment levels. However, the other site presented high-infection rates in 2011 on tanoak, and both tanoak sprouts re-growing after cutting in 2011 and their 7-year-old paired sprouts were infected in 2012.

Keywords: *Phytophthora ramorum*, sudden oak death, sporulation, stump sprout treatment

Introduction

Little is known about the role that stump sprouts of tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H. Oh) and California bay laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.) play in allowing *Phytophthora ramorum* to persist or re-invade stands that have been treated by silvicultural methods. In southern Oregon, where a long-term effort has been made to eradicate the pathogen from the region of infestation, standard protocol has included use of herbicide on sporulation-supporting hosts prior to cutting; this controls the sprouting response (Kanaskie et al. 2008). This protocol was initiated because of observation of infected stump sprouts from the treated stumps; 38 out of 43 sprout clumps sampled after initial treatment and resprouting displayed foliar symptoms (Hansen et al. 2006).

Phytophthora ramorum infestations in Humboldt County occur on small private parcels, industrial

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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ownerships, as well as a variety of public land types- each with distinct goals and management interest. Landowners vary in their interest in using herbicides to control stump sprouting and because of this, a better understanding of the role of stump sprouts have in pathogen persistence, re-invasion, and spread dynamics is needed. Manual control of sprouts is an option, but this requires considerable labor, and for many it is unfeasible.

In 2006 a series of treatments were installed to control *P. ramorum* from a variety of forest types in Humboldt County. In these treatments infected tanoak and California bay laurel were removed by chainsaw without herbicide application to control sprouting. The sprouts of these stumps were used as a bioassay to monitor potential re-establishment of *P. ramorum* over time (Valachovic et al., Suppression of *Phytophthora ramorum* infestations through silvicultural treatment in California's north coast, this Proceedings). Based on presence of symptoms at these tree removal treatment units, less than 10 percent of stump sprout materials had become infected by 2010 (4 years post-treatment). This symptom-based approach likely overestimates the actual infection rate, since only about 33 percent of symptomatic individuals actually yielded the pathogen in culture during 5 years of post-treatment monitoring. This low incidence of pathogen reestablishment in treated units occurred despite rapidly rising levels of infection in areas surrounding the treatment units (about 30 percent of plots in adjacent untreated areas had *P. ram*orum confirmed) by summer 2010. This suggested that young stump sprouts were either more resistant to becoming infected or that relatively low amounts of spring rainfall in 2007 through 2009 did not provide favorable conditions for *P. ramorum* to re-invade into the treatment units, particularly given that treatments likely produced drier microclimates in these stands.

Methods

In 2011 a secondary treatment was added to *P. ramorum* suppression management units that were established in 2006 on three sites in southern Humboldt County, California: "Connick Creek," "Jay Smith," and "Salmon Creek" (Valachovic et al., Suppression of *Phytophthora ramorum* infestations through silvicultural treatment in California's north coast, this Proceedings). In these treatment units where California bay laurel and tanoak were removed (one unit per site), pairs of stump sprouts were randomly selected from the peripheries of 0.04 ha (0.1 ac) permanent circular plots used to monitor the treatments; each stump sprout group within each pair was randomly assigned to either being untreated or having all stems manually cut (treated). At Connick Creek, 19 pairs of tanoak stump sprouts were selected, but California bay laurel was not included because of its paucity in the tree removal treatment unit. At Jay Smith, 15 tanoak and 20 California bay laurel sprout pairs were chosen. Five pairs of each species were selected at Salmon Creek. At this latter site, the only unit available was one in which only a thinning of infected hosts was completed in 2006 (and in many respects serves as a partially treated control because of the high number of uncut infected trees left to impact the site, as compared to the two other sites where treatment was thorough); complete removal of tanoak and bay laurel was conducted at the other two sites.

We examined stump sprouts for symptoms and sampled tissues where *P. ramorum* symptoms were present. This was done prior to the sprout cutting treatment in 2011 and again 1 year later. We plated symptomatic tissues on PARP media for isolation of *Phytophthora* spp.

Results and Discussion

At the time that the stump sprout pairs were established in 2011, infection levels varied among sites and by 2012 showed some interesting patterns following the creation of a new age cohort of sprouts. At Salmon Creek, 80 percent of selected California bay laurel and tanoak stump sprouts were infected in 2011 and in 2012, every tanoak individual of both untreated stump sprouts and one-year-old (treated) sprouts was infected. This is in contrast to the patterns observed at the Connick Creek site, where infection levels were initially low (10 percent or less) for tanoak. Infection levels were also low

for both tanoak and California bay laurel at Jay Smith. However, while infections were observed on 80 percent of the untreated California bay laurel sprouts at Salmon Creek in 2012, none of the treated California bay laurel sprouts were infected in the same year. A similar pattern was seen at Jay Smith with California bay laurel, where infection levels were observed on 50 percent of untreated sprouts, but were observed on only at 10 percent of treated sprouts (fig. 1). Infection rates of tanoak sprouts remained low at Connick Creek and Jay Smith. Untreated California bay laurel sprouts averaged over 4.6 m (15 ft) tall by 2012 at Jay Smith.

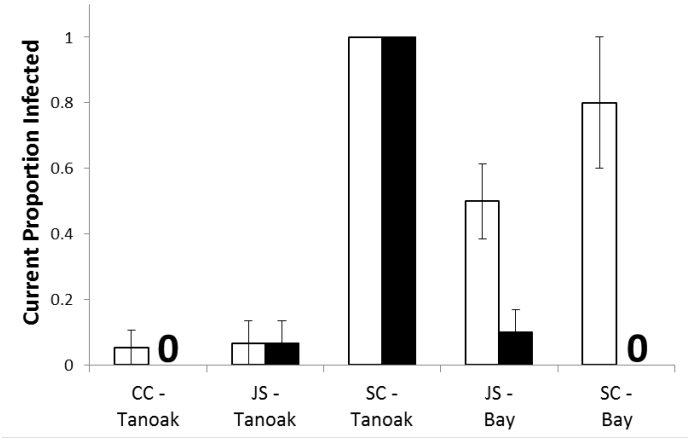

Figure 1—Proportion of stump sprouts infected by *Phytophthora ramorum* in 2012. Untreated–unfilled bars; cut in 2011–black-filled bars. Error bars plus/minus 1 SEM. Site names designated by first letter each of two-word names.

At the Salmon Creek site, the presence of infected mature California bay laurel and tanoak apparently resulted in high enough inoculum pressure that 1-year-old tanoak stump sprouts became heavily infected. We will need to monitor tanoak stump sprouts at other sites for a longer period, until more individuals become infected, to judge whether or not the age of these sprouts makes a difference to their susceptibility in units where inoculum pressure is lower due to absence of mature, sporulation-supporting hosts. On the other hand, our results to date suggest that younger California bay laurel stump sprouts are less likely to become infected than older ones. This pattern was strong at both the Salmon Creek site, where inoculum pressure was relatively high, and at Jay Smith, where low infection levels of tanoak stump sprouts in both cohorts suggest relatively low inoculum pressure. These results suggest that regular re-treatment of bay laurel sprouts may be warranted, although it may not be immediately necessary at the time of silvicultural treatments. At this time, however, data are preliminary, as this study is ongoing and requires a longer monitoring period to evaluate treatment effectiveness. We are collecting data on microclimate, weather variables, and sprout clump size, and we will examine their effects on infection rates in the future.

Acknowledgments

We thank property owners in southern Humboldt County, as well as California State Parks, for permission to conduct experiments on the properties. S. Underwood, T. Metz, H. Vaughn were especially helpful with permissions and logistics. Thanks to C. DeLong for assistance with data collection. This project was supported with funds from the USDA Forest Service, Pacific Southwest Research Station; funds were drawn from USDA Forest Service, Forest Health Protection, Pacific Southwest Region.

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The Current State of Knowledge on Operational Sanitation Measures to Lower Risk of *Phytophthora ramorum* Spread and the Need for Further Study[1]

Yana Valachovic,[2] Dave Rizzo,[3] and Brendan Twieg[2]

Abstract

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

Despite knowledge that there are distinct biological differences between *P. ramorum* and *P. lateralis*, POC RD-derived sanitation measures have been routinely recommended for SOD. While we know that *P. lateralis* has spread largely via soil-borne inoculum, the most often implicated spread mechanisms for *P. ramorum* do not include this pathway. However, infested soil has been linked to *P. ramorum* spread and persistence in nursery settings. Some epidemiology research suggests past spread in California via recreational activities, and *P. ramorum* has been isolated from boots and bike tires at several locations. Furthermore, streams carrying inoculum from wildland infestations can cause low rates of infection in nursery plants via irrigation. Still, we have little understanding of the potential of infested soil, plant debris, and water to spread the pathogen in forests and urban-wildland interfaces.

We are currently developing ideas for an integrated study that focuses on drafted water and potential soil and debris vectors associated with timber and fire management or arboriculture operations—operations that may present high-spread risk and also bear heavy costs in heavy equipment sanitation. The goal of this study is to identify sanitation measures that present the best combinations of application ease, cost effectiveness, and either elimination or minimization of spread risks.

In a small pilot study, we found that 100 percent of 400 ml samples of soil-vegetation samples (n=22) collected from heavy logging equipment operating in an infested area tested positive for *P. ramorum*. This type of equipment, particularly when transported on a trailer, can carry several hundred liters of soil from one harvest location to another. We also found that the pathogen could be isolated regularly (up to 40 percent of samples) from even very small amounts (<2 ml) of soil leftover after cleaning the majority of soil/debris from equipment. While it is difficult to translate amounts of soil/debris left on equipment to actual amounts of pathogen inoculum and corresponding spread risk, it is prudent that we make a more focused attempt at determining the best methods to quickly rid these potential vectors of viable *P. ramorum* inoculum and ensure that these methods are widely applied in forest operations.

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Monitoring the Effectiveness of *Phytophthora ramorum* Eradication Treatments in Oregon Tanoak Forests[1]

Ellen Michaels Goheen,[2] Alan Kanaskie,[3] Everett Hansen,[4] Wendy Sutton,[4] Paul Reeser,[4] and Nancy Osterbauer[5]

Abstract

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

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

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

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

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

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Effect of Phosphonate Treatments for Sudden Oak Death on Tanoaks in Naturally Infested Forests[1]

Matteo Garbelotto[2] and Doug Schmidt[2]

Abstract

Application of phosphonate compounds has been shown to be an effective preventive treatment for sudden oak death (SOD), caused by *Phytophthora ramorum*, in coast live oak (*Quercus agrifolia* Née) and tanoak (*Notholithocarpus densiflorus* Manos, Cannon & S.H. Oh). To test the effectiveness of these treatments in a natural setting, paired 400 m^2 sections of mixed evergreen/tanoak stands were randomly designated as either treatment or control plots, and topically treated with Agri-Fos® systemic fungicide. The experiment included 36 field plots in six California counties, including nearly 700 tanoak trees <8cm dbh. Both tree canopy and trunk conditions were visually assessed and scored for overall health and the presence of SOD symptoms each fall from 2006 until 2012. In the fall of 2009, five injection treatment plots, located near the existing plots, were added to the experiment.

Phosphonate treatments affected both tree mortality and spore production. The treatment plots had significantly lower mortality as well as reduced numbers of infected trees. Likewise, production of *P. ramorum* spores was reduced in the treatment plots. Since tanoaks can serve as a source of inoculum, once a stand is infested, it may be very difficult to prevent subsequent infestation of the entire stand. In general, phosphonate treatments do slow down the infection rate, even if they do not completely prevent infection.

The individual characteristics of the experimental plots also had an effect on the results. Factors such as slope, gradient, and the direction of the disease spread substantially affected disease incidence and mortality. In two cases, the experimental plots were established in areas that were already infested with *P. ramorum*, significantly reducing the effectiveness of the treatments. In addition, the results show that disease symptoms appear to advance in a punctuated rather than gradual fashion year to year.

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Long-Term Monitoring of *P. ramorum* Inoculum Identifies Spatio-Temporal Patterns of Pathogen Sporulation and Proves That Selective California Bay Laurel Removal Reduces Risk of Oak Infection[1]

M. Garbelotto,[2] S. Swain,[2] and D. Schmidt[2]

Abstract

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

1) Bait leaves were only infected when temperatures were approaching 19 °C and in the presence of rainfall.
2) Hotspots of infection (buckets with four to five leaves infected corresponding to 10^4 sporangia) were not constant, but shifted in place, indicating source trees produced high levels of inoculum at different times and only for a limited time period.
3) California bay laurel removal significantly reduced overall inoculum.
4) When California bay laurels were removed inside the treatment plots, no hotspots were ever found 20 m from the edges of any plot.
5) When California bay laurels were removed, significantly less hotspots were found 10 m from the edges of each plot.
6) California bay laurel removal never completely eliminated inoculum within treatment plots.

In 2010 and 2011, two oak (*Quercus*) inoculation experiments were performed. In both cases results showed that only suspensions of 10^4 sporangia could cause infection of oak stems, while inoculations using lower inoculum density were completely unsuccessful. We conclude that oak infection relies on warm temperatures and rainfall and that source California bay laurel trees do not produce high levels of inoculum for long periods of time. Elimination of California bay laurels 20 m around an oak will almost inevitably protect an oak from infection as inoculum density will never reach the required thresholds. However, even a buffer of 10 m will significantly decrease the number of instances where the threshold of inoculum necessary for oak infection will be reached.

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Posters

The Novel Interaction Between *Phytophthora ramorum* and Wildfire Elicits Elevated Ambrosia Beetle Landing Rates on Tanoak[1]

Maia M. Beh,[2] Margaret Metz,[2] Steven J. Seybold,[3] and David Rizzo[2]

Abstract

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

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

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

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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New Technologies to Detect and Monitor *Phytophthora ramorum* in Plant, Soil, and Water Samples[1]

Paul Russell,[2] Nathan McOwen,[2] and Robert Bohannon[2]

Abstract

The focus of our research efforts has been to develop methods to quickly identify plants, soil, and water samples infested with *Phytophthora* spp., and to rapidly confirm the findings using novel isothermal DNA technologies suitable for field use. These efforts have led to the development of a rapid Immunostrip® that reliably detects virtually all strains of *Phytophthora* spp. in plant, soil, and water samples within minutes. Two formats of a rapid molecular method were developed to accurately confirm the results. These methods are able to specifically identify *P. ramorum* in crude extracts prepared from distilled water, rain water, pond water, sandy soil and loamy soil at concentrations as low as 125pg/ul of sample. This detection paradigm allows for accurate monitoring of the location and spread of *P. ramorum*, giving field personnel the necessary tools required for mitigation actions with confidence.

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Episodic Abiotic Stress and *Phytophthora ramorum* Blight in Rhododendron: Impacts on Root Infection, Symptom Expression and Chemical Management[1]

Tatiana Roubtsova[2] and Richard Bostock[2]

Abstract

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

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

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

[1] A version of the paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Factors Influencing *Phytophthora ramorum* Infectivity on *Umbellularia californica* and Testing of a Defoliation-Based Control Method[1]

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Abstract

The primary foliar host for *Phytophthora ramorum* is California bay laurel, *Umbellularia californica* (Hook. & Arn.) Nutt., a main reservoir for the pathogen in California woodlands. We investigated environmental and pathogen-mediated influences on incidence and severity of *P. ramorum* infection of *U. californica*, as well as developing non-destructive means for controlling *P. ramorum* in woodlands.

Distribution and abundance of *P. ramorum* in California is typically assessed by counting symptomatic hosts and confirming by culturing the pathogen from field-collected samples. In 2010, extensive culturing was conducted within a previously established plot network in Sonoma County, California where *P. ramorum* has been studied since 2003. Symptomatic leaf tissues from 424 trees in 153 plots randomly distributed within a 275 km^2 region were collected. *Phytophthora ramorum* was successfully cultured from 138 trees (32.5 percent) and collected from 71 plots (46.4 percent). Culture success was greatest in the southwest portion of the study area and lowest in the northeast. Culture success was positively related to topographic moisture index and field count of symptomatic leaves at the site, and negatively related to average mean temperature at the site. Culture success in the laboratory could be used as an indicator of inoculum load in the field. Studies that rely solely on culture success to determine pathogen presence should use caution in interpreting results, as they may overlook some false negatives.

Additionally, a live plant model was developed to assess the validity of the commonly used detached leaf method for predicting interactions that occur between *P. ramorum* and foliar hosts. Specifically, infectivity of detached and attached leaves from the same *U. californica* trees in a growth chamber was assessed and compared to infectivity of detached leaves in an incubator. After 7 days, lesions were scored. Mean infection score did not differ between detached and attached leaves in the growth chamber. Detached leaves in the growth chamber and in the incubator also did not differ significantly. Despite differences in light and humidity between the growth chamber and the incubator, no differences in infection score were found between any treatments. These results suggest that the detached leaf assay is a good indicator of infectivity in live trees.

Controlled defoliation was examined as an alternative to the current practice of managing *P. ramorum* through the destruction of *U. californica* trees near symptomatic plants. Twenty-four *U. californica* seedlings were placed in six exclosures under infested canopies in the Fairfield Osborn Preserve in February 2011. Two trees in each exclosure were sprayed in May and July 2011 with ethephon, which releases ethylene upon decomposition, thereby inducing leaf abscission. Lesions were counted post treatment in January 2012. Leaves that received treatment developed significantly fewer lesions compared to the control group. Control saplings had 3.5 fold more infections than defoliated saplings. Defoliated saplings did experience excess lateral shooting, a side effect of ethephon, as well as a dead zone at the crown. Further testing could determine the long-term effects of ethephon on *U. californica* saplings and whether this dead zone is permanent or temporary. Ethephon does show promise as an alternate control method for *P. ramorum*.

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Forest Succession Following Wildfire and Sudden Oak Death Epidemic[1]

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Introduction

The Big Sur region of central California is a rugged, fire-prone area that has been severely affected by *Phytophthora ramorum*. Meentemeyer et al. (2008) estimated that over 200,000 oaks and tanoaks had been killed by *P. ramorum* across the Big Sur ecoregion by 2005. In June of 2008, a complex of lightning-initiated fires burned over 95,000 ha in Big Sur, including 43 percent of our long-term network of disease monitoring plots. Here we compile data collected in these plots before the fire in 2006 and 2007 (Metz et al. 2011), with 3 consecutive years of data following the fire (2009 through 2011).

Metz et al. (2011) demonstrated that fire severity was greater in areas recently invaded by *P. ramorum*. We further demonstrate how fire and *P. ramorum* have interacted to affect tree mortality and regeneration, and document the first stages of forest succession. The asymmetric impacts of these two disturbances across species may create significant conservation and management challenges in years to come (Metz et al. 2011).

Methods

During 2006 and 2007, we established 280 plots (each 500 m^2) to assess the ecological effects of *P. ramorum* across the Big Sur region (Metz et al. 2012). We then examined 42 plots that burned in the summer of 2008 and were re-surveyed once in 2009 and again between 2010 and 2011. Twenty plots were in redwood-tanoak forest, while 22 plots were in mixed-evergreen forest. About half of the plots (23) yielded positive culture results for *P. ramorum* at the time of establishment. This resulted in a fairly balanced study design in which disease, fire, and disease-fire interactions could be studied in natural ecosystems.

During a plot census, we recorded size and health for all stems with at least 1 cm diameter at breast height (DBH), and cultured symptomatic tissue to survey for *P. ramorum* infection. All tissue samples that were not positive in culture were genetically sequenced to screen for *P. ramorum*. We compared live and dead basal area, new stem recruitment, and plot-level *P. ramorum* infestation status among dominant tree species for all 3 survey years.

Results

Post-Fire Mortality

Grouping all host and non-host species together, live basal area was greater in uninfested plots across all years (MANOVA, P=0.0339, N=42). Regardless of infestation status, there was significant

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mortality in burned plots following the fires (P<0.0001). Total mortality was primarily driven by fire, with no discernible effect of *P. ramorum* infestation on mortality in burned plots (P=0.7152). A significant amount of standing dead basal area had fallen by our 2010/2011 sampling season, but *P. ramorum* infestation was not a significant driver of the recruitment of logs from snags (standing dead trees).

An analysis of tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H. Oh) alone yielded similar results. However, no significant difference in live tanoak basal area was observed between infested and uninfested plots (MANOVA, P=0.1299, N=42). Infestation status did not have a significant effect on the amount of standing dead tanoak overall, though a trend toward higher amounts in infested plots is suggested by the data (MANOVA, P=0.0862). Furthermore, mortality during the 2008 fires did not vary between infested and uninfested plots, but the rate of snag fall was significantly greater in infested plots (MANOVA, P=0.0376).

Post-Fire Recruitment

We analyzed the effect of *P. ramorum* infestation on the recruitment of new stems for each host species independently. New stem recruitment encompasses all stems which grew into our 1 cm minimum DBH criterion since the fire. While some of these new stems represent recruitment from saplings, most are basal sprouts from previously existing trees. For each species, we co-varied pre-fire live basal area with its corresponding density of new stems and compared them by infestation status using a GLM with a Poisson distribution.

The density of new stems was greater in 2010-2011 than in 2009 for all species, regardless of plot infestation status. All species with the exception of California bay laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.) have higher densities of newly recruited stems in uninfested plots. However, much of this difference is due to the co-linearity of tree species and pathogen distribution. In fact, *P. ramorum* infestation did not significantly affect stem recruitment for tanoak (P=0.3333, N=32). For oak (*Quercus* spp.), new stem density was higher in uninfested plots independent of initial basal area, but oak recruitment only occurred in ten plots, and was highly variable (P<0.0001, N=24).

Bay laurel had significantly higher recruitment density in infested plots, independent of pre-fire live basal area (P<0.0001, N=25). This relationship was particularly strong compared with the other species examined. The data for redwood (*Sequoia sempervirens* (Lamb. ex D. Don) Endl.) are more difficult to interpret. When we do not include pre-fire live basal area in our analysis, it appears as though new stem density is slightly higher in uninfested plots (P=0.0020, N=20). However, when initial live basal area is included as a factor, new stem density is higher in infested plots (P<0.0001). A more sophisticated analysis is needed to further separate the effects of species occurrence and pathogen impacts.

Conclusions

The collective effects of *P. ramorum* and wildfire have reduced live tree basal area across all species, but especially in oaks and tanoaks. It may be necessary to incorporate more sophisticated analyses and yet unexamined variables to conclusively explain the impact of *P. ramorum* infestation on mortality in burned plots. Plot-level infestation status may not be an adequate predictor of disease impacts due to heterogeneity in pathogen arrival date and disease severity (Metz et al. 2011). Furthermore, it may be inherently difficult to separate the effects of fire and *P. ramorum* because the reduction of live host material due to fire may temporarily diminish pathogen effects on live basal area (Beh 2011).

Higher fall rates of dead tanoak in infested plots may lead to increased woody debris accumulation and greater fire severity in the future (Metz et al. 2011). The prolific sprouting of bay laurel in

infested plots suggests it will have greater importance in the future, which may increase the long-term persistence of *P. ramorum* and increase its potential impacts.

Acknowledgments

Funding was provided by the USDA Forest Service Pacific Southwest Research Station and Pacific Southwest Region, Forest Health Protection. Special thanks to Maia Beh, Richard Cobb, Whalen Dillon, Andre Gauthier, Ashley Hawkins, Alex Hohl, Amelia Johnson, Heather Mehl, Lawrence Miracle, Akiko Oguchi, Emily Paddock, Kevin Pietrzak, and Allison Wickland for their tremendous help in the field. Additional thanks to Landel's Hill Big Creek Reserve, Los Padres National Forest, California State Parks, and the many concerned citizens and participating private landowners in Big Sur.

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Methods for Assessing *Phytophthora ramorum* Chlamydospore Germination[1]

Joyce Eberhart,[2] Elizabeth Stamm,[2] and Jennifer Parke[2]

Abstract

Germination of chlamydospores is difficult to accurately assess when chlamydospores are attached to remnants of supporting hyphae. We developed two approaches for closely observing and rigorously quantifying the frequency of chlamydospore germination *in vitro*. The plate marking and scanning method was useful for quantifying germination of large numbers of chlamydospores over a 7-day period. A method involving time lapse photography of microscope slide cultures was effective for visualizing the process of germination over shorter time periods.

Keywords: *Phytophthora ramorum*, chlamydospore, germination, assessment, banana slug

Introduction

Chlamydospores of *Phytophthora ramorum* are believed to contribute to the long-term survival of the pathogen. Understanding the factors that influence chlamydospore germination is necessary for determining their role in pathogen biology and epidemiology. Germination is difficult to assess when chlamydospores are attached to remnants of supporting hyphae. We developed two approaches to observe and quantify the frequency of chlamydospore germination *in vitro*. Colonies formed by hyphal fragments, sporangia, or zoospores were not counted.

Methods and Results

Plate Marking and Scanning Method

To separate chlamydospores from hyphal fragments and other propagules, 8- to 10-week-old broth cultures of *P. ramorum* were blended for 20 seconds, sonicated for 2 minutes, filtered through four layers of cheesecloth, and poured through a sieve (mesh opening = 106 µm). It was then filtered through 20 µm nylon mesh and the chlamydospores scraped off into V8 broth amended with 0.2 percent (w/v) agar. An aliquot of this chlamydospore suspension was spread evenly with a glass rod onto a CMA PARP plate. Using a fine-tipped marker under a dissecting scope, a small dot was made on the Petri dish next to each unattached, non-germinated chlamydospore. Marked spores were checked for germination at 3, 5, and 7 days after plating using a different color ink for each date (fig. 1). The plates were scanned and the scans saved as jpeg files. The open source image processing package FIJI (an ImageJ package) was used to count the dots. With this method we could be certain that the observed colonies were from germinating chlamydospores only, not from hyphal fragments or other propagules.

The plate marking method was used successfully to study how passage of chlamydospores through banana slugs (*Ariolimax columbianus*) affected germination of *P. ramorum* (Parke et al. 2010). Nine-

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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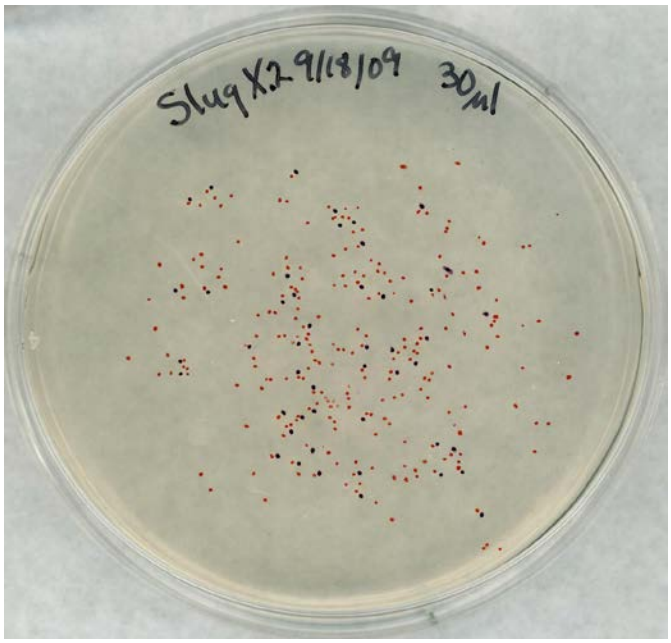

Figure 1—Plate marked with red dots to indicate initial non-germinated, unattached chlamydospores. Black dots indicate those that had germinated by a later date.

to eleven-week-old liquid cultures of *P. ramorum* isolate 4581 were processed as above, and the chlamydospore/V8-0.2 percent agar suspension was offered to banana slugs that had been deprived of solid food for 3 days. The slugs readily consumed the suspension and their feces contained large numbers of chlamydospores (fig. 2). The feces were diluted and spread on CMA PARP plates and germination was followed. Control plates of the same inoculum but without passage through slugs were made to compare germination frequencies. Results were pooled from three separate trials, each with the same three slugs.

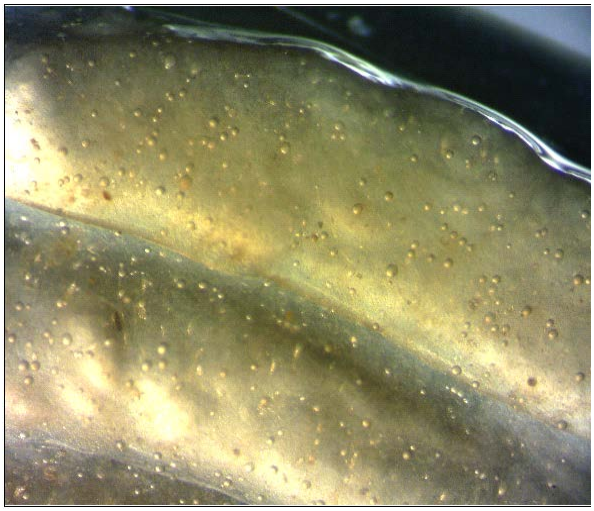

Figure 2—*Phytophthora ramorum* chlamydospores in slug feces.

After passage through slugs, average chlamydospore germination at day 7 was 22 percent, while germination on control plates was 12 percent (p = <0.0001). Results indicate that passage through slugs stimulates the germination of *P. ramorum* chlamydospores (table 1).

Table 1—Percent germination of marked chlamydospores passed or not passed through slugs

	Day 3	Day 5	Day 7
Slugs	17	21	22
Control	9	11	12

This method also gave us the ability to distinguish colonies not growing directly from a germinated chlamydospore. The percent of total colonies arising from marked chlamydospores was significantly different after passage through a slug (83 percent) than the control (68 percent) (p = <0.0001).

Time-Lapse Photography/Microscopy Method

We utilized a combination of time-lapse photography and microscopy to observe the early stages of chlamydospore germination. Chlamydospores scraped off of 2-week-old agar cultures of *P. ramorum* isolate 4581 were separated from hyphae and other structures with a blending/sonication/filtration method similar to the method described above. Chlamydospores were then placed on microscope slides dipped in CMA PAR agar. Slides were observed at 12-hour intervals for 36 hours with brightfield microscopy, using a 425 nm wave length filter. With this method it was easy to distinguish between emerging germ tubes and regrowth from subtending hyphae, allowing an accurate assessment of germination (fig. 3). Observations were discontinued after 36 hours due to hyphal growth obscuring further chlamydospore germination. At 36 hours, approximately 5 percent of the chlamydospores had germinated.

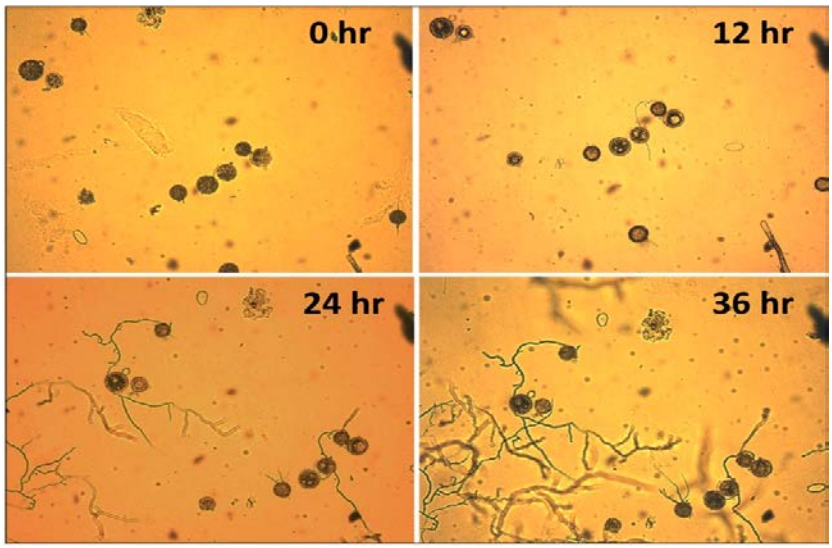

Figure 3—Time sequence of *Phytophthora ramorum* chlamydospore germination at 0, 12, 24, and 36 hours after placement on microscope slides coated with CMA PAR.

Discussion

Both methods were useful for quantifying chlamydospore germination without interference from hyphal fragments or other propagules. Time-lapse photography of microscope slide cultures was well-suited for closely observing the germination process during the first 36 hours. The plate-marking method was useful for monitoring larger populations of chlamydospores over a longer time period.

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Biological Control of Tanoak and Bay Laurel Resprouts Using the Fungus, *Chondrostereum purpureum*[1]

Marianne Elliott,[2] Gary Chastagner,[2] Simon Shamoun,[3] Grace Sumampong,[3] Ellen Goheen,[4] and Alan Kanaskie[5]

Introduction

In southwest Oregon, an aggressive program of cutting and burning host plants in an effort to eradicate *Phytophthora ramorum* was initiated. It was soon apparent that tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H. Oh) resprouts were highly susceptible to *P. ramorum* and that infected sprouts hamper eradication efforts by maintaining inoculum on site (Hansen and Sutton 2006). The basidiomycete fungus, *Chondrostereum purpureum* causes a white rot of mostly broadleaf trees and has a wide host range. It invades through fresh wounds in the xylem or cut stumps and is a weak pathogen that can survive as a saprophyte. After the host tree is weakened or killed, *C. purpureum* is quickly replaced by other, more competitive decay fungi that are naturally occurring in the environment. This fungus is used as a biological control agent for woody vegetation all over the world (Becker et al. 2005, Bourdot et al. 2006). A preparation of mycelium of the fungus *C. purpureum* is registered under the trade name "Chontrol™ Paste" in the United States and Canada for use as a biological control agent and has been tested as a stump treatment on many hardwood species (EPA Registration No. 74200-1, 2004; and PMRA Registration No. REG. 2004-09, 2004). Chontrol™ is not registered for use in California, so indigenous isolates of *C. purpureum* are being obtained and formulated for use on California bay laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.), the host responsible for *P. ramorum* inoculum buildup in California.

In fall 2009, our research team established field trials near Brookings, Oregon to assess the bioherbicidal efficacy of the fungus *C. purpureum* on tanoak to inhibit resprouts. *Chondrostereum purpureum* was found occurring naturally on tanoak logs and stumps at other sites in the Brookings area. Laboratory testing of three California isolates of *C. purpureum* indicate that the fungus can colonize bay laurel stems, and further testing under natural conditions is planned in California. If a formulated product of *C. purpureum* and/or its mixture with other stem and wood decay fungi applied to tanoak and bay laurel does inhibit the trees from growing new sprouts, this *P. ramorum* inoculum reservoir would be reduced or eliminated in the ecosystem. In areas where the application of herbicides is not prudent or permitted, this biocontrol treatment would be an indispensable alternative to chemical herbicides.

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Methods

Stump Treatments

Tanoak trees with a range of stem diameters from 5 to 45 cm (mean 20 cm) were felled in November 2009. Seven treatments (table 1) were applied to three blocks of between 18 and 21 trees per treatment.

The treatments were assessed approximately 1 and 2 years after application in September 2010 and 2011. Rather than single sprouts, the sprouting tended to occur in clumps of sprouts emerging from a single origin. These were treated as a unit. Number of live sprout clumps, number of sprout clumps dead or with dieback, height of the tallest sprout, sprout clump width, and stump diameter were measured. In addition, presence of *C. purpureum* or other decay fungi was noted.

Similar methods will be used in the bay laurel study. Depending on laboratory results, other fungi such as *Ganoderma applanatum*, in addition to *C. purpureum*, will be tested.

Table 1—Treatments applied to cut tanoak stumps in fall 2009; treatment efficacy against resprouting was evaluated in fall 2010 and 2011

Treatment	Description
Control	No treatment.
Chontrol™[a] liquid w/inoculum	Peat spray formulation containing *Chondrostereum purpureum* isolate PFC2139 10^5 to 10^7 Colony Forming Units (CFU) per L.
Chontrol™ liquid wo/inoculum	Peat spray formulation only.
Chontrol™ paste w/inoculum	Paste formulation containing *Chondrostereum purpureum* isolate PFC2139 1 x 10^2 CFU per gram.
Chontrol™ paste wo/inoculum	Paste formulation only.
Garlon 3A[b]	Apply triclopyr (Garlon® 3A (Amine)), cut 50-50 with water, plus dye to all exposed cambium immediately after cutting (within 30 minutes). Exposed cambium includes the stump surface and bark tears that occurred during falling.
Hack and squirt[c]	Inject imazapyr (Arsenal®) cut 50-50 with water, 1 hack (1 ml solution/hack) per 7.6 cm (3 inches) diameter) plus dye using the hack-and-squirt method. Hacks will be made at or below stump height of 0.46 m (1.5 ft).

[a] Chontrol™ produced by Mycologic, Inc., c/o IDC, The University of Victoria, Victoria, BC Canada V8W 2Y2. EPA Reg. No. 74200-2, EPA Est. No. 074200-CAN-001.
[b] Garlon® 3A Herbicide produced by Dow AgroScien ces LLC, 9330 Zionsville Rd., Indianapolis, IN, 46268, USA. EPA Reg. No. 62719-37.
[c] Arsenal® Herbicide produced by BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709. EPA Reg. No. 241-346.

Laboratory Testing of Isolates on Bay Laurel

The University of California (UC) Riverside sent three California native *Chondrostereum purpureum* isolates to the Pacific Forestry Center in Victoria, British Columbia. The UC Riverside identification numbers for the isolates are 2249, 2434, and 2367. These isolates were cultured on potato-dextrose-agar media. Bay laurel stems were cut and trimmed of all leaves, to a length of 8 cm. The stems were inoculated with the UC Riverside isolates and the inoculum site was sealed with parafilm. Stems were incubated in the laboratory in a moist chamber under ambient temperature and light conditions. New leaves were observed in all four groups of stems (2249, 2367, 2434, and control). Some of the stems have shown a subtle discoloration around the point of inoculation. The fungus has been re-isolated from discolored tissue.

Testing of other decay and sapwood-rotting fungi isolated from bay laurel and other hosts is ongoing. These fungi include *Ganoderma applanatum*, *G. lucidum*, and *Trametes versicolor.*

Results

There was a positive correlation between stump diameter and number of live sprout clumps (R^2= 0.685 in 2010, 0.553 in 2011). There was no significant difference in stump diameter among the treatments. Mean stump diameter was 20 cm (range 5 to 45 cm). Fewer live sprout clumps were found on tanoak stumps that received the inoculum treatments in 2011, but these differences were not significant. The two herbicide treatments had the fewest live sprout clumps.

Stumps treated with Chontrol™ formulations with and without inoculum had more dead sprouts than in the Garlon spray treatment, where there were more live sprouts in 2011. These results suggest that even though Garlon greatly inhibited sprout production compared to Chontrol™, the sprouts that were present on Garlon-treated stumps (presumably because of herbicide "misses") had less dieback than the Chontrol™ sprouts. With incomplete coverage of herbicide, fewer and healthier sprouts form than with an application of Chontrol™.

Chontrol™ formulations appear to have some effect on reducing resprouting in tanoak, but the most effective treatment is the hack and squirt method of applying the herbicide imazapyr. Over time, applications of Chontrol™ may be a more permanent solution as the stumps become decayed. Monitoring these field trials for the bioherbicidal efficacy of Chontrol™ on tanoak resprouts for a third year is planned.

Acknowledgments

The authors wish to thank Katie Coats, Katie McKeever, and Lucy Rollins, Washington State University, for molecular identification of fungi and assistance in the lab; Paul de la Bastide, MycoLogic Inc., Helga Forester, UC Riverside, for isolates; South Coast Lumber for use of the treatment site in Oregon; and Ed Orre and Angela Bernheisel, CalFire, for their assistance at the California site. Funding for this project was provided by USDA Forest Service Forest Health Protection grant #09-DG-11052021-185.

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Management of *Phytophthora ramorum* at Plot and Landscape Scales for Disease Control, Tanoak Conservation, and Forest Restoration – Insights From Epidemiological and Ecosystem Models[1]

João A.N. Filipe,[2] Richard C. Cobb,[3] Maëlle Salmon,[4] David M. Rizzo,[3] and Christopher A. Gilligan[2]

Introduction

Phytophthora ramorum has continued to spread in forests in the western United States, the United Kingdom, and the Republic of Ireland, and continues to challenge vegetation and ecosystems in temperate regions (Brasier and Webber 2010, Grünwald et al. 2012). Disease management in the wild has been applied with some success in localized outbreaks in northern California and in Oregon, and in trial treatments; for example, using host removal and host protection (phosphonate application) at plot and stand levels. However, there is still very limited observational data on the efficacy of these treatments, both at the individual-tree level and at community and landscape scales. A central question to decision-making and the deployment of resources, is how to design management strategies that have the greatest and most durable impact and the least expenditure. In order to address this question, we need to gain a better understanding of which treatments work best for a specific scale, forest composition, and set of resources available. We also need to use population models that integrate epidemiology and community ecology. In this context, two main action goals arise: short-term management to reduce disease damage and pathogen spread, and long-term management for species conservation and forest restoration. How to deploy existing tools most efficiently depends on which goal applies, on the spatial scale of the outbreak (e.g., stand with single landowner or watershed), and on practical constraints. As observation data on the efficacy of treatments at individual-tree and community levels are still very limited, we use parameterized mathematical models (Cobb et al. 2012, Filipe et al. 2012) that represent the spatial spread of the pathogen, the competing recruitment of tree species, and the changing forest composition in order to assess the efficacy of management strategies at two spatial scales.

Models Used and Scenarios Explored

Stand-Scale Management – Conservation of Tanoak

We considered the stand scale, represented by a hypothetical plot with a few hectares and a single landowner. The management goals are 1) to reduce disease in the short term, and 2) to retain the tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H. Oh) population in the long term. We used a lattice-structured metapopulation model (Cobb et al. 2012) characterized by (fig.1): 1) units (20m) of differing and changing species composition, 2) trees species that have natural death and compete for recruitment, 3) pathogen spread at local scale in bay laurel

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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(*Umbellularia californica* (Hook.& Arn.) Nutt.) and tanoak, and 4) tanoak trees that are killed by the pathogen and can sprout. We added to the model (Cobb et al. 2012) the following management strategies with treatments applied once or twice across the whole stand: 1) removal of bay laurel (curative and pre-emptive), 2) protection of tanoak (preventive, e.g., Agri-Fos®), and 3) a mixed/combined strategy.

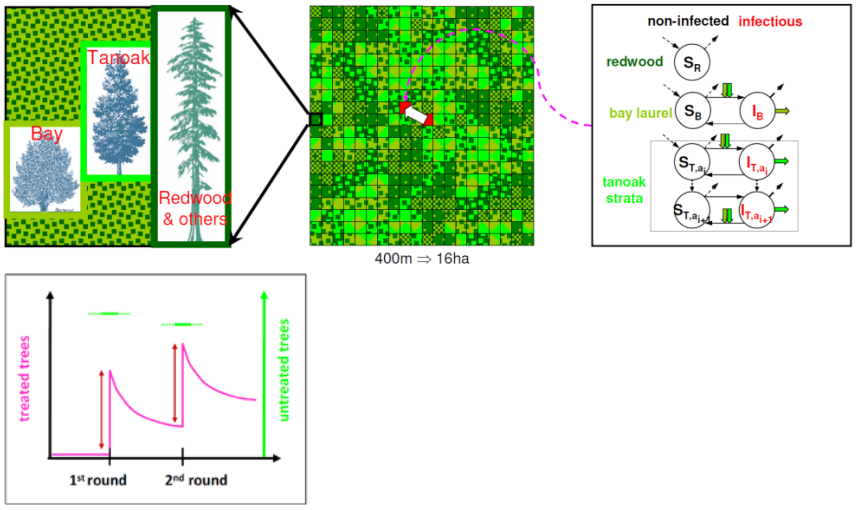

Figure 1—Stand-level epidemiological and ecosystem model, and management schedule.

Watershed-Scale Management – Restoration of Forest Services

Second, we considered the watershed scale, represented typically by a landscape with tens of km^2 and multiple landowners. We used the Mattole watershed in Humboldt County, California, as an example where a positive sample has been found and there is an opportunity to inform disease management and control. The management goals are 1) to contain the pathogen outbreak in the short term, and 2) to restore forest services in the long term through partial replacement of hosts with conifers. We used a lattice-structured metapopulation model (Filipe et al. 2012) characterized by (fig. 2): 1) units (250 m) of differing but constant species composition (CALVEG dataset), 2) pathogen spread over small and large distances, 3) specific weather conditions, and 4) cryptic infection prior to detection of symptoms (mortality) in aerial surveys. We added the following management strategies to the model (Filipe et al. 2012) with treatments applied annually in a confined zone of the host landscape: 1) detection and removal of hosts in symptomatic and adjacent units (curative and pre-emptive, with partial efficacy) (figs. 4.2-4.4), 2) forest restoration (fig. 4.5) by replacing 50 percent of the hardwood hosts with conifers ahead of the pathogen, and 3) a mixed/combined strategy (fig. 4.5). In this watershed, the outbreak develops in an area 30 km x 35 km since 2011, and management was implemented since 2012.

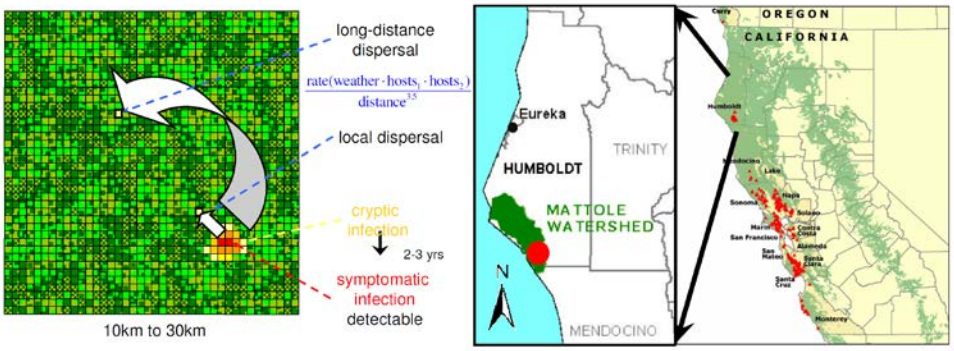

Figure 2—Watershed-level epidemiological model.

Conclusions

Stand-Scale Management – Conservation of Tanoak

Disease management that is effective and promotes tanoak conservation (increased durability[5]) requires:

1) Long-term follow-up (fig. 3A) and large coverage (percent of population treated) (figs. 3B, 3D).
2) Accompanying removal of bay laurel with herbicidal application – adding herbicide improves the durability of tanoak dramatically by preventing stump re-sprouting; alternatively, the application of follow-up treatments is the next best option (fig. 3B).
3) Specificity to forest composition (tanoak and bay laurel) prior to pathogen invasion – the extra durability of tanoak due to protective treatments declines rapidly with bay laurel prevalence (fig. 3C).
4) Combining preventive and curative treatments – offers the greatest increase in tanoak durability by combining the benefits of each treatment in a given pre-invasion forest composition (fig. 3A, 3D).

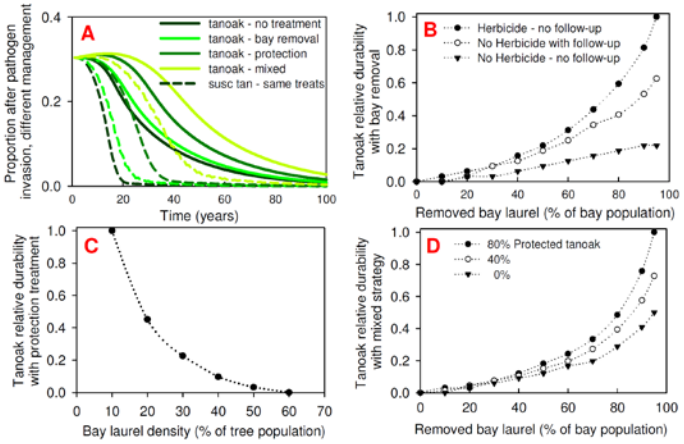

Figure 3—Results from the stand-level epidemiological and ecosystem model.

Watershed-Scale Management – Restoration of Forest Services

1) The mixed strategy, combining curative treatment and partial replacement of hosts with non-host species (fig. 4.1), is the most effective in delaying, and possibly containing, the pathogen.
2) The differing payoff of the control scenarios only becomes apparent after several years (figs. 4.2-4.5).

[5] Tanoak relative durability = rescaled time to ½ population decline.

3) Forest restoration with non-host species may be a viable long-term, commercially fruitful approach to containing pathogen spread and restoring ecological functions.
4) Cryptic infection and long-distance dispersal of generalist pathogens pose very challenging conditions to forest and disease management – it is critical to act early, to treat cryptic infections, and to protect the wide surrounding landscape (figs. 4.4-4.5).
5) We are investigating the implications of heterogeneous patterns of landownership and cooperation for the effectiveness of these forest management strategies.

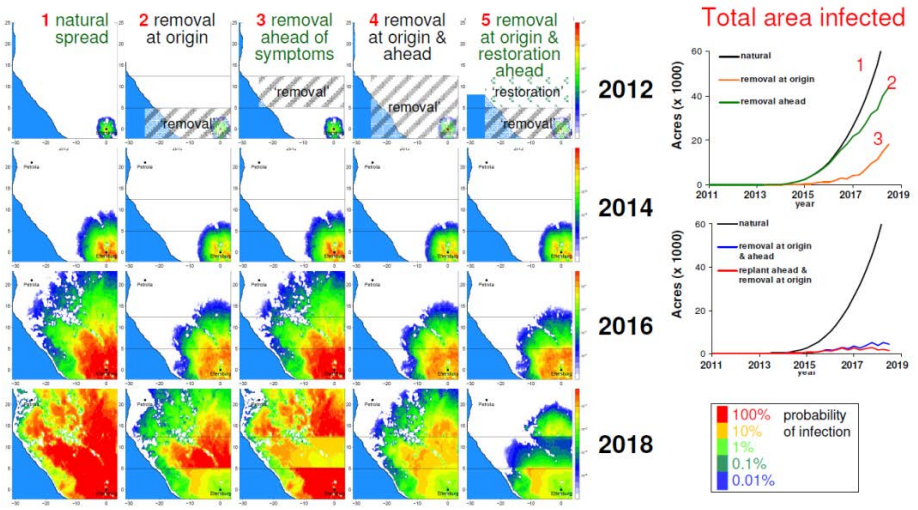

Figure 4—Results from the watershed-level epidemiological model.

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Reducing the Spread of *Phytophthora ramorum* on the Redwood Nature Trail, Rogue River-Siskiyou National Forest, Curry County, Oregon: A Case Study[1]

Ellen Michaels Goheen[2]

Abstract

In late August 2009, a 20.3 cm (8 in) diameter tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H. Oh) adjacent to a popular hiking trail on the Rogue River-Siskiyou National Forest was found infected with *Phytophthora ramorum*. The trail was immediately closed to the public. An eradication treatment consisting of injecting herbicide and cutting, piling, and burning tanoaks and other selected hosts in a 91.4 m (300 ft) radius around the infected tanoak was prescribed and completed by early winter.

Close to 487.7 m (1600 ft) of trail lies within or on the boundary of the treatment area while approximately 61 m (200 ft) of trail pass through the heart of the infested zone. Knowing the potential for *P. ramorum* to persist in soils after treatment, options to reduce the risk of human-assisted spread of the pathogen via infested trail soil were discussed. Closing the trail permanently was not considered a viable option. A previous study suggested that, due to their antimicrobial activity, western red cedar (*Thuja plicata* Donn ex D. Don) heartwood chips placed on trails could help limit the number of *P. ramorum* spores in soils and the potential for new infections from splash dispersal. As a result, a 10.2 cm (4 in) thick layer of western redcedar heartwood chips was placed on the trailhead and through the center of the treated area. The trail was reopened to public use after the chip treatment was completed.

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

Soil samples from the trail surface were wetted and baited for *P. ramorum* at Oregon State University. *Phytophthora ramorum* was recovered from at least one of the 11 samples on all occasions except July 2010 and June 2011. The number of *P. ramorum*-positive soil samples from each date tested declined from 2 out of 11 (October 2009), 5 out of 11(February 2010), and 6 out of 11 (May 2010) samples collected before-chip treatment to 1out of 11 in November 2010 and 1out of 11 samples in March 2011 after-chip treatment. All *P. ramorum*-positive samples were found within approximately 7.6 m (25 ft) from the infected tree. The majority of positive soil samples were collected within what would have been the dripline of the infected tree or "down trail" from there.

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

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Host and Habitat Index for *Phytophthora* Species in Oregon[1]

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Abstract

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

We compiled existing records from all available sources of reliably identified *Phytophthora* species from forests and forest trees in Oregon, United States. The results are summarized by host, habitat, and *Phytophthora* species in table 1. Details of specifically documented isolates, including locations, available cultures, and Genbank acquisitions (table 2), as well as citations, are included in the interactive paper available at the website ForestPhytophthoras.org. We have included isolations from soil and streams in forests that are often not associated with any specific disease symptoms. Our goal is to inventory forest *Phytophthora* species, not forest diseases. On the other hand, we have included records from forest trees growing outside the forest, as in Christmas tree plantations or in the urban landscape, for example. The result is, we hope, a more accurate representation of the ecological amplitude of *Phytophthora* and a more complete record of the sources from which they may be spread.

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

Thirty-two *Phytophthora* species, including described but not formally named taxa, have been identified associated with 25 host species from Oregon forests or forest trees. This total includes 17 species recovered from streams and 18 from forest soils, generally in the absence of noticeable disease on associated vegetation (table 3). The sampling that produced these lists is not systematic, however, and is very uneven. Only in tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H. Oh) forests of Curry County have the full range of habitats been sampled. Two large studies have focused on forest stream sampling, so the list from that habitat is perhaps most complete, but this work still covers only a small portion of the state. In contrast, there are relatively few records of *Phytophthora* associated with root rot or bole cankers of trees in the forest apart from the invasive *P. ramorum* and *P. lateralis*. This reflects the relative health of Oregon forests despite the potential susceptibility of the trees evident from nurseries and Christmas tree plantations.

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Table 1—Sample summaries sorted by host (A), habitat (B) or *Phytophthora* species (C)

(A) Host

Host	*Phytophthora* species	Habitat	Plant part
Alnus rubra	*alni* subsp. *uniformis*, *gonapodyides*, *gregata*, *pseudosyringae*, *siskiyouensis*, taxon Oaksoil, taxon *Pgchlamydo*, taxon Salixsoil, *gallica*, *siskiyouensis*	Forest	Bole, root
Notholithocarpus densiflorus	*cactorum*, *cambivora*, *cinnamomi*, *gonapodyides*, *nemorosa*, *pseudosyringae*, *psychrophila*, *ramorum*, *siskiyouensis*, taxon *Pluvialis*, taxon *Pgchlamydo*	Forest	Bole, leaf/twig
Umbellularia californica	*nemorosa*, *ramorum*, *siskiyouensis*	Forest	leaf/twig

(B) Habitat

Habitat	*Phytophthora* species	Host
Christmas tree plantation	*cactorum*	*Abies procera*
	cambivora	*Abies procera*
	cinnamomi	*Abies procera*
	megasperma	*Abies procera*
	pseudotsugae	*Pseudotsuga menziezii*
	taxon *Pgchlamydo*	*Abies procera*
Landscape	*ilicis*	*Ilex aquifolium*
	lateralis	*Chamaecyparis lawsoniana, Thuja occidentalis*

(C) Species

Phytophthora species	Hosts	Habitats	Plant parts	Counties
alni subsp. *uniformis*	*Alnus rubra*	Forest	Root	1
cambivora	*Abies procera*, *Alnus* sp., *Chrysolepis chrysophylla*, *Fagus grandifolia*, *Notholithocarpus densiflorus*	Christmas tree plantation, forest, forest soil, forest stream, urban	Bole	9
lateralis	*Chamaecyparis lawsoniana*, *Taxus brevifolia*, *Thuja occidentalis*	Forest, forest research nursery, forest tree nursery, landscape	Root	12

Table 2—Sample entries from the searchable index

Phytophthora species	Notes	Isolate	Host	Habitat	Plant part	County	Year Isolated	Culture collection	GenBank	Citation
alni spp. *uniformis*		118-R-1J3	*Alnus rubra*	Forest	Root	Lane	2011			Sims
cambivora	A1	143	*Abies procera*	Christmas tree plantation	Root	Benton	1987			Saaverdra, Chastagner
cambivora	A1	150	*Abies procera*	Christmas tree plantation	Bole	Marion	1987			Saavedra
cambivora	A2	P592	*Abies procera*	Christmas tree plantation		Oregon		ATCC46719 MYA-4076 CB114087	HQ261516	Gallegly ME, Hong C. 2006; Robideau
lateralis		T4P3	*Chamaecyparis lawsoniana*	Forest	Root	Josephine	2000		HQ643176 AY369361	Martin and Tooley, Oh
lateralis		43-3	*Chamaecyparis lawsoniana*			Oregon	1974	ATCC28511		Trione, Phytopath. 64: 1531-1533, 1974

Table 3—Thirty-two species of *Phytophthora* associated with forests in Oregon

Phytophthora species	Forest plants[a]	Forest soil	Forest streams	Cultivated and urban[b]
alni subsp. *uniformis*				
cactorum				
cambivora				
cinnamomi				
cryptogea				
europaea				
gallica				
gonapodyides				
gregata				
hydropathica				
ilicis				
lateralis				
megasperma				
multivora				
nemorosa				
nicotianae				
pini				
plurivora				
pseudosyringae				
pseudotsugae				
psychrophila				
ramorum				
riparia				
sansomeana				
siskiyouensis				
syringae				
taxon Ceanothus				
taxon Morella				
taxon Oaksoil				
taxon Pgchlamydo				
taxon Pluvialis				
taxon Salixsoil				

[a] Includes canopy drip from baited raintraps.
[b] Includes Christmas tree plantations, seed orchards, landscape plantings, forest tree and native plant restoration nurseries, and urban forests.

Scaling up from Greenhouse to Field Resistance in Tanoaks[1]

Katherine J. Hayden,[2] Richard S. Dodd,[2] Catherine Eyre,[2] Matteo Garbelotto,[2] and Jessica W. Wright[3]

Abstract

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

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

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Ethanol Attracts Scolytid Beetles to *Phytophthora ramorum* Cankers on Coast Live Oak[1]

Rick G. Kelsey,[2] Maia Beh,[3] Dave Shaw,[4] and Daniel K. Manter[5]

Abstract

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

Insect traps were installed at all three sites and baited with ethanol, ethanol+ (-)-α-pinene, or ethanol+ 4-allylanisole (4AA) lures. The three most abundant scolytids captured were *Xyleborinus saxesenii, Pseudopityophthorus pubipennis*, and *Monarthrum scutellare*, with considerable variation among sites. These species have all been trapped previously on *Q. agrifolia* trees inoculated with *P. ramorum*. Traps baited with ethanol only captured significantly more total scolytid beetles, *X. saxesenii*, and *P. pubipennis* than the traps where ethanol was combined with (-)-α-pinene or 4AA, except for *P. pubipennis* captured in traps with ethanol+4AA.

In another experiment, a 50 percent aqueous ethanol solution was sealed in the sapwood of *Q. agrifolia* logs. The bark surface immediately above the ethanol infused sapwood then received one of six treatments: 1) sprayed with an antitranspirant (Moisturin) solution to block ethanol release, 2) sprayed with (-)-α-pinene, 3) attached an ultrahigh release pouch of (-)-α-pinene, 4) sprayed with both antitranspirant and (-)-α-pinene, 5) sprayed with antitranspirant plus attached an ultrahigh release pouch of (-)-α-pinene, and 6) no bark treatment control. The bolts were placed at two of the study sites for 8 weeks (26 May to 21 July, 2011), and the number of combined bark and ambrosia beetle attacks were counted within the area of treated bark. In control logs, the number of beetles attacking the bark above the ethanol infused sapwood was 4.4 times greater than on the opposite side of the log where ethanol was absent in the sapwood. The attachment of an ultrahigh release pouch of (-)-α-pinene was the only treatment on the bark surface that impacted the beetles, reducing their densities to 19.1 percent of the attacks on logs without these pouches.

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

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Relationship Between Precipitation and Tree Mortality Levels in Coastal California Forests Infested with Sudden Oak Death[1]

Brent Oblinger,[2] Zachary Heath,[2] Jeffrey Moore,[2] and Lisa Fischer[2]

Abstract

*Phytophthora ramoru*m has caused extensive oak (*Quercus*) and tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H. Oh) mortality in portions of the central and north coasts of California. In conjunction with stream and terrestrial surveys, aerial detection surveys have played a critical role in detection and monitoring efforts associated with sudden oak death (SOD) throughout these regions. Aerial surveys conducted by the U.S. Department of Agriculture Forest Service, Forest Health Protection, have consistently documented the extent and intensity of hardwood mortality across forested areas affected by SOD since 2005. The main objective of this analysis is to determine whether oak and tanoak mortality levels, within regions infested with SOD, are related to precipitation.

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

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

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

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Verifying Critical Control Points for *Phytophthora* Introduction into Nurseries[1]

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Abstract

The Oregon Department of Agriculture implemented the Grower Assisted Inspection Program (GAIP) for nurseries in 2007. Participants in GAIP adopted best management practices (BMP) for five critical control points (CCP) (used containers, irrigation water, soil substrate, potting media, and incoming plants), where foliar *Phytophthora* can be introduced into nurseries. The goal of this study was to determine the presence or absence of *Phytophthora* at four CCP in GAIP nurseries 3- to 4-years after implementation of the program. From January to March 2011, samples were collected from irrigation water, potting media, used containers, and soil substrate at 13 GAIP nurseries. Irrigation water samples were collected from each nursery's water source. Potting media samples were collected from individual media components and from finished media. Potting media and debris were scraped from the insides of 25 used containers to create a composite used container sample per nursery. Transects were walked within each nursery, with inspectors collecting soil subsamples at three points located equidistant along each transect to create one composite soil substrate sample per transect. All samples were tested by baiting with healthy *Viburnum davidii* Franch. leaves followed by plating on PARP. A total of 354 samples were collected from all CCP checked in this study, with 30.2 percent testing *Phytophthora* positive. *Phytophthora* was detected in 10.3 percent, 30.4 percent, 36.4 percent, and 45.5 percent of potting media, used container, soil substrate, and irrigation water samples, respectively. *Phytophthora* incidence in irrigation water and soil substrate samples was significantly different from the incidence in potting media samples ($p < 0.05$), although there was no significant difference between soil substrate and used container samples. When looking at the number of nurseries with *Phytophthora* detected at each CCP, soil substrate (92.3 percent of nurseries) and irrigation water (66.7 percent of nurseries) were significantly more likely to be sources of potential contamination than potting media (30.8 percent of nurseries) and used containers (33.3 percent of nurseries) ($p < 0.05$).

Keywords: Grower Assisted Inspection Program, *Phytophthora*, nurseries, critical control points

Introduction

In 2007 and 2008, the Oregon Department of Agriculture implemented the Grower Assisted Inspection Program (GAIP) for nurseries. Participants in GAIP must adopt best management practices (BMP) for critical control points (CCP) where foliar *Phytophthora* species can be introduced into their nursery. These CCP were identified previously as used containers, irrigation water, soil substrate, potting media, and incoming plants (Parke et al. 2009). The goal of this study was to determine the presence or absence of *Phytophthora* at four of the CCP in GAIP nurseries 3- to 4-years after implementation of the program.

Materials and Methods

Samples were collected from irrigation water, potting media, used containers, and soil substrate once at each nursery from January through March, 2011 (table 1). Irrigation water samples (3.7 L or 1 gal each) were collected from each water source and tested within 48 hours of collection. Potting media samples (1000 cm^3 or 1 qt each) were collected from individual media components and from finished media. Used container samples were collected by scraping potting media and debris from inside 25 used containers to create a composite sample of 1000 cm^3 (1 qt). Soil substrate samples were

[1] A version of this paper was presented at the Sudden Oak Death Science Fifth Symposium, June 19-22, 2012, Petaluma, California.

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collected by walking transects within each nursery and collecting 350 cm^3 (0.37 qt) subsamples at three points located equidistant along each transect to make one composite sample per transect. The number of transects walked depended upon nursery size, ranging from six in nurseries <0.4 ha (1 ac) in size to 36 in nurseries 202 to 405 ha (500 to 1,000 ac) in size.

Table 1—The number of samples collected from 13 nurseries at four critical control points for *Phytophthora* introduction into nursery production systems

	Critical control points			
Nursery	Irrigation water	Used containers	Potting media	Soil substrate
86	0	1	3	13
36	8	0	2	39
53	5	1	5	32
77	5	0	6	13
10	1	1	1	6
38	2	0	1	6
88	2	1	1	8
9S	3	1	4	19
9G	3	2	4	24
9D	3	1	4	20
75	4	2	4	30
28	5	1	2	30
84	3	0	2	20

All samples were tested by baiting with healthy *Viburnum davidii* Franch leaves followed by plating on PARP (USDA APHIS PPQ 2010b, 2010c). Statistical analyses were performed using analysis of variance for a completely randomized design with unequal replication and by calculating the least significant difference between means.

Thirteen nurseries were surveyed. None adopted BMPs specifically for soil substrate, although practices adopted for other CCP could affect *Phytophthora* populations in soil. The nurseries ranged widely in size; three were ≤2 ha (5 ac), five were 4 to 40 ha (10 to 100 ac), four were 40 to 202 ha (100 to 500 ac), and one was >202 ha (>500 ac). Irrigation water sources varied by nursery, with three nurseries using well water only, two using well and river water, two using well and recycled water, two using river and recycled water, two using recycled water only, and two using water from all three sources.

Each nursery adopted BMPs for the four CCPs that worked best for their production system (table 2). For used containers, BMPs included using new containers on host and associated host plants for *Phytophthora ramorum* (USDA APHIS PPQ 2010a), steaming or chemically sanitizing pots, and recycling used pots. For potting media, BMPs included storing media on a concrete pad or other barrier, using dedicated or cleaned potting equipment, testing the media, using commercially-produced media, or steam sanitizing used media before re-use. For irrigation water, BMPs included using well water, using chemical or biological treatments, and testing the water quarterly for *P. ramorum*. Several nurseries used multiple BMPs for each CCP.

Results and Discussion

A total of 354 samples were collected from all CCPs for this study, with 30.2 percent testing *Phytophthora* positive. *Phytophthora* was detected in 10.3 percent, 30.4 percent, 36.4 percent, and 45.5 percent of potting media, used container, soil substrate, and irrigation water samples, respectively. *Phytophthora* incidence in potting media was significantly lower, and in irrigation water was significantly higher, than *Phytophthora* incidence in used containers and soil substrate samples ($p < 0.05$).

When looking at the number of nurseries with *Phytophthora* detected at each CCP, soil substrate and irrigation water were significantly more likely sources of potential contamination (fig. 1). Nine nurseries had no positive detections in their potting media samples.

Table 2—Number of nurseries adopting specific best management practices (BMP) for four critical control points (CCP) for *Phytophthora* introduction into nursery production systems

CCP	BMP	No. of nurseries adopting BMP
Irrigation water	1. Use well water	9
	2. Use chemically/biologically treated water	5
	3. Test water for *P. ramorum*	5
	4. Use multiple listed BMPs	10
Potting media	1. Store on concrete or other barrier	12
	2. Use cleaned/dedicated equipment	11
	3. Test for *Phytophthora* before use	6
	4. Steam before use	1
	5. Media from a commercial source	2
	6. Do not re-use potting media	1
	7. Use multiple listed BMPs	13
Used containers	1. Use new pots on HAP[a]	12
	2. Recycle used pots	3
	3. Steam/sanitize pots before re-use	8
	4. Used pots for non-HAP only	1
	5. Use multiple listed BMPs	11

[a] HAP = host and associated host plants for *P. ramorum*

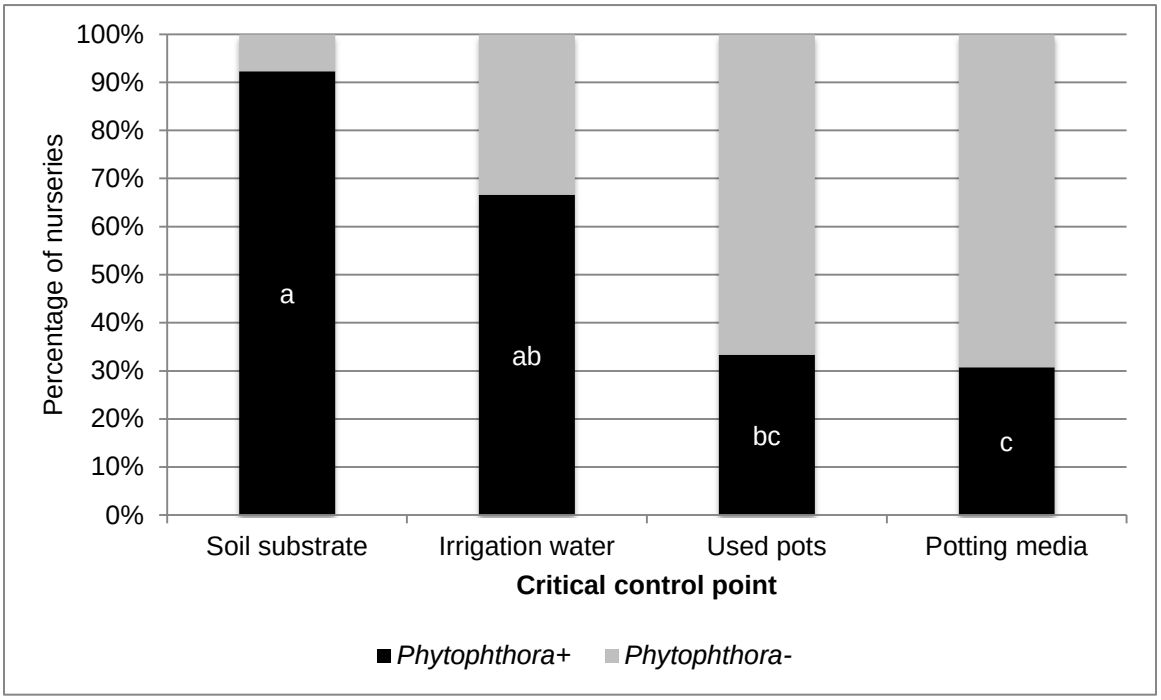

Figure 1—Percentage of nurseries with *Phytophthora* detected at four critical control points; statistical significance (p≤0.05) is indicated by different letters.

Forty-four water samples were collected for testing, with *Phytophthora* detected in 20. When examined by water source, river water and water in recycling ponds were significantly more likely to have a *Phytophthora* detection (fig. 2).

Most samples collected for testing were from soil substrate. Two hundred sixty samples were collected, with 30.4 percent positive for *Phytophthora* (fig. 3). *Phytophthora* incidence was significantly different between the nurseries, with incidence tending to be higher in larger nurseries.

Eleven samples were collected from used containers, with four positive for *Phytophthora*. Of the positive samples, *Phytophthora* was detected once after the pots had reportedly been sterilized.

Thirty-nine samples were collected from potting media components and mixtures, with *Phytophthora* detected in four. One positive sample was collected from media stored on a bark layer. Two of eight samples collected from media stored on gravel were positive. One of 27 samples collected from media stored on a concrete pad tested positive. This latter sample was collected from a nursery that did not use cleaned or dedicated potting equipment.

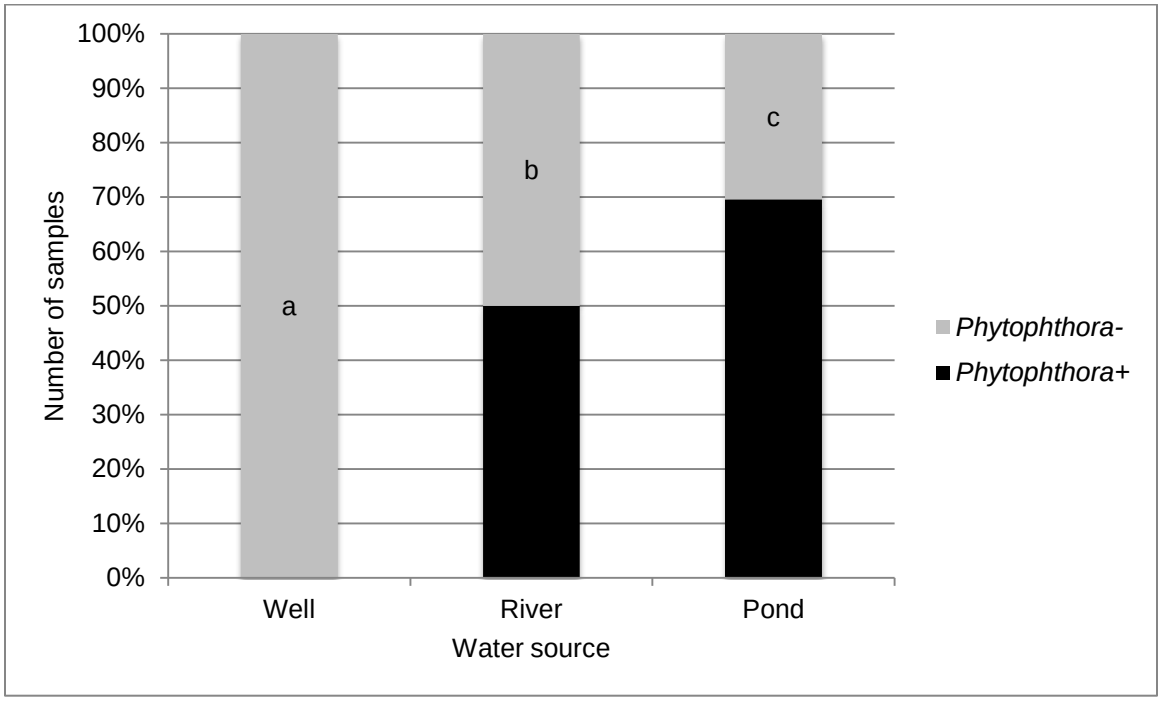

Figure 2—The number of samples testing positive for *Phytophthora* by water source; statistical significance (p≤0.05) is indicated by different letters.

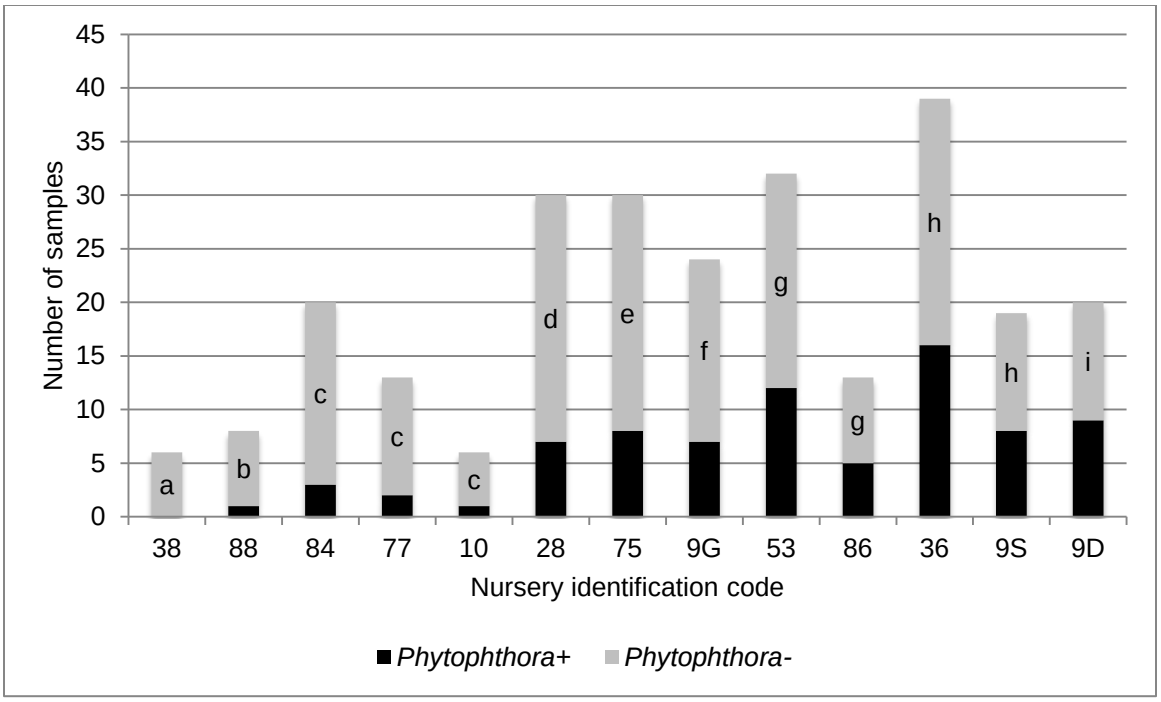

Figure 3—*Phytophthora* incidence in soil substrate samples collected and tested from each nursery; statistical significance (p≤0.05) is indicated by different letters.

The results of this study underscore the importance of these CCPs as sources of *Phytophthora* contamination within nurseries. It also highlights BMPs that effectively mitigate the risk presented by each CCP and the importance of performing BMPs correctly or using multiple BMPs for a CCP to achieve maximum protection. Although all four CCPs are important, directing resources at irrigation water and soil substrate may provide the greatest opportunity for risk mitigation in nurseries with limited resources.

Acknowledgments

The authors thank G. Roemhildt for assisting with field work and Dr. N. Grünwald for critical review of this manuscript. This work was supported by Specialty Crop Grant #ODA-20232-GA.

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Comparison of the Recovery of *Phytophthora ramorum* From Tanoak and California Bay Laurel, and the Potential Recovery of Inoculum in Fog[1]

E.K. Peterson,[2] E.M. Hansen,[3] W. Sutton,[3] P.W. Reeser,[3] and J.M. Hulbert[3]

Introduction

Oregon's sudden oak death (SOD) eradication program has focused its efforts upon the aggressive treatment of tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H. Oh) over all other host species in its efforts to control the spread of *Phytophthora ramorum*. Despite its known importance to the epidemiology of SOD as described in California, bay laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.) has been retained at some eradicated sites due to apparent lack of infection. Some of these trees have since been identified as harboring *P. ramorum*. Along with the retention of California bay laurel at some recently identified SOD sites, these circumstances have allowed us to compare rates of infection and sporulation of *P. ramorum* from tanoak and California bay laurel in Oregon. In this study, we compared incidence of *P. ramorum* infection between tanoak and California bay laurel located within one forest stand identified as positive for SOD in 2011; additionally, we compared rates of inoculum capture with open, baited buckets set underneath either host. While the collection of inoculum in baited buckets has proven to be a useful measure of detection during periods with rain, we also sought to assess the feasibility of monitoring sporulation and spore movement in precipitation resulting from fog.

Methods

Recovery From Foliage and Baited Buckets

The recovery of *P. ramorum* from foliage was assessed at an extensively infested and untreated SOD site in which both California bay laurel and tanoak were present. Between May and September 2011 we sampled 20 to 25 symptomatic tanoak sprouts from random trees at 2-week intervals. One lesion per sprout was plated in *Phytophthora*-selective media. On the last collection period, we also gathered symptomatic California bay laurel leaves in the understory of infected tanoak to determine the extent by which *Phytophthora* spp. were infecting California bay laurel within this stand.

The recovery of *P. ramorum* from rain splash was assessed by placing bait leaves of rhododendron and tanoak in plastic bags secured in screened, 4 L buckets set in SOD-positive areas. Baits have been recovered and plated in *Phytophthora*-selective media every 2 weeks since the buckets were first deployed. To monitor sporulation from California bay laurel, buckets were placed under bay laurel trees retained at multiple SOD-positive sites; sporulation from tanoak was assessed for all baits placed underneath untreated, infected tanoaks.

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Recovery From Fog Traps

Traps designed to monitor fog quantity (Schemenauer and Cereceda 1994) were adapted to monitor potential spore movement in blowing fog: three 1.5 m by 1.5 m traps were constructed using a pvc frame stretched with tan-colored, 70 percent polymer shade cloth. The bottom of the shade cloth was contained in a round 12.7 cm diameter trough. The trough was connected via 30.5 cm irrigation tubing to a closed, translucent bucket baited with rhododendron and tanoak leaves. Each screen was suspended in the opening between two Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) or alder (*Alnus* sp.) trees approximately 10 to 20 m above the ground and 15 to 25 m away from the nearest live tanoak canopy within drainages known to contain SOD. The bait leaves were processed as done for the bucket traps every 2 weeks between 16 July 2011 and 8 March 2012.

Results

Recovery From Foliage and Baited Buckets

Recovery of *P. ramorum* from emergent and aged tanoak sprouts was consistently high throughout the summer months, exceeding 90 percent for all collection dates (fig. 1). Infection by *P. ramorum* could account for only 43 percent of the lesions found on California bay laurel at this site (fig. 1). Other *Phytophthora* spp. were found infecting California bay laurel. If these species are infecting tanoak at this study location, their relative frequency is too low to be detected with our sample size (fig. 1).

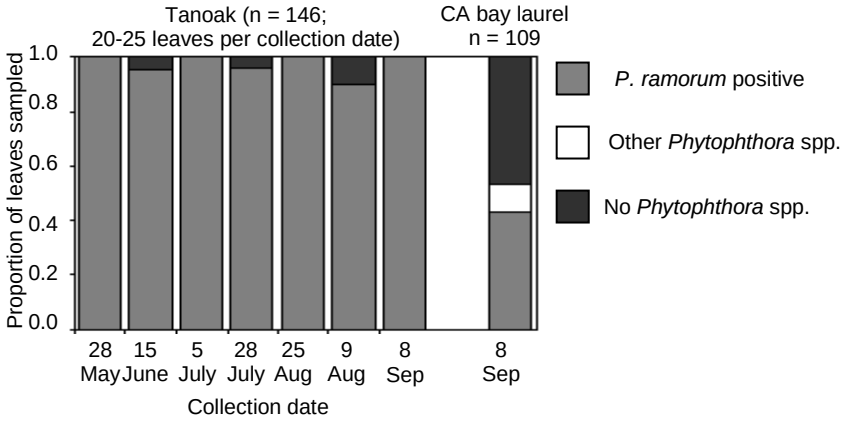

Figure 1—Recovery of *Phytophthora ramorum* or other *Phytophthora* species from symptomatic tanoak sprouts (collected between 28 May and 8 September 2011) or California bay laurel leaves (collected 8 September 2011). All samples were collected from a single untreated sudden oak death site first identified in spring 2011.

The proportion of *P. ramorum* -positive buckets ranged from 0 to 0.89 and was positively correlated to the amount of precipitation over the collection period (Pearson's r = 0.49) (fig 2a). The proportion of *P. ramorum*-positive buckets placed underneath California bay laurel ranged from 0 to 0.42, never exceeding 0.5, even during spring rains (fig. 2a). A greater diversity of *Phytophthora* spp. were recovered from buckets placed underneath California bay laurel; in contrast to previous observations in Oregon, we recovered no other *Phytophthora* spp. from buckets underneath tanoak within this study period and locations (fig. 2b).

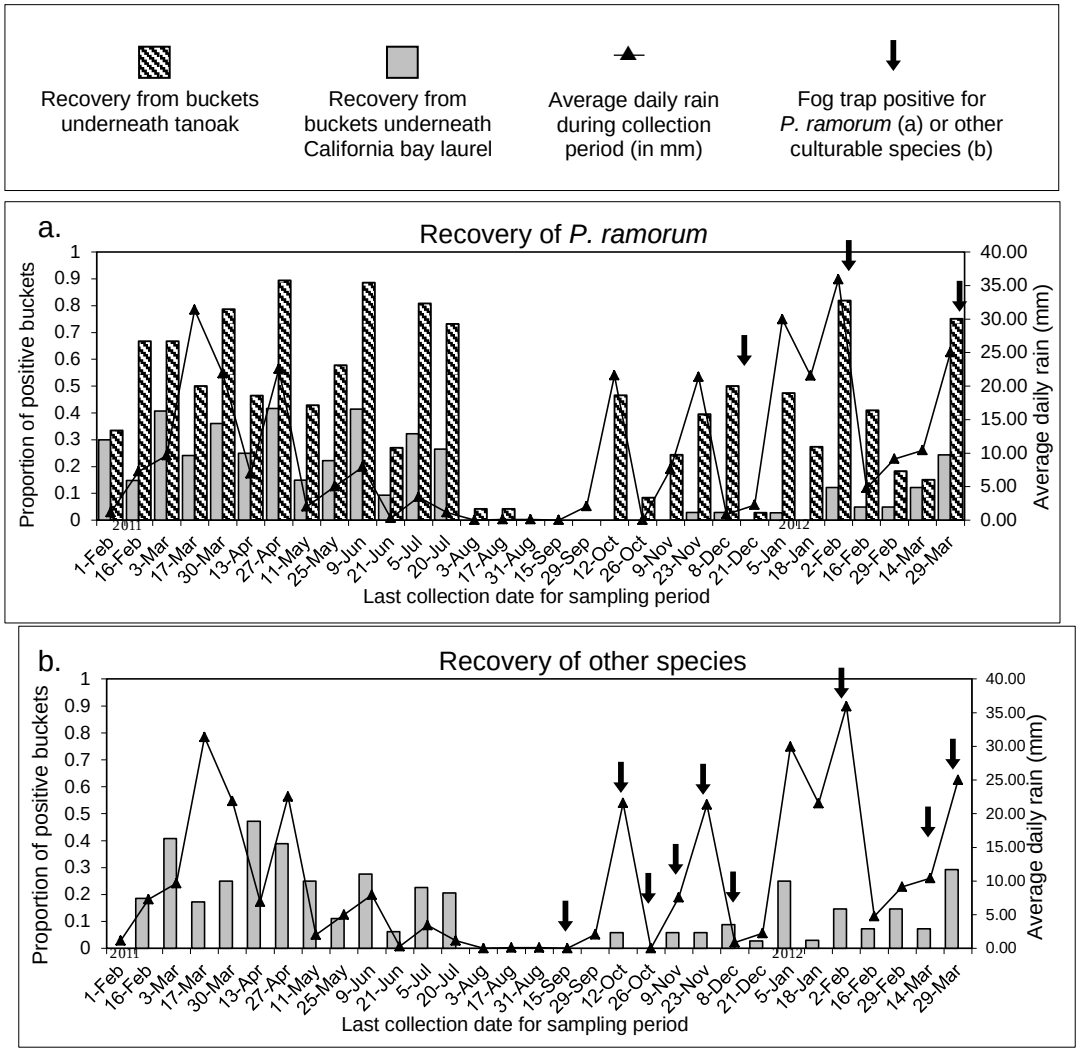

Figure 2—Recovery of *Phytophthora ramorum* (a) or other *Phytophthora* spp. (b) from baited buckets placed underneath tanoak or California bay laurel. Arrows indicate the recovery of *P. ramorum* (a) or any culturable non-*P. ramorum* species (b) from at least one fog trap. Weather data: Redmound RAWS weather station.

Recovery From Fog Traps

A low amount of precipitation reached the buckets during periods without any rain, although recovery of culturable species (*Phytophthora* or *Pythium* spp.) coincided with the onset of seasonal rains (fig. 2b). *Phytophthora ramorum* was recovered on three dates over the baiting period from two of the three fog traps (fig. 2a).

Discussion

In describing the epidemiology of SOD in California, most research has focused upon the importance of *U. californica*, predominantly because of this host's capacity to produce large quantities of sporangia (Davidson et al. 2008). In contrast, our preferential recovery of *P. ramorum* from tanoak is consistent with prior observations that *N. densiflorus* is an important contributor to the establishment and spread of SOD in Oregon. It remains unclear if the differences we observed in infection and sporulation between California and Oregon can be attributed to differences in forest composition, environment, host phenology, or the retention rates of infected foliage on either host (Davidson et al. 2011, Hüberli et al. 2011).

Despite the differences in recovery rates from buckets underneath either host, the temporal pattern of recovery is similar between this and prior studies. Davidson et al. (2008) noted that spore quantity increased over the rainy season for California bay laurel, but not tanoak. Our consistent recovery of inoculum in the autumn months from tanoak but not California bay laurel corroborates these findings. Due to differences between the methods employed in California and Oregon, however, a direct comparison is difficult. Baiting buckets with water and leaves provides a crude estimate of spore quantity. This method may increase our sensitivity during the drier months, although it is prone to saturation during times of high inoculum production.

As we only recovered *P. ramorum* in fog traps during periods of rain, we cannot conclude that movement in fog, specifically, has contributed to the capture of inoculum via this new method. While we did observe fog condensing on the screens, very little moisture was recovered in the buckets in the absence of rain. This is most likely due to rapid evaporation once the fog lifted at the sampling locations. An improvement upon our design would include the ability to periodically wash the traps and capture any rinsate, which may then be baited. Nevertheless, we have confirmed the capture of *P. ramorum* 25 m away from the nearest inoculum source.

Acknowledgments

Thanks to the USDA Forest Service, Pacific Northwest Region (Region 6) Forest Health Monitoring Program and the Pacific Southwest Research Station for funding.

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The Effects of Salinity on *Phytophthora ramorum* Viability and Infectivity[1]

Jason Preuett,[2] Daniel Collins,[2] Douglas Luster,[3] and Timothy Widmer[3]

Abstract

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

[1] A version of the paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Stream Baiting in Southern Louisiana for *Phytophthora ramorum*[1]

Jason Preuett,[2] Daniel Collins,[2] Ashley Williams,[2] Kenneth Deahl,[3] and Richard Jones[3]

Abstract

The use of stream monitoring is an important method for early detection of *Phytophthora ramorum*. Five different waterway locations representing different ecosystems and potential *P. ramorum* inoculum sources across southern Louisiana were monitored for *P. ramorum* using bait bags containing whole *Rhododendron* 'Cunningham's White' leaves from December 2010 to January 2011. After 1 week, the leaves were retrieved and 30 leaf disks (11 mm diameter) per bait bag were taken from necrotic areas of the exposed leaves and placed on a *Phytophthora*-selective agar medium (PARPH+V8) or 2 percent water agar and incubated in the dark at 20 ºC. Plates were monitored for mycelial growth, and suspected *Pythium* and *Phytophthora* species were transferred individually to V8 agar to obtain pure cultures. The pure cultures were identified using internal transcribed spacer polymerase chain reaction (ITS PCR). Thirty-four cultures containing 10 different Oomycete species were positively identified from all locations, including: *Phytophthora* sp. (2.9 percent), *P. cryptogea* (11.8 percent), *P.* taxon *sylvatica* (11.8 percent), *Pythium* sp. (14.7 percent), *Py. aphanidermatum* (2.9 percent), *Py. diclinum* (14.7 percent), *Py. litorale* (29.4 percent), *Py. sterilum* (2.9 percent), *Py. tumidum* (5.9 percent), and *Py. undulatum* (2.9 percent). The Amite River was the only stream baiting study area to contain species of *Phytophthora*. *Phytophthora ramorum* was not found.

[1] A version of the paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Germination of *Phytophthora ramorum* Chlamydospores: A Comparison of Separation Method and Chlamydospore Age[1]

Justin P. Shaffer[2] and Jennifer L. Parke[2]

Abstract

Phytophthora ramorum characteristically produces large amounts of chlamydospores *in vitro*, but the role of these propagules in the disease cycle remains unclear. Germination is difficult to observe and quantify if chlamydospores are not free of mycelium, and the low frequency of germination commonly reported suggests that requirements for germination may not have been entirely met. Here, we conducted germination experiments factoring for chlamydospore age, chlamydospore isolation method, and nutrient medium. Chlamydospore germination frequency in each of the treatments ranged from 0 to 70 percent. Results suggest that chlamydospore age, isolation method, and nutrient media significantly affect chlamydospore germination.

Keywords: *Phytophthora ramorum,* chlamydospores, germination, Oomycete, ecology.

Introduction

Chlamydospores are thick-walled, resistant structures produced by many *Phytophthora* species. These structures can be a key component in the ecology and epidemiology of *Phytophthora* species by ensuring pathogen survival during adverse conditions. While *P. ramorum* characteristically produces large amounts of chlamydospores *in vitro*, the role of these propagules in the disease cycle in both natural and managed systems remains unclear. Germination is difficult to observe and quantify if chlamydospores are not free of mycelium. Moreover, the low frequency of germination commonly reported suggests that endogenous and exogenous requirements for germination may not have been entirely met. Finally, findings of high-germination frequency by some researchers have not been universally reproducible. Reports of germination frequency vary from 5 to 10 percent on V8 (Smith and Hansen 2008) to 40 to 47 percent on V8 agar + PARPH (Tooley et al. 2008). These differences may be due to variation in inoculum type, media, or chlamydospore maturity. Given the potential importance of chlamydospores to the life cycle of *P. ramorum*, there is a need for comparison and refinement of methods regarding chlamydospore germination.

Methods

Phytophthora ramorum isolate 4581 (NA1, A2 mating type) was grown on 10 percent V8 agar to obtain 1-month- and 8-month-old cultures. Plates were sealed with parafilm to reduce formation of sporangia. Each plate was marked at 3 and 6 days to demarcate growth of similar, defined colony age. For both culture ages, two types of inoculum were prepared from within the marked areas: chlamydospores in agar and chlamydospores in suspension.

Aqueous media consisted of corn meal broth + PAR (PAR), reverse osmosis (R/O) water (H_2O), and 20 µm-filtered creek water. Each treatment was replicated five times. Treatment plates were randomized and incubated at room temperature (19 to 21 °C). Germination was quantified at 24 hours

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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by counting the number of germinated chlamydospores out of 100 using a stereomicroscope. The experiment was repeated once.

A second experiment was conducted using an identical design, but comparing only agar plug chlamydospore inoculum of young (2-week-old) and old (6-month-old) cultures, between R/O water and the same creek water. Each treatment was replicated 16 times. Treatment plates were randomized and incubated at room temperature (19 to 21 °C). Germination was quantified at 96 hours as above. The experiment was repeated once.

Results

In the first experiment (8 months vs. 4 weeks), chlamydospore germination ranged from 7 to 78 percent (figs. 1, 2). There was no significant effect of inoculum type on chlamydospore germination; however, age, aqueous media type, and the interaction between age and aqueous media significantly affected germination ($F_{1,45} = 3.79$, $p = 0.058$; $F_{2,45} = 21.44$, $p < 0.001$; $F_{2,45} = 10.06$, $p < 0.001$, respectively). In creek water and R/O water, germination frequencies for old and young chlamydospores were similar (62 to 78 percent), but in PAR germination, frequencies of young chlamydospores were reduced relative to old ones (fig. 2). In the second experiment (6 months vs. 2 weeks), chlamydospore germination ranged from 34 to 68 percent (fig. 3). Age and aqueous media type significantly affected germination ($F_{1,55} = 7.73$, $p = 0.007$; $F_{1,55} = 16.17$, $p < 0.001$).

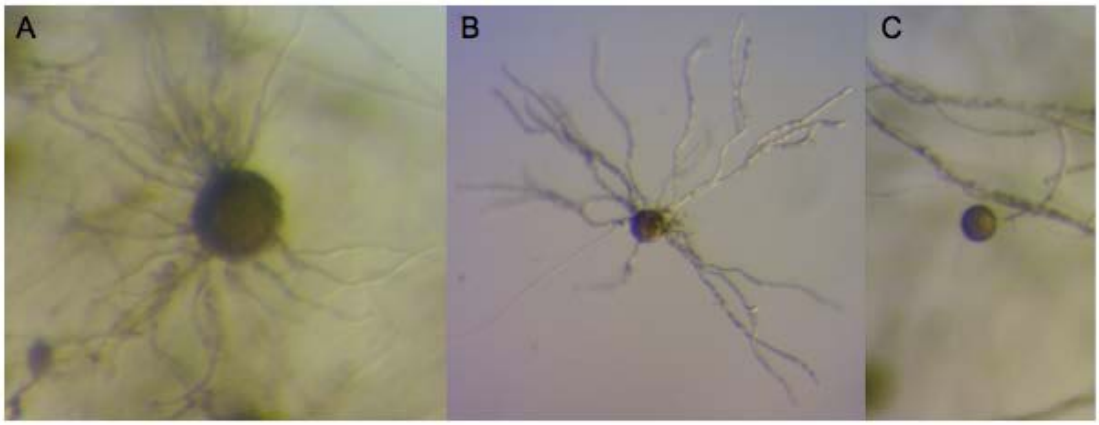

Figure 1—Chlamydospore germination at 24 hours. (A) Old (8-month) chlamydospore germinating in an agar plug submerged in creek water. (B) Old (8-month) chlamydospore from a suspension germinating in PAR broth. (C) Young (1-month) chlamydospore in an agar plug not germinated.

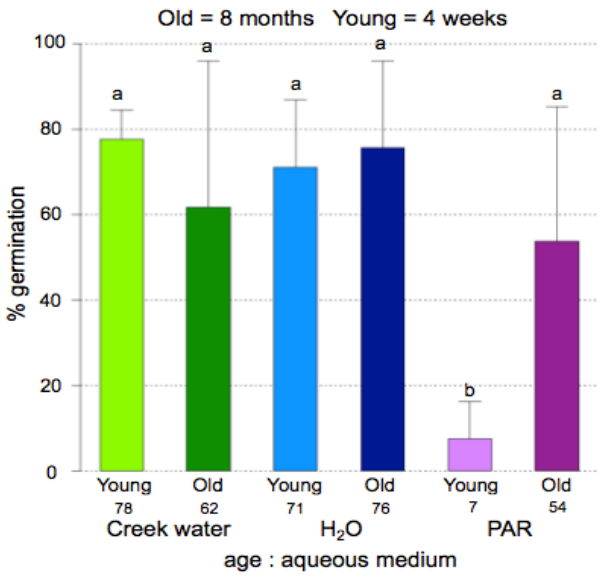

Figure 2—Chlamydospore germination (percent) among treatments after 24 hours. Data for agar plug and suspension treatments were pooled among age and liquid media combinations. Letters represent significant differences among means displayed below bars (Tukey's HSD, $p < 0.0001$).

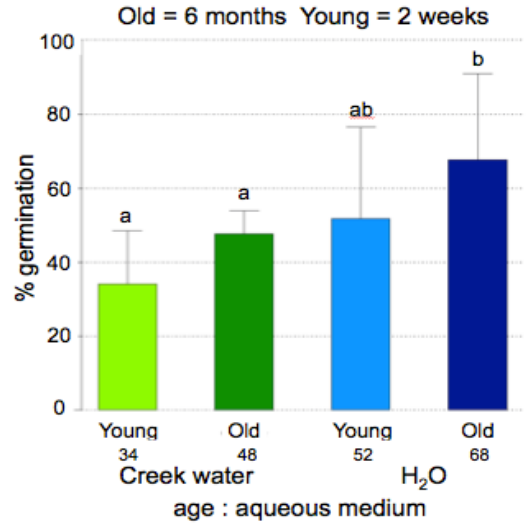

Figure 3—Chlamydospore germination (percent) among treatments at 96 hours. Letters above bars represent significant differences among means displayed below bars (Tukey's HSD, $p < 0.05$).

Discussion

Chlamydospore germination in R/O water and creek water was greater than has been reported previously for *P. ramorum* (Smith and Hansen 2008, Tooley et al. 2008). Germination in corn meal agar + PAR was similar to levels reported by Tooley et al. (2008) working with 11- to 12-week-old chlamydospores, in which 40 to 47 percent of chlamydospores germinated on V8 agar + PARPH. Germination was somewhat lower for comparable treatments in the second experiment relative to the first (figs. 2, 3), perhaps due to the younger chlamydospore ages. Chlamydospore age has been shown to influence germination frequency of other *Phytophthora* species (Tsao 1971), and chlamydospore wall thickness, which is a function of age, has been related to germination of *P. ramorum* (Smith and Hansen 2008).

Interestingly, the germination frequency of young chlamydospores incubated in creek water and R/O water was significantly greater than that of young chlamydospores incubated in corn meal broth + PAR. These results demonstrate that exogenous nutrients are not required for chlamydospore germination, but suggest that some component of corn meal broth + PAR (antibiotics and/or nutrients) reduces the germination of young (4-week-old) chlamydospores. Germination of older chlamydospores incubated in corn meal broth + PAR was not affected, implying that germination ability may be related to chlamydospore maturity.

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Effect of Herbicides on Production of Inoculum and Root Colonization of Plants Infected With *Phytophthora ramorum*[1]

Nina Shishkoff[2]

Abstract

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

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Effect of Environmental Conditions and Lesion Age on Sporulation of *Phytophthora ramorum* on California Bay Laurel, Rhododendron, and Camellia[1]

Steve Tjosvold,[2] David Chambers,[2] and Sylvia Mori[3]

Abstract

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

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

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

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Effect of Fungicides and Biocontrol Agents on Inoculum Production and Persistence of *Phytophthora ramorum* on Nursery Hosts[1]

Steve Tjosvold,[2] David Chambers,[2] Gary Chastagner,[3] and Marianne Elliott[3]

Abstract

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

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

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

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Infection of Five *Phytophthora ramorum* Hosts in Response to Increasing Inoculum Levels[1]

Paul Tooley,[2] Marsha Browning,[2] and Robert Leighty[3]

Abstract

The objective of this work was to establish inoculum density relationships between *Phytophthora ramorum* and selected hosts based on whole plant inoculations. Knowledge of levels of initial inoculum needed to generate epidemics is needed for disease prediction and development of pest risk assessments. Sporangia of six *P. ramorum* isolates representing the NA1 and EU1 clonal lineages were produced by incubating 20 percent V8-juice agar plugs containing mycelium, in 1 percent soil extract for 48 hours and adjusting the suspensions to 0, 50, 100, 500, 1,000, 2,000, and 3,000 sporangia/ml. Whole plants (2- to 3-year-old) of chestnut oak (*Quercus prinus* L.), northern red oak (*Q. rubra* L.), red maple (*Acer rubrum* L.), mountain laurel (*Kalmia latifolia* L.), and *Rhododendron* 'Cunningham's White' were dip-inoculated and incubated in a 20 °C dew chamber in darkness for 5 days. The total number of diseased and healthy leaves was recorded and leaves were scanned. A linear model, as well as, a two-parameter asymptotic regression analysis through the origin were fit to the data. For all five species, the percentage of infected leaves increased from 0 to 2,000 sporangia/ml and then leveled off. Calibration threshold estimates for obtaining 50 percent infected leaves based on the linear analysis ranged from 36 to 750 sporangia/ml for the five hosts. Half-life (LD50) estimates from the asymptotic regression analysis ranged from 94 to 319 sporangia/ml. Multiple regression analysis revealed statistically significant differences ($p = 0.0076$) among hosts in increases in infection in response to increased inoculum density. Our results provide estimates of initial inoculum levels necessary to cause disease on these five *P. ramorum* hosts and will be useful in disease prediction and for development of pest risk assessments. Spore concentrations occurring in nature have rarely been determined experimentally. Thus, it is not known whether the level of spores determined experimentally to result in a given level of disease occurs commonly in native ecosystems.

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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First Results With a Lab-on-a-Chip System for a Fast *Phytophthora* Diagnosis[1]

Sonja Horatzek,[2] Stephan König,[2] Stefan Wagner,[2] Sabine Werres,[2] Lydia Schwenkbier,[3] Karina Weber,[3] and Jörg Weber[4]

Abstract

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

Industry and academics collaborated to develop a chip-based technical platform that miniaturized hybridization and PCR on a chip (Julich, S.; Riedel, M.; Kielpinski, M.; Urban, M.; Kretschmer, R.; Wagner, S.; Fritzsche, W.; Henkel, T.; Möller, R.; Werres, S. 2011. Development of a lab-on-a-chip device for diagnosis of plant pathogens. Biosensors and Bioelectronics. 26(10):4070–4075). This technology will be improved and different techniques for sample preparation will be tested. First results on the specificity of the developed probes tested with *in vitro* samples will be presented. Both projects were funded by the German Federal Office for Agriculture and Food.

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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Survey of Eastern U.S. Native *Rhododendron* spp. for Antagonistic Endophytes Towards *Phytophthora ramorum*[1]

Timothy L. Widmer[2]

Abstract

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

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

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

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[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

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